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The Pseudorabies Virus gII Gene Is Closely Related to the gB Glycoprotein Gene of Herpes Simplex Virus

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We have looked for conserved DNA sequences between four herpes simplex virus type 1 (HSV-1) glycoprotein genes encoding gB, gC, gD, and gE and pseudorabies virus (PRV) DNA. HSV-1 DNA fragments representing these four glycoprotein-coding sequences were hybridized to restriction enzyme fragments of PRV DNA by the Southern blot procedure. Specific hybridization was observed only when HSV-1 gB DNA was used as probe. This region of hybridization was localized to a 5.2-kilobase (kb) region mapping at approximately 0.15 map units on the PRV genome. Northern blot (RNA blot) analysis, with a 1.2-kb probe derived from this segment, revealed a predominant hybridizing RNA species of approximately 3 kb in PRV-infected PK15 cells. DNA sequence analysis of the region corresponding to this RNA revealed a single large open reading frame with significant nucleotide homology with the gB gene of HSV-1 KOS 321. In addition, the beginning of the sequenced PRV region also contained the end of an open reading frame with amino acid homology to HSV-1 ICP 18.5, a protein that may be involved in viral glycoprotein transport. This sequence partially overlaps the PRV gB homolog coding sequence. We have shown that the PRV gene with homology to HSV-1 gB encoded the gII glycoprotein gene by expressing a 765-base-pair segment of the PRV open reading frame in *Escherichia coli* as a protein fused to β-galactosidase. Antiserum, raised in rabbits, against this fusion protein immunoprecipitated a specific family of PRV glycoproteins of apparent molecular mass 110, 68, and 55 kilodaltons that have been identified as the gII family of glycoproteins. Analysis of the predicted amino acid sequence indicated that the PRV gII protein shares 50% amino acid homology with the aligned HSV-1 gB protein. All 10 cysteine residues located outside of the signal sequence, as well as 4 of 6 potential N-linked glycosylation sites, were conserved between the two proteins. The primary protein sequence for HSV-1 gB regions known to be involved in the rate of virus entry into the cells and cell-cell fusion, as well as regions known to be associated with monoclonal antibody resistance, were highly homologous with the PRV protein sequence. Furthermore, monospecific antibody made against PRV gII immunoprecipitated HSV-1 gB from infected cells. Taken together, these findings suggest significant conservation of structure and function between the two proteins and may indicate a common evolutionary history.

The surface glycoproteins of herpesviruses play pivotal roles in the infectious process, viral pathogenesis, and the interaction of the virus with the immune system of the host (43). The constraints imposed by structural requirements of the virus, as well as needs for the unique parasitism of cells by alphaherpesviruses, suggests that certain protein domains, or even entire glycoprotein-coding sequences, may be conserved among certain herpesviruses. Considerable evidence is accumulating that supports this hypothesis (12, 18, 28–30, 33, 37, 41, 45).

Pseudorabies virus (PRV) is an alphaherpesvirus that causes a disease of economic importance in swine (13). While sharing overall similarity in structure to the general family of herpesviruses, PRV is morphologically related to varicella-zoster virus (44) and equine herpesvirus type 1 (15).

PRV has been reported to synthesize several major and minor glycoproteins that are localized in viral envelopes as well as the plasma membrane of the infected cell (1–3, 14, 18, 34, 38). In addition, one PRV-encoded glycoprotein is released into the medium (36). Several of these glycoproteins are apparently linked through disulfide bonds (14, 21). To date, six PRV glycoprotein genes have been localized on the genome (25, 26, 33, 34, 36, 37, 47).

Evidence that PRV shares some homology with other herpesviruses at the DNA and protein level has been reviewed (1, 4). Recently it has been reported that the glycoprotein C and D genes of herpes simplex virus (HSV) share homology with the PRV gIII gene (37) and PRV gp50 gene (33), respectively. Of particular interest for this report are the observations that the HSV glycoprotein B gene shares considerable homology with certain glycoproteins of Epstein-Barr virus (30), varicella-zoster virus (12), bovine mammilitis virus (41), and equine herpesvirus type 1 (41).

In this report we describe our search for homology between PRV and HSV type 1 (HSV-1) glycoproteins by using four cloned HSV-1 glycoprotein genes as probes. Only the HSV-1 glycoprotein B gene hybridized to PRV DNA under stringent conditions. The PRV DNA showing this homology was localized and demonstrated to encode the PRV gII gene.

(A preliminary report of these findings was presented at the 1985 Herpesvirus Meeting, Ann Arbor, Mich.)

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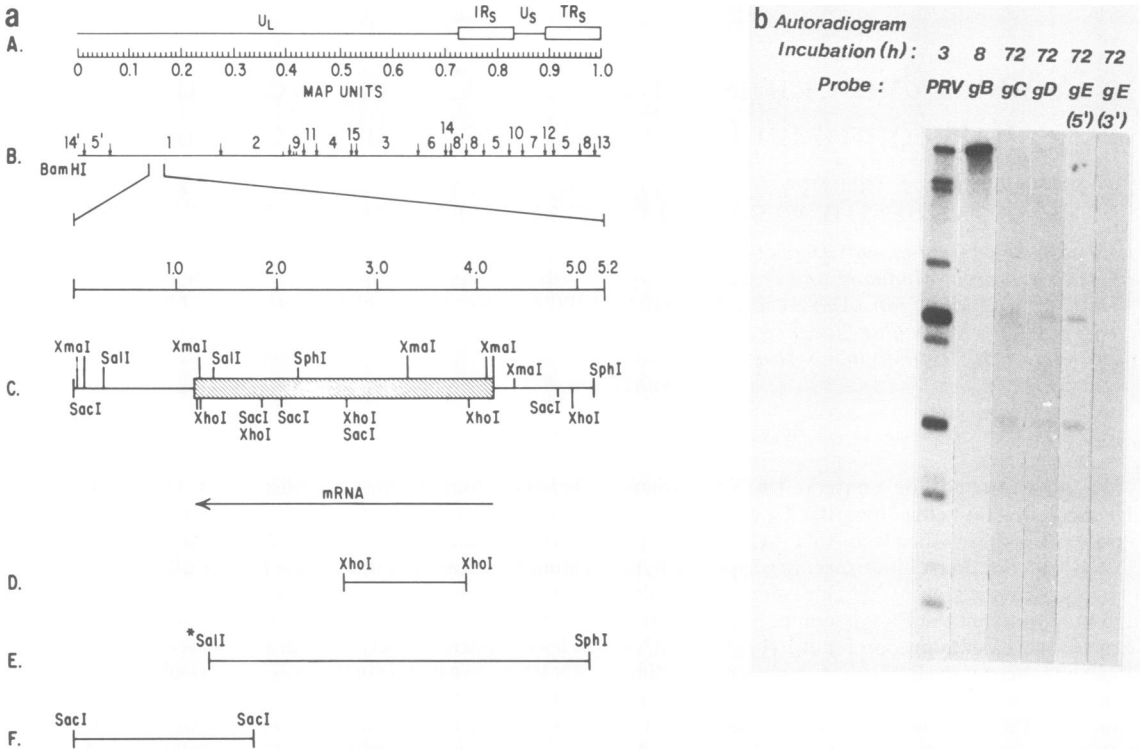

