JOURNAL OF VIROLOGY, June 1995, p. 3863–3867 Vol. 69, No. 6
0022-538X/95/$04.00+0

Characterization of Bovine Herpesvirus 1 UL49 Homolog Gene and Product: Bovine Herpesvirus 1 UL49 Homolog Is Dispensable for Virus Growth†

XIAOPING LIANG,[1]* BETTY CHOW,[1] YUANHAO LI,[1,2] CAM RAGGO,[1,2] DONGWAN YOO,[1] SAM ATTAH-POKU,[1] AND LORNE A. BABIUK[1,2]

The Veterinary Infectious Diseases Organization[1] and Department of Veterinary Microbiology,[2] University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5E3

Received 3 February 1995/Accepted 28 February 1995

The sequence of the bovine herpesvirus 1 (BHV-1) gene that is homologous to the herpes simplex virus UL49 gene was determined. The BHV-1 UL49 homolog open reading frame consists of 774 bp and is capable of encoding 258 amino acids. Northern (RNA) blot analysis showed that the BHV-1 UL49 homolog is transcribed into a 1.1-kb RNA which is coterminal with the transcripts of an upstream UL49.5 homolog gene. Rabbit antisera produced against synthetic peptides of the predicted UL49 homolog gene product recognized a polypeptide of 33 to 35 kDa in both virus-infected cells and isolated virions. Further analysis by unionic-detergent partition of isolated virions suggested that the UL49 homolog gene product is a virion tegument protein. Indirect immunofluorescence assay revealed that the UL49 homolog gene product was predominantly localized in the nuclei of BHV-1-infected cells. A mutant virus with the UL49 homolog gene deleted was produced, and it was able to replicate in noncomplementing cells. Nevertheless, the yield of mutant virus was significantly reduced. The results from this study suggest that the BHV-1 UL49 homolog gene encodes a nuclear protein which constitutes a tegument component in mature virions and that it is dispensable for virus growth in cell culture.

Virions of alphaherpesviruses contain a number of tegument proteins that are situated between the nucleocapsid and envelope (30). In addition to being virion structural components, these tegument proteins play important roles in regulating viral gene expression. For example, the herpes simplex virus type 1 (HSV-1) major tegument protein (α-transinducing factor [α-TIF], VP16, or Vmw65) forms a complex with cellular transcription factors and binds to a consensus sequence in the promoters of viral immediate-early genes, by which it induces viral immediate-early gene expression (9, 11), the first step in viral replication. Mutant virus that lacked α-TIF *trans*-inducing activity exhibited significantly impaired infectivity, establishing infection only at a high multiplicity of infection (MOI); furthermore, such a mutant was avirulent in vivo (1). HSV-1 α-TIF is encoded by the UL48 gene, which is surrounded by two additional tegument protein genes (UL47 and UL49), which encode VP13/14 and VP22, respectively (6, 19, 31). VP13/14 has been shown to participate in modulating the α-TIF-dependent transcription process; mutant virus with a VP13/14 deletion was found to have reduced replication efficiency (33). The function of VP22 and whether it is essential for virus growth are unknown.

Bovine herpesvirus 1 (BHV-1) is a member of the *Alphaherpesviridae* subfamily and an important pathogen in cattle (8). The virion of BHV-1 contains 25 to 33 proteins (23, 24). The most abundant virion protein, VP8, has been identified as an HSV-1 UL47 homolog (3, 13). The UL48 homolog of BHV-1 has been mapped and sequenced (4). In addition, a partial sequence of the UL49 homolog (UL49h) has also been reported (4). The gene product of the BHV-1 UL48 homolog has recently been shown to transactivate immediate-early gene expression, indicating that it plays a similar role to that of its HSV-1 counterpart in virus replication (22). In an effort to gain further insight into the functions of virion tegument proteins in virus replication, in this study we characterized the BHV-1 UL49h gene and its product.