FIG. 1. Map of the PRV genome indicating relevant restriction enzyme sites and DNA fragments used to study the PRV gII glycoprotein gene. (a) Line A, Genome of PRV depicting the U_L and U_S regions as well as the inverted terminal repeat sequences bracketing U_S (IR_S and TR_S, respectively). The genome is divided into map units as indicated. Line B, *Bam*HI restriction map of the PRV gemone. Line C, The region encompassing the PRV gII gene is expanded and detailed as a 5.2-kb *Sac*I-to-*Sph*I fragment with relevant restriction enzyme sites noted. ▭, gII glycoprotein gene, the sequence of which is given in Fig. 3. The mRNA transcript for this gene and its orientation are given below the line. Line D, Specific nick-translated probe used to identify the size of the mRNA transcript in this region of the PRV genome. Line E, The specific end-labeled probe used to establish the 5′ mRNA termini and direction of transcription. Line F, The 1.8-kb *Sac*I PRV DNA fragment containing the 3′ end of the gII gene that was cloned into polink 26 (37) to facilitate DNA sequencing. b. Southern blot analysis of PRV DNA. PRV DNA was digested with *Bam*HI and fractionated by electrophoresis on a 1.2% agarose gel. Southern blot analysis was done as described in Materials and Methods. Individual nitrocellulose strips and hybridized at 42°C in 40% formamide with nick-translated DNA containing HSV-1 glycoprotein gene sequences (Table 1) and subsequently prepared for autoradiography. The lane labeled PRV contained nick-translated PRV DNA as probe. After hybridization, the strips were washed and exposed to Kodak X-Omat AR film for the indicated times. Note that the last four lanes were exposed nine times longer than the second lane.

MATERIALS AND METHODS

Cell culture and viruses. Swine kidney cells (PK15) were grown in Dulbecco modified Eagle medium containing 10% heat-inactivated fetal bovine serum as described previously (37, 38). The Becker strain of PRV has been described previously (38). Vero cells were grown in Eagle minimum essential medium supplemented with 10% fetal calf serum as described peviously (23). HSV-1 KOS 321 was described previously (23).

Preparation of cell extracts for immunoprecipitations. The methods described by Robbins et al. (38) for preparation of cell extracts for immunoprecipitation were followed.

Analysis of nucleic acids. The methods for Southern blot analysis of PRV DNA and Northern blot (RNA blot) analysis of viral RNA in infected cells have been described previously (37).

Bacterial strains. *Escherichia coli* NF1829 is strain MC1000 carrying an F′ plasmid with the *lacI*^{q} mutation that results in overproduction of the Lac repressor. The F′ also carries a *lac* operon with a Tn*5* transposon in *lacZ*.

Plasmid construction and expression of the PRV open reading frame in *E. coli*. The vectors and methods used in this report for plasmid construction are identical to those used by Robbins et al. (37, 38).

Plasmid pALM45 was constructed as follows. Plasmid pALM46 containing the 9.5-kilobase (kb) *Sal*I 3 fragment of PRV was partially digested with the restriction enzyme *Sph*I and subsequently digested to completion with *Sal*I. The resultant DNA was fractionated on a 1% agarose gel, and a 3.4-kb *Sal*I-to-*Sph*I DNA fragment was purified and ligated into the *Sal*I-to-*Sph*I sites of pBR322. After $CaCl_2$ transformation, a plasmid designated pALM45 was isolated that contained the 3.4-kb *Sal*I-to-*Sph*I PRV DNA fragment shown in Fig. 1a, line E.

Plasmid p1800T was constructed as follows. Plasmid pALM47 containing the *Bam*HI 1 fragment of PRV genomic DNA inserted into the *Bam*HI site of pBR322 was digested with *Sac*I. The resulting DNA digest was fractionated on a 1% agarose gel, and an 1,800-base-pair (bp) *Sac*I DNA fragment was purified and subsequently inserted into the *Sac*I site of polink 26 (37). A plasmid designated p1800T was isolated and was shown to carry the desired 1,800-bp PRV *Sac*I fragment shown in Fig. 1, line F.

Expression plasmid pALM9 was constructed in two steps as follows. First, pALM45 DNA was digested with the

TABLE 1. HSV-1 clones used as probes

Gene[a]	Clone	Ends	Size (kb)	Map units	Reference
gB	pSG18-St11	*Sst*I	2.8	0.351–0.368	17
gC	pFH-60	*Sal*I	3.7	0.620–0.645	18
gD	pSG25-St27	*Sst*I	2.9	0.907–0.923	21
gE-5′	pSG25-St23	*Sst*I	1.9	0.923–0.935	21
gE-3′	pSG25-B362	*Bam*HI	1.9	0.938–0.952	21

[a] The gD and gE clones described in this table are equivalent to pRB309 (gD), pRB308 (gE-5′), and pRB124 (gE-3′) described in reference 21.

restriction enzyme *Xma*I and fractionated on a 1% agarose gel. A 765-bp DNA fragment located between 211 and 976 bp (see Fig. 3) was purified and subsequently inserted into the *Xma*I site of the pJS413 expression vector (38). Plasmids resulting from this ligation were introduced into strain NF1829 by $CaCl_2$ transformation followed by selection for ampicillin resistance. A plasmid was chosen that had the PRV DNA fragment inserted in the correct orientation such that the 5′ portion of the PRV open reading frame was in phase with the *cro* leader sequence. Subsequently this plasmid was digested with the restriction enzymes *Bgl*II and *Bam*HI, and the 765-bp fragment now having *Bgl*II and *Bam*HI ends was inserted into the expression vector pHK412. Following $CaCl_2$ transformation of NF1829 cells, a plasmid was isolated that expressed a Cro-PRV-β-galactosidase fusion protein. This plasmid was designated pALM9.

Preparation of antisera directed toward the Cro-gII-β-galactosidase fusion protein. The Cro-gII-β-galactosidase fusion protein was partially purified from a culture of *E. coli* carrying the plasmid pALM9 and then injected into rabbits as described previously (37).