Sequence determination and transcript analysis of the BHV-1 UL49h gene. By using a probe derived from the HSV-1 UL48 gene, we had previously mapped and sequenced the BHV-1 gene that is homologous to HSV-1 UL48 (13a). In a subsequent study, we identified a BHV-1 dUTPase gene (UL50 homolog) and a gene that is homologous to HSV-1 UL49.5 (16). The genomic locations of these BHV-1 homolog genes were found to be colinear with those of their HSV-1 counterparts. On the basis of these findings, the UL49h gene was predicted to be located between the UL48 and UL49.5 homolog (UL49.5h) genes. Therefore, we determined the sequence between these two genes. Complete sequencing of this region revealed a 774-bp open reading frame (ORF) that is separated from the UL48 homolog ORF and the UL49.5h ORF by 183 and 123 bp, respectively (DNA sequence not shown). While this study was in progress, a partial sequence of the UL49h gene was reported (4); it was found to be identical to the corresponding portion of the sequence determined in this study. Computer-assisted analysis revealed a putative TATA box 89 bp upstream of this ORF, which is preceded by a putative GC-rich sequence, and a polyadenylation signal sequence, AATAAA, 66 bp after the translation termination codon. The deduced amino acid sequence exhibits significant homology (40.7%) with that of the HSV-1 UL49 gene product. Taken together, these features suggest that the ORF that we have identified represents a BHV-1 homolog of the HSV-1 UL49 gene.

Figure 1A shows the map location of the BHV-1 UL49h gene and restriction endonuclease recognition sites at the

* Corresponding author. Phone: (306) 966-7482. Fax: (306) 966-7478.

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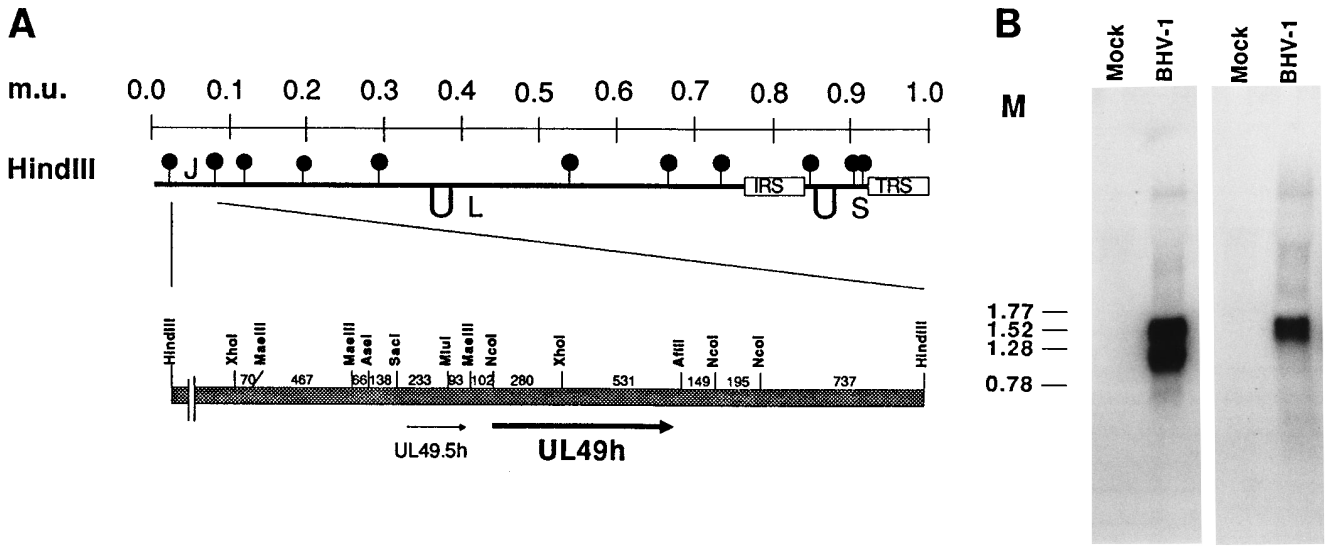

FIG. 1. Schematic representation of the genomic location of the BHV-1 UL49h gene and Northern blot analysis of UL49h transcripts. (A) Map location of UL49h. The genome of BHV-1 (Cooper strain) consists of a unique long (U_L) and a unique short (U_S) region. The U_S is flanked at its ends by an internal repeat segment (IRS) and a terminal repeat segment (TRS). *Hin*dIII recognition sites within the genome are indicated by solid circles. The *Hin*dIII J fragment, in which the UL49h ORF (bold arrow) is located, is depicted with relevant restriction enzyme recognition sites. Also shown is the UL49.5h ORF (thin arrow). (B) Northern blot analysis of UL49h transcripts. Total cytoplasmic RNA from BHV-1-infected cells or mock-infected cells was isolated at 10 h postinfection. Two sets of RNA samples were separated in parallel on a 1.2% agarose–formaldehyde gel and blotted to nitrocellulose paper. One set of samples were probed with a 280-bp *Nco*I-*Xho*I fragment of the UL49h ORF (left), and the other set of samples were probed with a 530-bp *Mae*III-*Mae*III fragment located 102 bp upstream of the UL49h ORF (right). The molecular size (in kilobases) was determined by an RNA ladder (GIBCO/BRL Canada, Burlington, Ontario, Canada) run in parallel with the samples (M).