Antibody reagents. The monoclonal antibodies M2 and M3, specific for gII, are described in reference 14 and were provided by T. Ben-Porat, Vanderbilt University. The monoclonal antibodies B3, B4, B6, and B7, specific for HSV-1 gB, are from the laboratory of J. Glorioso, University of Michigan, Ann Arbor. The polyvalent anti-PRV gII serum 284 against purified gII proteins was made in goats by using the M2 monoclonal antibody for purification. The polyvalent anti-HSV-1 gB sera 790 and R1156 were kindly provided by R. L. Burke, Chiron Corp., and R. Courtney, Louisiana State University Medical Center, Shreveport, respectively.

DNA sequence. The DNA sequence of PRV Becker DNA fragments was determined by the method of Maxam and Gilbert (24). The DNA sequence of HSV-1 KOS 321 DNA fragments was determined by the dideoxy method (40) with ^{35}S-labeled nucleotides (5). An alternate precursor, dITP (27), was substituted for dGTP to eliminate band compressions through G + C-rich segments of the sequence.

RESULTS

Identification of PRV genomic DNA encoding the HSV-1 gB homolog. DNA from PRV virions was digested with *Bam*HI, fractionated on a 1.2% agarose gel, transferred to nitrocellulose (42), and hybridized with ^{32}P-labeled plasmid DNA samples containing HSV-1 DNA sequences for the glycoprotein genes gB, gC, gD, and gE (Table 1 as described in Materials and Methods. Specific hybridization was observed only when using the plasmid pSG18-St11, which contains HSV-1 gB DNA sequences. The hybridization was localized to the *Bam*HI 1 fragment of PRV genomic DNA (Fig. 1a). Further Southern blot analysis with the same probe demonstrated hybridization predominantly to the 9.5-kb *Sal*I 3 fragment (data not shown). The *Sal*I 3 fragment was subsequently cloned into the *Sal*I site of pBR322 and designated pALM46. Plasmid DNA from pALM46 was further analyzed by Southern blotting (42). For these experiments, the probe was a purified DNA fragment from pSG18-St11 containing HSV-1 gB DNA sequences (Table 1). The plasmid containing the *Sal*I 3 fragment was digested (*Sal*I, *Pst*I-*Sal*I, *Pvu*I-*Sal*I, or *Pvu*II-*Sal*I) and fractionated on a 1% agarose gel. Southern blot analysis showed that the ^{32}P-labeled gB probe hybridized near the left end of the *Sal*I 3 fragment (data not shown).

Analysis of gII RNA from PRV-infected cells. To determine what RNA species were transcribed from this region, we constructed plasmid pALM45, which contained the 3.4-kb *Sal*I-to-*Sph*I DNA fragment present within the 9.5-kb *Sal*I 3 fragment (see Materials and Methods). RNA from PRV-infected cells was then analyzed by Northern blot analysis with a nick-translated PRV fragment carried by pALM45 as a probe.

Total cytoplasmic RNA was extracted from PRV-infected cells at 4, 8, and 16 h postinfection and analyzed by the Northern blot technique as described in Materials and Methods. This RNA was probed with a 1,200-bp *Xho*I DNA fragment from pALM45 (Fig. 1a, line D) labeled with ^{32}P by nick translation. The results are shown in Fig. 2. The probe hybridized predominantly to an RNA species of approximately 3 kb that appeared most abundant at 8 h postinfection but was clearly identified as early as 4 h postinfection. No hybridization to uninfected cell RNA was observed.

The direction of transcription and the approximate location of the 5′ and 3′ ends of the RNA transcript delineated by the previous hybridization experiments was defined by S1 analysis as described previously (37). Plasmid pALM45

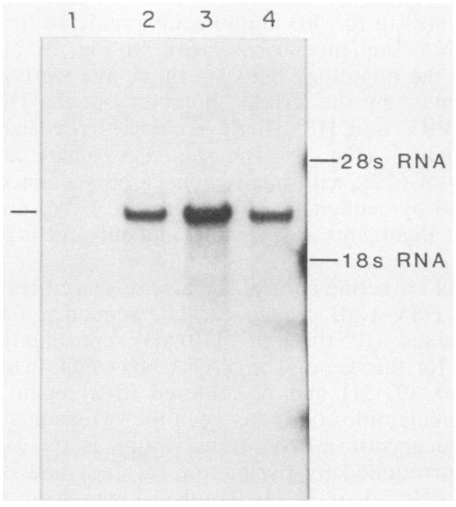

FIG. 2. Northern blot analysis of RNA extracted from PRV-infected PK15 cells. Total cytoplasmic RNA was extracted from uninfected and PRV-infected cells at various time points postinfection and fractionated on an agarose-formaldehyde gel as described in Materials and Methods. The RNA was transferred to a nitrocellulose membrane and hybridized with nick-translated DNA as shown in Fig. 1a, line D. Lanes: 1, RNA extracted from uninfected cells; 2, 3, and 4, RNA extracted from cells 4, 8, and 16 h, respectively, after infection with PRV. The hybridized blots were washed and prepared for autoradiography as described previously (37, 39). The size of the RNA fragment (indicated by the bar) hybridizing to the radioactive probe was estimated by using ^{32}P-labeled RNA markers as indicated.

DNA was cut with *Sal*I to produce a linear fragment, and the 5′ and 3′ termini were labeled with ^{32}P and subsequently cut with *Sph*I to yield 3.4-kb *Sph*I-*Sal*I fragments labeled at the 5′ or 3′ end (Fig. 1a, line E). The end-labeled DNA was then hybridized to total cytoplasmic RNA extracted from PRV-infected cells 8 h after infection. RNA-DNA hybrids were treated with S1 endonuclease and fractionated by electrophoresis on a 1.4% alkaline agarose gel. Only DNA with the 5′ label was protected from S1 digestion, and the protected fragment was approximately 2.7 kb (data not shown). From these experiments, we inferred that the direction of transcription of the gene encoding this RNA is from the *Sph*I site toward the *Sal*I site (Fig. 1a, lines C and E). Moreover, the 5′ terminus of this RNA is located 2.7 kb from the *Sal*I site (Fig. 1a, lines C and E), and the 3′ end of the transcript is just beyond the *Sal*I site and is not contained in pALM45.

On the basis of this RNA analysis, we then devised a sequencing strategy to analyze the gene(s) homologous to HSV-1 gB that are present in this PRV fragment. Because pALM45 appeared not to contain the 3′ end of this gene, it was necessary to clone a fragment that was likely to contain these sequences. To do so, a 1.8-kb *Sac*I PRV DNA fragment was cloned into polink 26 (37), and the resulting plasmid was designated p1800T (Fig. 1a, line F).

DNA sequence analysis of the transcribed region showing homology to the HSV-1 gb gene. The DNA sequence of the PRV region defined by Northern blot analysis and S1 mapping was determined by the method of Maxam and Gilbert (24). We determined the sequence of laboratory strain HSV-1 KOS 321 gB by the method of Sanger et al. (40) with ^{35}S-labeled nucleotides (5).