UL49h gene locus. To ascertain that the UL49h gene was functional, we first examined UL49h gene transcripts in virus-infected cells by Northern (RNA) blot analysis (Fig. 1B). Total cytoplasmic RNA from BHV-1 infected cells or mock-infected cells was isolated at 10 h postinfection. Two sets of RNA samples (10 μg per sample) were separated in parallel on a 1.2% agarose–formaldehyde gel and blotted to nitrocellulose paper. One set of samples were probed with a 280-bp *Nco*I-*Xho*I fragment of the UL49h ORF, and the other set of samples were probed with a 530-bp *Mae*III-*Mae*III fragment located 102 bp upstream of the UL49h ORF. The probe that consisted of the 280-bp *Nco*I-*Xho*I fragment hybridized to two RNA species, about 1.1 and 1.5 kb in length. The probe that consisted of the 530-bp *Mae*III-*Mae*III fragment hybridized to the 1.5-kb transcript only. According to sequence analysis, the TATA box of the UL49h gene and that of UL49.5h gene are separated by 458 bp, which appears to be comparable to the difference observed between the two transcripts. These results suggest that the BHV-1 UL49h gene is transcribed into a 1.1-kb RNA species which is coterminal with a 1.5-kb UL49.5h RNA. Of note, it has previously been shown that the transcripts of HSV-1 UL49 and UL49.5 are also 3′ coterminal (10).

Construction and characterization of a UL49h gene deletion mutant. In order to facilitate characterization of the UL49h gene, we attempted to construct a UL49h gene deletion mutant by homologous recombination. To generate the transfer vector, a 1.5-kb *Xho*I fragment which contained 280 bp of the 5′ coding sequence and 1.2 kb of the 5′ flanking sequence of the BHV-1 UL49h gene was isolated from plasmid pSD57 (17) and cloned into the *Xho*I site of plasmid Bluescript II KS+ (Stratagene, La Jolla, Calif.). The resulting plasmid was digested with *Eco*RI and *Nco*I and treated with Klenow fragment; the plasmid backbone was ligated. From this plasmid, a 0.6-kb *Xma*I-*Sal*I fragment which contained the UL49h gene 5′ flanking sequence was isolated and cloned into the *Xma*I and *Sal*I sites of polink5′gIII (17) to generate p5′UL49C. A 0.7-kb *Sac*II-*Xho*I fragment that contained the UL49h 3′ flanking sequence was isolated from pSD57, blunted with Klenow fragment, and cloned into the *Hpa*I site of p5′UL49C, resulting in pdUL49C. The *Escherichia coli lacZ* gene with a simian virus 40 polyadenylation signal sequence was isolated from pgIII/LacZ (15) by *Nco*I and *Hin*dIII digestion and cloned into the *Nco*I and *Hin*dIII sites of p5′UL49C. This resulted in pdUL49Z (Fig. 2A). According to the design of the transfer vector, the mutant virus contains a 280-bp deletion at the 5′ end of the UL49h ORF. Recombinant viruses were generated by cotransfecting MDBK cells with the transfer vector and naked BHV-1 genomic DNA and were identified by their production of β-galactosidase. One of the resulting mutant viruses, vdUL49Z, was plaque purified and used for subsequent studies.

The genomic configuration of this recombinant virus was verified by Southern blot analysis. Total cellular DNA from virus-infected cells was digested with *Ase*I and *Nco*I, separated on a 1% agarose gel, transferred to a nitrocellulose filter, and probed with a 1.4-kb *Afl*II-*Ase*I fragment (Fig. 1A). As shown in Fig. 2B, two fragments of approximately 0.9 and 0.5 kb were detected in wild-type (wt) virus DNA and two fragments of approximately 4 and 1 kb were detected in mutant virus DNA. This pattern is consistent with the prediction based on the construct of the transfer vector, thus confirming deletion of the UL49h gene in this mutant virus.