A single 2,976-bp open reading frame was found in the PRV sequence with an ATG codon 262 bp downstream from the beginning of the sequenced region. The predicted translation product of the PRV open reading frame would correspond to an unmodified protein of 913 amino acids, which is similar in size to the 903 amino acids predicted for gB.

The DNA sequences are shown in Fig. 3, aligned to maximize the homology between them. We were guided in this alignment by the striking homology at the DNA level between PRV and HSV-1 gB sequences revealed by dot matrix analysis (Fig. 4). The sequences share an overall homology of 62%, with highly conserved sequence regions punctuated by sequences of low similarity. We note that a number of alignments are possible, and only one is presented in Fig. 3.

Potential *cis*-acting control signals are shared between the PRV and HSV-1 gB sequences. The sequence TATATCC (PRV residues −125 through −119) may represent the TATA sequence for this gene. The TATA box aligns with the gB TATA box (9, 31) and is centered in a region with 23 identical nucleotides of 24 between the two sequences. The start of eucaryotic mRNA transcription is typically an A residue surrounded by pyrimidine residues and is located approximately 24 to 32 nucleotides downstream from the TATA box (7). Accordingly, the PRV transcript is likely to start at −96 or −89 with respect to the ATG codon. Another conserved sequence is the CCAAT box motif characteristic of many eucaryotic promoters. The sequence 5′-ATTG-3′ (residues −208 through −205 in the PRV sequence and residues −398 to −395 in the gB sequence) reads CAAT on the opposite strand.

A potential polyadenylation signal, AATAAA, was present downstream from the coding sequence of the PRV open reading frame beginning at nucleotide 2760. This site would be consistent with the observed size of the transcript.

Primary structure of the PRV protein and comparison with HSV-1 gB. We aligned the predicted amino acid sequences from the PRV open reading frame with that deduced from the HSV-1 gB sequence as shown in Fig. 5. Both proteins are similar in molecular mass: the PRV protein is 100.4 kilodaltons (kDa) compared with 100.3 kDa for gB. More than 50% of the amino acid sequence of the PRV and gB proteins is identical in the alignment shown in Fig. 5. Codon usage is similar between the two proteins and, not surprisingly, reflects the base composition bias of the two sequences (PRV, 71.2% G+C; gB, 66.2% G+C).

The PRV protein predicted from the DNA sequence has several features in common with the envelope glycoproteins from PRV and other herpesviruses, including a characteristic (although unusual) signal sequence at the amino terminus and a membrane-spanning hydrophobic region near the carboxy terminus. These features are often predicted by the hydropathic analysis of Kyte and Doolittle (19) (Fig. 6); however, the PRV protein was more complicated to interpret than expected, as discussed below.

Overlap of predicted signal sequence and an upstream open reading frame. Upon analysis of the deduced PRV amino acid sequence, it was difficult to find sequences that corresponded to a typical signal sequence in the first 25 to 30 codons. In fact, the first 31 amino acids were decidedly hydrophilic, contained little potential for formation of alpha- or beta-sheet structures, and also contained five proline residues, features not typical of signal sequences. One predominant characteristic of a signal sequence is a hydrophobic core (46, 48). Upon further inspection, a 15-amino-acid segment with strong hydrophobicity and with the potential to form an alpha-helix was found from amino acids 39 to 53, although the highly conserved Val-Val sequence at the consensus hydrophobic core positions 7 and 8 was absent. In addition, the sequence Ala-Ala-Ala (residues 51 to 53), followed by Pro-Pro-Cys-Gly (residues 54 to 57, may correspond to the Ala-X-Ala-turn sequence predicted to be a common motif at signal peptidase cleavage sites (32, 46, 48). The mature PRV protein would then be predicted to begin with Pro-54. We recognize that this is an unusually long signal sequence, although a 62-amino-acid signal sequence has been reported for the Rous sarcoma virus membrane glycoprotein (48).

The unusual amino-terminal region of the PRV protein was further analyzed as follows. In HSV, an open reading frame encoding ICP 18.5, a protein reported to be involved in viral glycoprotein transport (29), terminates 10 nucleotides prior to the gB ATG codon. Upon examination of the PRV sequence, we found an open reading frame with 42% amino acid homology to ICP 18.5 that overlaps the gB homolog coding sequence and terminates at a position corresponding roughly to amino acid 44 of the PRV protein signal sequence. The presence of the unusually long predicted signal sequence in the PRV protein may reflect the constraints imposed upon the sequence to accommodate both an upstream coding region and the signal sequence requirements (Fig. 7). Further work is necessary to prove that the first 53 amino acids of the PRV sequence actually function as a signal sequence and that a homolog to ICP 18.5 is encoded upstream from the PRV gB homolog.

Prediction of the transmembrane domain. Alignment of the hydropathic profiles of the PRV sequence with gB-1 (Fig. 6) suggests the location of a transmembrane domain in PRV (amino acids 740 to 808) that may traverse the membrane three times as proposed for gB-1 (31). The amino acid sequences of the predicted transmembrane domain are

FIG. 3. DNA sequence of the PRV glycoprotein gII gene and homologous HSV-1 KOS 321 gB. The top line is the DNA sequence of HSV-1 KOS 321 gB. The bottom line is the homologous PRV DNA sequence. Nucleotides are numbered with reference to the translation start codon ATG assigned as 1. Gaps have been introduced to maximize homology between the two sequences. Equivalent residues are marked with a colon. Relevant sequence features (CAT, TATA, mRNA initiation, translation start [start], and translation termination [TER]) are noted either above or below the sequence and are discussed in the text.

highly homologous between the proteins (62% identical amino acids).