Next we compared the growth properties of the UL49h deletion mutant and wt BHV-1. To ascertain that the observed phenotype of the mutant virus was indeed due to this specific deletion rather than a secondary mutation during construction of the mutant virus, the UL49h gene was reintroduced into the mutant virus genome by cotransfection of a plasmid that contained a 2.2-kb *Hin*dIII-*Ase*I fragment of pSD57 (Fig. 1A) and UL49h gene deletion mutant DNA. Marker-rescued virus was selected on the basis of its white phenotype on the blue plaque assay (15). The UL49h deletion mutant showed a delay in growth and produced a lower virus yield than did wt BHV-1.

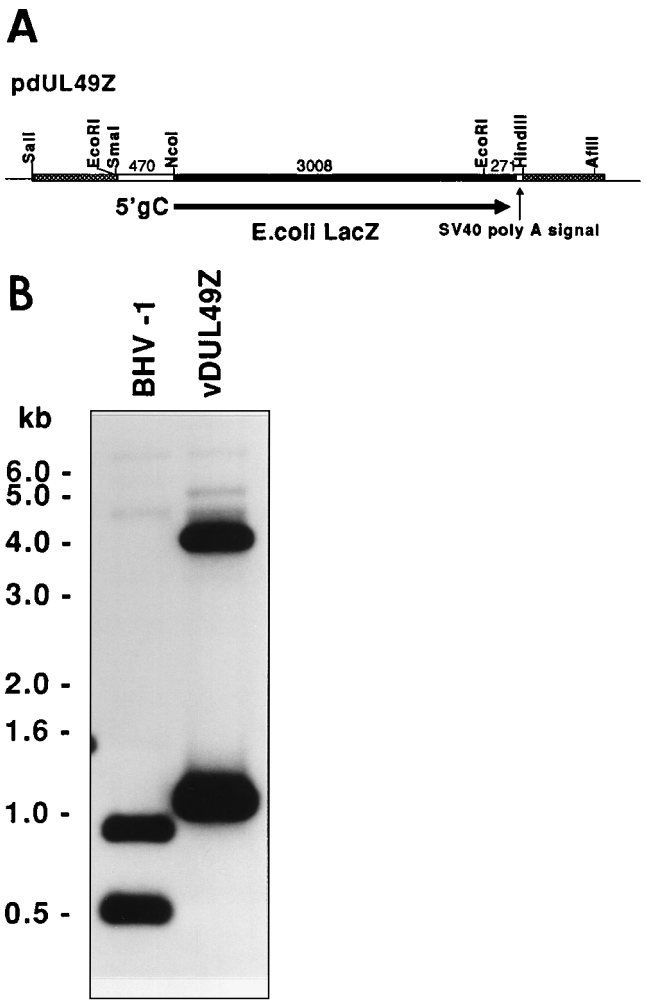

FIG. 2. Schematic representation of the transfer vector used for construction of the UL49h deletion mutant and Southern blot analysis of the BHV-1 UL49h deletion mutant genome. (A) Transfer vector pdUL49Z. Hatched bars, BHV-1 genomic DNA; solid lines, backbones of cloning vectors; SV40 polyA signal, simian virus 40 polyadenylation signal. (B) Southern blot analysis to verify the genomic configuration of vdUL49Z. Cellular DNA was isolated from virus-infected cells at 10 h postinfection. DNA was digested with *Ase*I and *Nco*I, separated on a 1% agarose gel, transferred to a nitrocellulose filter, and probed with a 1.4-kb *Afl*III-*Ase*I fragment that encompassed the UL49h gene. DNA markers are presented on the left.

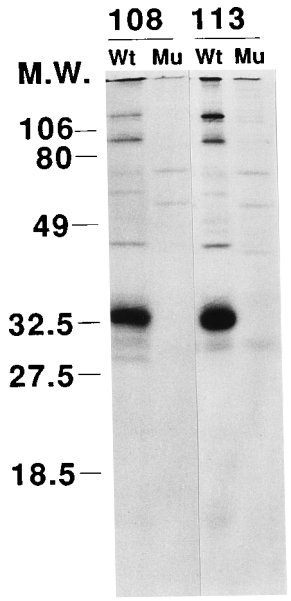

FIG. 3. Immunoprecipitation assay of the UL49h gene product in virus-infected cells. MDBK cells were infected with either wt virus or mutant vdUL49Z at an MOI of 1 and labelled with 25 μCi of [^{35}S]methionine per ml of medium. After being labelled for 18 h, cells were lysed and immunoprecipitated with a UL49h-specific rabbit antiserum. Samples were separated by SDS–12% PAGE under reducing conditions. 108, antiserum that recognized peptide$_{66–85}$; 113, antiserum that recognized peptide$_{235–258}$; Wt, wt BHV-1; Mu, UL49h deletion mutant; M.W., molecular markers (in kilodaltons).