The carboxy-terminal anchor sequence. The remaining 95 amino acids of the PRV protein are hydrophilic with strong alpha-helix-forming potential. This carboxy-terminal segment could function at least in part as an anchor sequence. This region shares primary amino acid sequence homology of 50% with gB-1; however, secondary structure analysis suggests that the overall structural homology may be higher, with 77% identity in the predicted secondary structure (data

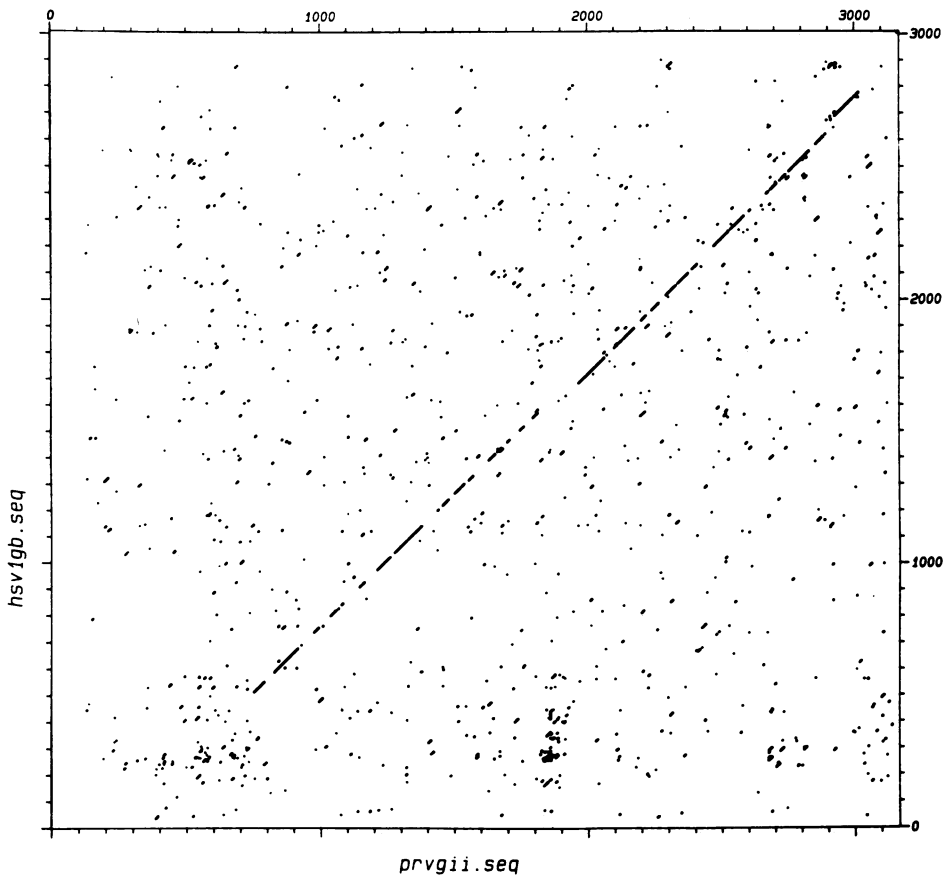

FIG. 4. Dot homology matrix of PRV DNA and HSV-1 gB DNA. The analysis was done with COMPARE and DOTPLOT from the University of Wisconsin Genetics Computer Group programs on the Dupont Experimental Station Vax cluster. The PRV sequence is given on the horizontal axis, and the HSV-1 gB sequence is given on the vertical axis. The putative PRV ATG start codon occurs at position 262, and the stop codon occurs at position 2977. The HSV-1 gB ATG start codon occurs at position 193, and the stop codon occurs at position 2896. A window of 20 bases was used in this analysis with a dot plotted only if 14 or more bases matched. A total of 4,381 points were plotted. Similar results were obtained with the Pustell and Kafatos (35) dot matrix homology analysis (data not shown).

not shown). The mutation in an HSV-1 gB mutant whose phenotype is extensive cell fusion (syncytial plaque morphology) is an Arg-to-His substitution at residue 858 (denoted s in Fig. 5) (31). This mutation is located in a highly ordered stretch of alpha-helices and beta-sheets (gB-1 residues 836 to 864). The analogous PRV sequence is 73% identical in primary sequence, suggesting a conserved function in PRV.

The hydrophilic external domain. The bulk of the PRV sequences are likely to lie on the surface of infected cell membranes and virion envelopes. Six potential N-linked glycosylation sites (Asn-X-Ser/Thr) are present in the PRV external region. Four of these sites are shared with gB-1 as we have aligned the sequences. Three are identical, and one is off by 1 amino acid. It is noteworthy that all 10 cysteine residues located outside of the signal sequence are shared with gB-1, suggesting the potential for common secondary and tertiary structure elements between the two molecules.

Alterations in the gB-1 external domain occur in conserved PRV regions. A domain in gB-1 believed to be involved in rate of virus entry into cells is highly conserved in a colinear segment of the predicted PRV protein. A rapid rate of entry mutation (roe) in HSV-1 HFEM *ts*B5 has been identified as a Val (GTC)-to-Ala (GCC) substitution at amino acid 553 of gB-1 (8) (roe in Fig. 5). The amino acid corresponding to this substitution in gB-1 is found in the PRV sequence. The gB roe mutation is located in an extremely conserved region between the two homologs (69% identity, corresponding to gB-1 amino acids 527 to 574), as may be expected for a domain affecting virus entry into host cells.

Of the HSV-1 gB mutants isolated as resistant to neutralization with gB-specific monoclonal antibodies, four have been sequenced (22, 31). The substitutions occur at gB-1 residues 303, 313, 315, 335, and 473 (m in Fig. 5). The high amino acid homology between gB and PRV in the vicinity of those residues suggests the possibility that the gB-1 epitopes share cross-reacting PRV determinants.

Identification of the PRV gene showing homology to HSV-1 gB. We identified the PRV gene corresponding to this open reading frame by expressing a 765-bp segment in *E. coli* as a β-galactosidase fusion protein (Fig. 8), producing antiserum against the bacterially produced protein, and using the sera so obtained for immunoprecipitating specific PRV polypeptides. The unknown protein was then identified by comparing the PRV proteins immunoprecipitated from infected cells by this antiserum with proteins immunoprecipitated by specific PRV monoclonal antibodies.

Cells carrying pALM9 were induced, and the insoluble fusion protein was partially purified (37). The fusion protein was then partially solubilized with NaOH, neutralized, and

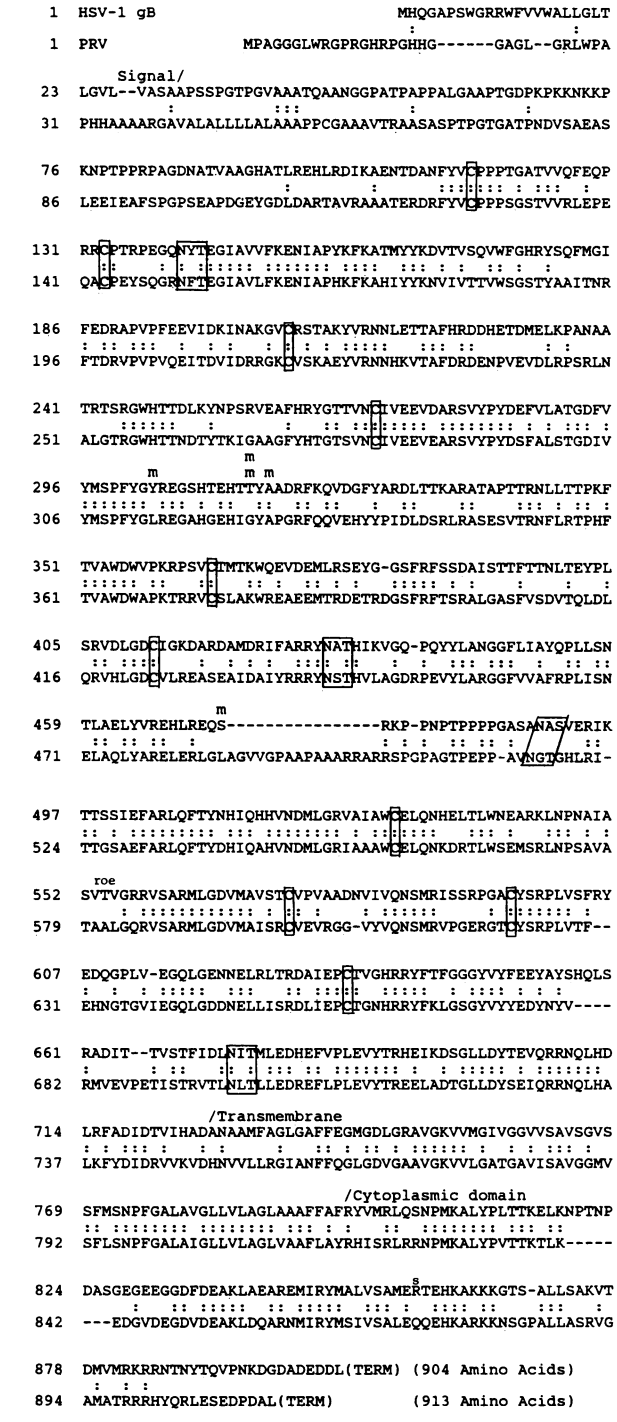

FIG. 5. Alignment of the amino acid sequences of HSV-1 KOS 321 gB with PRV DNA. The top line is the HSV-1 gB amino acid sequence, and the bottom line is the PRV amino acid sequence. Dashes were inserted to increase the homology between the two proteins. Equivalent residues are marked with a colon. Shared cysteine residues and N-linked glycosylation sites are boxed. The predicted location of the signal sequence, transmembrane, and cytoplasmic domains are marked. gB mutants are indicated: rate of virus entry (roe) (8); syncytial formation mutant(s) (8); monoclonal antibody-resistant mutants (m) (22, 31). Intertypic strain differences between KOS 321 gB and gB KOS (9) with KOS gB sequence in parentheses: position 515 His (515 Arg); position 756 Ile (756 Leu). It should be noted that the amino acid positions for KOS 321 gB shown differ by one from the positions given for the HSV-1 F sequence (31), because the predicted signal sequence for KOS 321 gB contains 30 amino acids, and strain F gB contains 29 amino acids.

injected into rabbits to raise antibodies by the regimen described in Materials and Methods.

Extracts prepared from [^{3}H]glucosamine-labeled PRV-infected PK15 cells were reacted with two monoclonal antibodies directed against the gII family of PRV glycoproteins, M2 and M3 (14), or antiserum raised in rabbits against the fusion protein. Monoclonal antibodies M2 and M3 specifically immunoprecipitated PRV glycoproteins of apparent molecular mass 110, 68, and 55 kDa (Fig. 9). M3 also immunoprecipitated a PRV glycoprotein of apparent molecular mass 100 kDa, previously shown to be a precursor form of the mature 110-kDa gII protein (21, 26). Two antisera raised in different rabbits against the fusion protein immunoprecipitated the same pattern of PRV-specific glycoproteins as the M3 monoclonal antibody (Fig. 9), although both rabbit antisera appeared to be more reactive with the 100-kDa precursor form of the gII protein family. These data strongly suggest that the open reading frame expressed in pALM9 is part of the PRV gII gene. This is consistent with the work of Mettenleiter et al. (26), who localized the gII gene to the same DNA region by using RNA hybridization and in vitro translation techniques.

Anti-PRV gII sera reacts with HSV-1 gB. Since PRV gII and HSV-1 gB shared significant homology at the amino acid level, it was of interest to ascertain whether antibodies directed against either protein would cross-react in an immunoprecipitation assay. PK15 or Vero cells were infected with 10 PFU of PRV or HSV KOS per cell, respectively. Glycoproteins were labeled with [^{3}H]glucosamine for 16 h, after which the cells were harvested and extracts were prepared for immunoprecipitation as described in Materials and Methods.

Cell extracts were reacted with antisera directed against PRV gII (M3 and 284) or HSV-1 gB (B6 and 790). Antibodies M3 and B6 are murine monoclonal antibodies, whereas 284 and 790 are polyvalent antisera prepared by injecting purified viral glycoproteins into goats (284 serum) or guinea pigs (790 serum).

Monoclonal antibody M3 and 284 antiserum specifically immunoprecipitated the PRV gII family of glycoproteins of 110, 100, 68, and 55 kDa from a PRV-infected PK15 extract (Fig. 10, lanes 1 and 5). Both B6 and 790 antibodies specifically immunoprecipitated a glycoprotein of approximately 105 kDa from HSV-1-infected Vero cells (Fig. 10, lanes 4 and 8).

Neither M3 nor B6 monoclonal antibodies cross-immunoprecipitated glycoproteins from HSV-1- or PRV-infected cells (Fig. 10, lanes 2 and 3). This negative result was true for a number of monoclonal antibodies directed against either PRV gII or HSV-1 gB (data not shown). However, 284 polyvalent serum (anti-PRV gII) specifically immunoprecipitated an HSV-1 glycoprotein of approximately 105 kDa (lane 6) that comigrated with the HSV-1 gB species brought down by either the B6 monoclonal antibody or the 790 polyvalent serum (lanes 4 and 8, respectively). In contrast, 790 polyvalent serum (anti-HSV-1 gB) showed no corresponding cross-reaction with any PRV glycoproteins (lane 7). This negative result was obtained by using several independent sources of polyvalent sera directed against HSV-1 gB (data not shown). Similarly, antiserum made against the fusion protein expressed by pALM9 did not react