For example, by 12 h postinfection, the wt virus titer had increased by about 4 logs, whereas the mutant virus titer had increased by less than 1 log; the maximum yield of mutant virus (titer at 24 h postinfection) was about 10-fold lower than that of wt virus. In contrast to mutant virus, marker-rescued virus exhibited a growth curve that was similar to that of wt virus.

UL49h encodes a virion protein. To characterize the UL49h gene product, oligopeptides of the predicted UL49h gene product were synthesized and used to produce UL49h-specific antisera in rabbits. Two oligopeptides that corresponded to amino acid residues 66 to 85 (peptide$_{66–85}$) and 235 to 258 (peptide$_{235–258}$), respectively, of the predicted UL49h protein sequence were synthesized and conjugated to keyhole limpet hemocyanin by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. Rabbits were immunized intramuscularly with 200 μg of conjugated peptide in complete Freund's adjuvant and given booster injections 4 weeks later with 200 μg of conjugated peptide in incomplete Freund's adjuvant; this was repeated four times at 2-week intervals. One week after the final immunization, serum samples were collected. The antiserum that recognized peptide$_{66–85}$ and the one that recognized peptide$_{235–258}$ were labelled 108 and 113, respectively. For immunoprecipitation, MDBK cells were infected with either wt virus or mutant vdUL49Z at an MOI of 1 and labelled with 25 μCi of [^{35}S]methionine per ml of medium. After being labelled for 18 h, cells were lysed and immunoprecipitated with a UL49h-specific rabbit antiserum. Samples were separated by sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis (SDS–12% PAGE) under reducing conditions. Immunoprecipitation with either antiserum detected a distinct band with an apparent molecular mass of about 33 kDa for wt BHV-1-infected cells, but not for mutant virus-infected cells (Fig. 3). This suggests that the 33-kDa polypeptide is the gene product of BHV-1 UL49h.

To assess whether UL49h protein was also present in virions, wt virus and the UL49h deletion mutant were purified via Na-K tartrate gradient (14) and analyzed by Western blotting (immunoblotting) (12). The results of this experiment showed that the UL49h gene product is present in virions (data not shown). It has previously been shown for HSV that envelope proteins can be released by treating virions with an unionic detergent, whereas tegument proteins such as VP22 can be released by treating virions with detergent plus high concentrations of salt (21). Accordingly, to assess the relative location of the BHV-1 UL49h protein in virions, purified wt BHV-1 virions were treated with either 1% Nonidet P-40 (NP-40) or 1% NP-40 plus 1 M NaCl at 4°C for 30 min, with subsequent centrifugation at 178,000 × *g* for 30 min. After centrifugation, the supernatant and pellet were collected separately, separated by SDS–12% PAGE under reducing conditions, and analyzed by Western blot with a mixture of anti-UL49h antiserum and

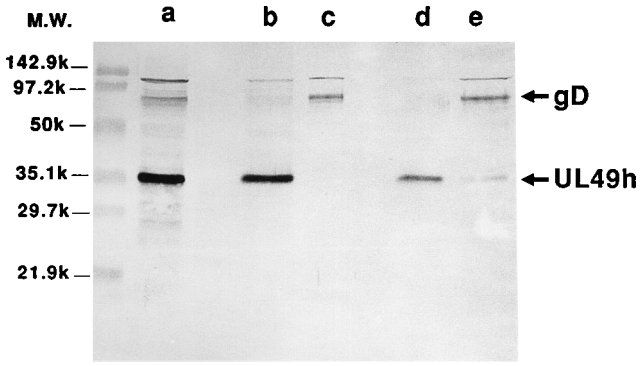

FIG. 4. Western blot analysis of the UL49h gene product in isolated virions. Purified wt BHV-1 virions were treated with either 1% NP-40 (lanes b and c) or 1% NP-40 plus 1 M NaCl (lanes d and e) at 4°C for 30 min, with subsequent centrifugation at 178,000 × *g* for 30 min. After centrifugation, the supernatant (lanes c and e) and pellet (lanes b and d) were collected separately and separated by SDS–12% PAGE under reducing conditions. Lane a, virions without detergent treatment. gD- and UL49h-specific bands were detected by using a mixture of gD MAbs and UL49h-specific rabbit antiserum 108. M.W., molecular markers (in kilodaltons).