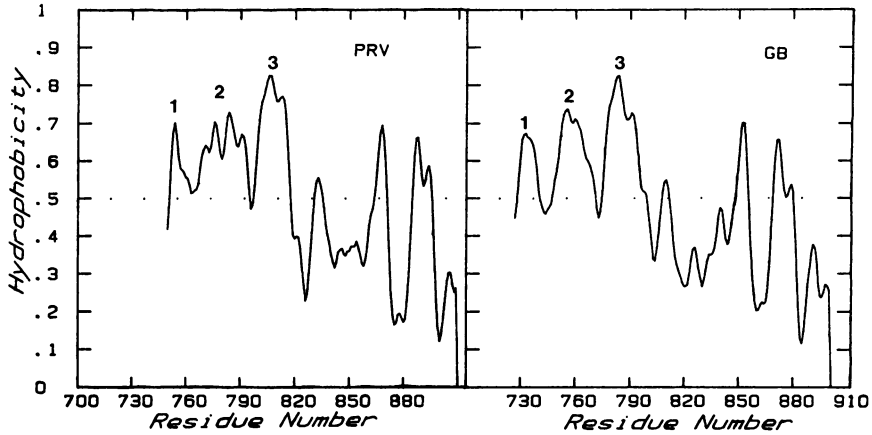

FIG. 6. Hydropathic analysis of the PRV gII and HSV-1 gB transmembrane and carboxy-terminal regions. Hydropathy at each position was computed by the method of Kyte and Doolittle (19) with a window of 9 amino acids with smoothing. The residue numbers are indicated on the horizontal axis, and relative hydrophobicity is plotted on the vertical axis. Points above 0.5 are hydrophobic, and those below 0.5 are hydrophilic. The numbers 1, 2, and 3 above the main hydrophobic peaks are the predicted membrane-spanning segments as discussed in the text.

with any glycoprotein in HSV-1-infected cell extracts, but did react with the entire PRV gII family of glycoproteins from PRV-infected cell extracts (data not shown). A more detailed study of the cross-reactivity between PRV gII and HSV-1 gB involving a greater number of specific antibodies will be required to determine what regions share common antigenic domains.

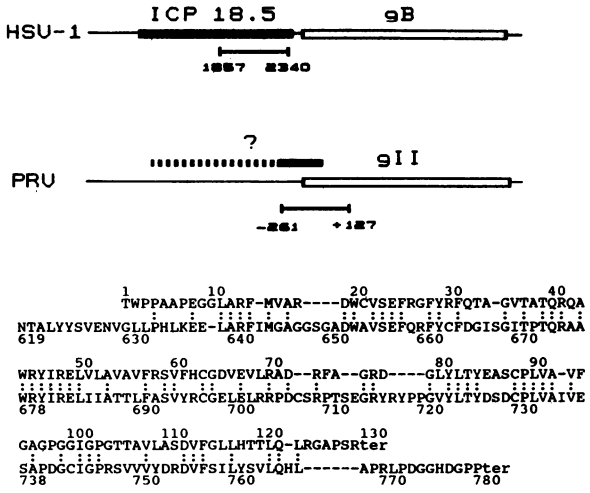

FIG. 7. Identification of HSV-1 ICP 18.5 homolog upstream of the PRV gII gene. The upper diagram describes the orientation of the ICP 18.5 gene and the gB gene as deduced for HSV-1 (29). The predicted orientation of the PRV putative ICP 18.5 homolog and the gII gene are shown below. Note that the genes overlap. The brackets below each diagram indicate the DNA sequence used to derive the protein sequence in the comparison below. For HSV-1 the residue numbers are those used by Pellett et al. (29); for PRV the residue numbers are taken from Fig. 3, in which −261 is the first nucleotide. The deduced amino acid sequence of the HSV-1 ICP 18.5 protein from residue 619 to the carboxy-terminal proline at residue 780 is shown below the line. The deduced amino acid sequence upstream of the PRV gII gene starting at nucleotide −262 (Fig. 3) is given on the top line. The ACG codon (threonine) is arbitrarily numbered 1 for simplicity. A colon denotes identical amino acids; a dash denotes a gap introduced to maximize the homology.

DISCUSSION

In this report we describe and characterize a region of the PRV genome that specifically cross-hybridized with the HSV-1 gB glycoprotein gene. Other HSV-1 glycoprotein genes encoding gC, gD, and gE showed no hybridization to PRV DNA under the same conditions. The PRV sequences with homology to the HSV-1 gB probe were cloned and further analyzed. We found that the PRV segment corresponding to the gB-1 homology encoded a single, abundant RNA species in PRV-infected PK15 cells.

The DNA sequence of this region defined an open reading frame with significant homology with the HSV-1 gB gene. In

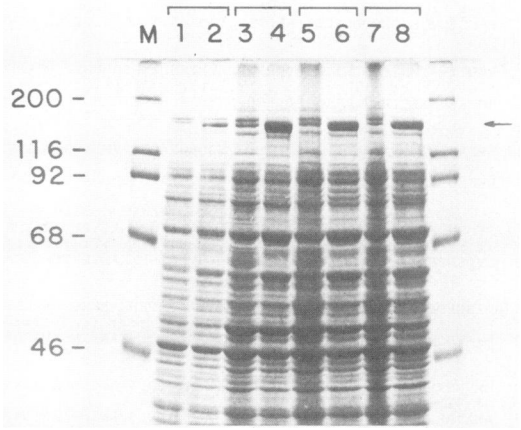

FIG. 8. Induction of fusion protein expressed by *E. coli* NF1829 carrying plasmid pALM9. Bacteria were grown at 37°C in L-broth containing 100 μg of ampicillin per ml. Synthesis of fusion protein was induced by addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 1 mM. Samples (1.5 ml) of uninduced (lanes 1, 3, 5, and 7) or induced (lanes 2, 4, 6, and 8) culture were harvested at 1 h (lanes 1 and 2), 2 h (lanes 3 and 4), 4 h (lanes 5 and 6), or 8 h (lanes 7 and 8) of incubation with inducer. The bacteria were pelleted by centrifugation and lysed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer and fractionated by electrophoresis on a 10% polyacrylamide-sodium dodecyl sulfate gel followed by staining with Coomassie blue. The position of the Cro-PRV-β-galactosidase fusion protein is indicated by the arrow. The location of molecular mass standards (M) is shown).

addition, a portion of the PRV DNA sequence also contained an open reading frame that was homologous to the HSV-1 ICP 18.5 gene (29).

The deduced amino acid sequence of the PRV gB-1 homolog shared significant homology with the large external domain of gB-1, including a region known to contain several monoclonal antibody-resistant alterations as well as a domain believed to be involved in virus entry into the cell (8). Similarly we found significant homology in the carboxy-terminal segment of the protein thought to be the cytoplasmic anchor sequence. Because one suggested role of this sequence for HSV-1 gB is to interact with virion tegument proteins as perhaps other membrane proteins (31), our results suggest conservation of these functions in PRV and should aid in the identification of the common structural requirements of this anchor sequence.

The potential conservation of primary and secondary structure is a consistent finding in the comparison of the two proteins. For example, Pellett et al. (31) predicted that the transmembrane region of gB passes through the membrane three times. The PRV gII protein shares a strikingly similar region with HSV-1 gB when compared by hydropathic analysis. In addition, all 10 cysteine residues of the predicted external domain, as well as four glycosylation sites, are conserved between the two proteins. This may indicate that the two proteins share similar tertiary structure as well. It is known that a fraction of gB-1 is found in infected cells in oligomeric form and may also provide a distinct structural component of the virion (e.g. spikes) (11). Further work is necessary to determine whether the PRV protein is involved in similar structures.