gD-specific monoclonal antibodies (MAbs) (Fig. 4). In the absence of detergent treatment (Fig. 4, lane a), there are three major bands; the second and third bands correspond to gD and UL49h, respectively. The identity of the top band is not clear. However, it appears to be related to gD as this band was not detected in the absence of gD MAbs (data not shown). Upon treatment of virions with detergent, gD was released but UL49h was not (Fig. 4, lanes b and c). In contrast, treatment with detergent and NaCl resulted in partial release of UL49h. These results suggest that the UL49h gene product is a virion tegument component. It was noted that the molecular mass of the UL49h gene product, as estimated by Western blotting, is about 35 kDa, slightly larger than the estimate from immunoprecipitation. This is most likely due to the different molecular weight markers used in these two assays.

Nuclear localization of UL49h protein. To examine the intracellular distribution of UL49h, indirect immunofluorescence assays were performed with BHV-1-infected MDBK cells. MDBK cells that had been grown on glass chamber slides (Miles Laboratories, Inc., Naperville, Ill.) were infected with BHV-1 at an MOI of approximately 0.001. At 16 h postinfection, cells were fixed with 2% paraformaldehyde for 10 min at 24°C and permeabilized with absolute methanol at −20°C for an additional 10 min. Cells were reacted with a mixture of rabbit antisera 108 and 113 and then with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G at a dilution of 1:80 (Becton Dickinson, Mountain View, Calif.). Slides were examined for fluorescence with a microscope that was equipped with a UV attachment. As shown in Fig. 5, UL49h protein was predominantly localized in the nucleus (Fig. 5), indicating that the UL49h gene product is synthesized primarily as a nuclear protein.

Genes homologous to HSV-1 UL49 have been identified for a number of other herpesviruses, including varicella-zoster virus (5), equine herpesvirus (29), and Marek's disease virus (32), indicating that UL49 homologs are highly conserved among members of the *Herpesviridae* family. Prior to this study, HSV-1 was the only virus whose UL49 gene product had been identified. While it is known that the UL49 gene product, VP22, is a tegument protein, the function of this protein in virus replication has yet to be characterized. VP22 is a phosphoprotein (21), and it is ADP ribosylated (2, 27). In addition,

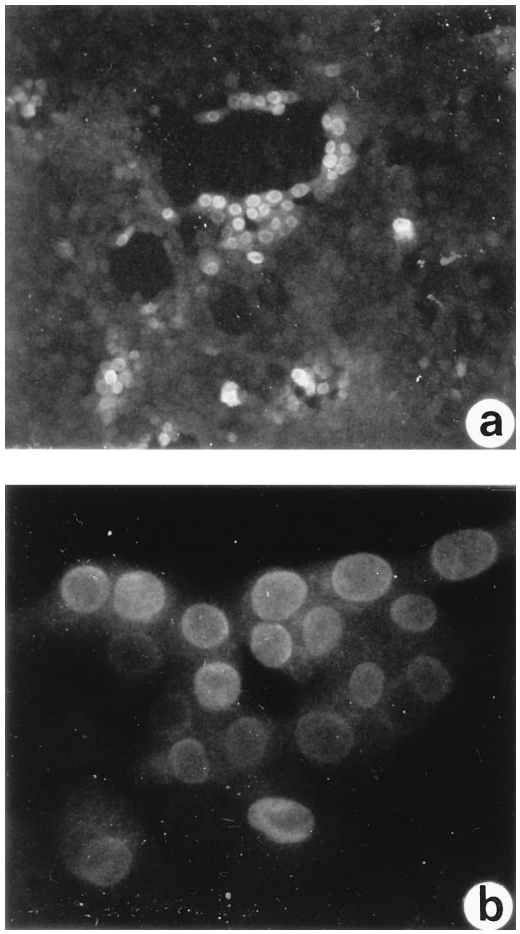

FIG. 5. Indirect immunofluorescence assay of subcellular distribution of the UL49h gene product. MDBK cells were infected with an MOI of approximately 0.001, and assays were performed at 16 h postinfection. Magnifications, ×25 (a) and ×100 (b).