An unusual finding was the predicted 53-amino-acid signal sequence of the PRV protein, an uncharacteristic length for a signal sequence. Our analysis revealed significant homology of this sequence to the HSV-1 ICP 18.5 gene (29). Perhaps the unusual length of the signal sequence reflects the constraints of overlapping coding regions between the upstream PRV analog of ICP 18.5 and the PRV gB-1 homolog coding sequence. At this time, it is not clear whether the upstream gene of PRV is expressed or functional.

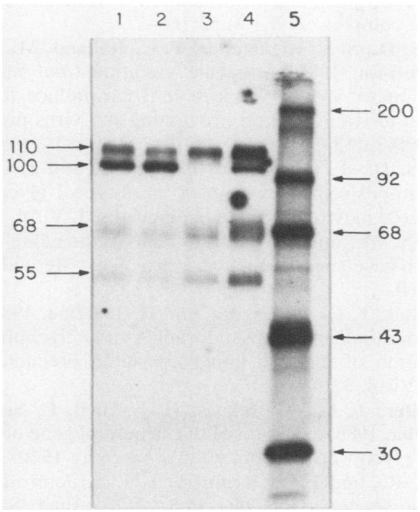

FIG. 9. Immunoprecipitation of PRV-specific proteins with antisera raised against the Cro-PRV-β-galactosidase fusion protein produced in *E. coli* carrying plasmid pALM9. PRV-infected cells were labeled with [^{3}H]glucosamine as described in Materials and Methods. Infected cell extracts were reacted with either rabbit anti-Cro-PRV-β-galactosidase serum (lanes 1 and 2), M2 monoclonal antiserum against gII (lane 3), or M3 monoclonal antiserum against gII (lane 4). Immune complexes were collected by adsorption to Pansorbin (*Staphylococcus* A cells) and fractionated by electrophoresis on a 7 to 17% polyacrylamide-sodium dodecyl sulfate gradient gel. ^{3}H-labeled polypeptides were detected by fluorography. The apparent molecular masses of the PRV gII complex of glycoproteins are indicated by arrows. The positions of molecular mass standards (lane 5) are indicated.

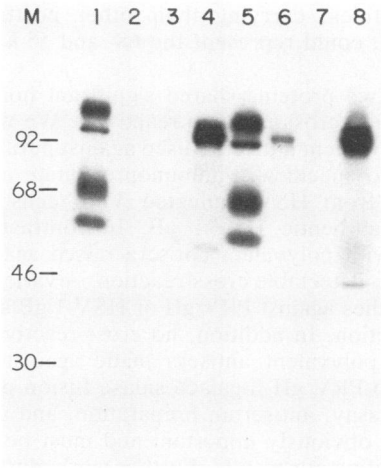

FIG. 10. Immunoprecipitation of PRV and HSV-1 glycoproteins from infected cell extracts. PK15 or Vero cells were infected with PRV and HSV-1 KOS, respectively, in the presence of [^{3}H]glucosamine as described in Materials and Methods. PRV-infected cell extracts (lanes 1, 3, 5, and 7) or HSV-1-infected cell extracts (lanes 2, 4, 6, and 8) were reacted with anti-gII M3 monoclonal antibody (lanes 1 and 2), anti-gB B6 monoclonal antibody (lanes 3 and 4), anti-gII polyvalent serum 284 (lanes 5 and 6), or anti-gB polyvalent serum 790 (lanes 7 and 8). Immune complexes were collected by adsorption to Pansorbin (*Staphylococcus* A) and fractionated by electrophoresis on a 7 to 17% polyacrylamide-sodium dodecyl sulfate gradient gel. ^{3}H-labeled polypeptides were detected by fluorography. The positions of molecular mass standards in kilodaltons are indicated.

We identified the PRV gB-1 homolog as the gII glycoprotein complex (14, 21, 26) by expressing a portion of the PRV open reading frame in *E. coli* as a Cro-PRV-β-galactosidase fusion protein. Antisera raised in rabbits against this fusion protein immunoprecipitated glycoproteins from PRV-infected PK15 cells with indistinguishable mobilities in polyacrylamide gel electrophoresis to those found for two PRV monoclonal antibodies that define the gII glycoprotein family. From this we concluded that the PRV gB-1 homolog is gII. Our predicted amino acid sequence of gII compares well with results reported by Mettenleiter et al. (26) and Lucaks et al. (21) that indicated that the primary gII translation product was a 110-kDa form that was subsequently post-translationally modified to a higher-molecular-mass form of approximately 120 kDa. This form was then cleaved to two lower-molecular-mass species of 68 and 55 kDa. The 120-kDa form was linked by disulfide bridges to both the 68- and 55- kDa form in the virus envelope (21). Since the HSV-1 gB-1 gene is not proteolytically processed in this fashion, we would expect that the gII processing site(s) would have no counterpart in the HSV-1 gB sequence. In the absence of direct protein sequencing, we are unable to define such site(s); however, the unusual sequence including the Arg-Arg-Ala-Arg-Arg sequence (PRV residues 498 to 502) has no counterpart in gB and may represent a processing site. If the PRV gII protein were cleaved in this region, the resulting

two polypeptides, carrying their other posttranslational modifications, could represent the 68- and 55-kDa gII proteins.

Since the two proteins shared significant homology, we expected to find cross-immuno reactivity. We were able to show that polyvalent antisera raised against purified PRV gII proteins could specifically immunoprecipitate a species of glycoprotein from HSV-1-infected Vero cells that comigrated with authentic HSV-1 gB. In contrast, a similar experiment with polyvalent antisera raised against HSV-1 gB showed no detectable cross-reaction. A variety of monoclonal antibodies against PRV gII or HSV-1 gB also showed no cross-reaction. In addition, no cross-reactivity was observed with polyvalent antisera made against bacterially produced Cro-PRV gII-β-galactosidase fusion protein. The methods of assay, antiserum preparation, and antigen presentation are obviously important and must be considered when interpreting our results. Further work must be done to identify the epitope(s) on PRV gII that stimulate the cross-reacting antibody.

In conclusion, we have identified a PRV glycoprotein with significant homology at the DNA and amino level with HSV-1 gB. The implication of our finding is that both PRV and HSV use this protein for similar functions, even though the viruses themselves are quite distinct overall. This is consistent with the recent observations that HSV-1 gB is a highly conserved glycoprotein among a variety of herpesviruses (12, 18, 30).

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