VP22 has been shown to be associated with the nuclear matrix during virus replication (25). These features have been taken as indications of a regulatory role for this viral protein in virus replication (6). For HSV, UL49 is classified as an essential gene (7). In this study, we constructed a partial UL49h gene deletion mutant. This mutant virus was completely defective in producing the UL49h gene product; it produced no UL49h protein in BHV-1-infected cells. However, the mutant was able to replicate and produce infectious progeny in noncomplementing cells. This establishes that BHV-1 UL49h is not essential for virus growth in cell culture. Nevertheless, this mutant virus appears to be impaired in replication. Marker rescue of the UL49h gene restored the wt phenotype, indicating that the designated mutation at the UL49h gene locus was responsible for impaired growth of the mutant virus. The observation that deletion of UL49h reduced virus replication appears to be consistent with the contention that UL49 homolog genes play a role in virus replication. Since transcripts of UL49h overlap with those of UL49.5h, deletion and foreign-gene insertion at the UL49h ORF would probably affect transcription of the UL49.5h gene, which may also partially contribute to impaired growth of the mutant virus. It has previously been reported that the HSV-1 UL49.5 ORF mutation per se does not exert any apparent effect on virus replication either in cell culture or in mice (28). This may indirectly imply that the defect associ-

ated with the UL49h deletion mutant is primarily caused by the UL49h gene deletion. Studies to further define the role of UL49h in virus replication by more specific mutagenesis of the UL49h gene and characterization of the UL49.5h gene product are under way.

The UL49h gene product was found to be predominantly localized in the nuclei of BHV-1-infected cells. According to computer-assisted analysis, no consensus nuclear localization signal was identified within the UL49h amino acid sequence. However, there are discernible clusters of basic amino acids, which may serve as a nuclear localization signal (26). Nuclear localization of the BHV-1 UL49h gene product is in contrast to the distribution of HSV VP22 and other HSV tegument proteins, including VP1/2 and UL37 gene products, which have been shown to be diffusely distributed throughout the nucleus and cytoplasm (6, 18, 20). The distinct subcellular distribution of the BHV-1 and HSV-1 UL49 homologous gene products may imply that these homologous proteins have different functional properties. The subcellular compartments in which incorporation of tegument proteins into mature virions takes place have not been defined. In this respect, the finding that the BHV-1 UL49h gene product, a tegument protein, was synthesized as a nuclear protein is of interest. This, in conjunction with the fact that UL49h is not essential for virus growth, may suggest a means by which to define the compartmentation of tegument assembly. For example, it may be possible to identify and mutate the nuclear localization signal of UL49h and then investigate assembly of the mutated UL49h into virions.

In summary, we have identified and characterized the BHV-1 UL49h gene and its product. Our results suggest that the BHV-1 UL49h gene encodes a polypeptide of about 33 to 35 kDa, which is synthesized as a nuclear protein during virus replication and constitutes a tegument component in mature virions. In addition, by producing a viable UL49h-negative mutant, we have shown that BHV-1 UL49h is not essential for virus growth in cell culture.

Nucleotide sequence accession number. The nucleotide sequence of the BHV-1 UL49h gene has been deposited in the GenBank database under accession number U21137.

This work was supported by the Medical Research Council of Canada and the Natural Science and Engineering Research Council of Canada. D.Y. is the recipient of a grant from HSURC of Saskatchewan.

REFERENCES

1. **Ace, C. I., T. A. McKee, J. M. Ryan, J. M. Cameron, and C. M. Preston.** 1989. Construction and characterization of a herpes simplex virus type 1 mutant unable to transinduce immediate-early gene expression. J. Virol. **63:**2260–2269.
2. **Blaho, J. A., C. Mitchell, and B. Roizman.** 1994. An amino acid sequence shared by the herpes simplex virus 1 alpha regulatory proteins 0, 4, 22, and 27 predicts the nucleotidylation of the UL21, UL31, UL47, and UL49 gene products. J. Biol. Chem. **269:**17401–17410.
3. **Carpenter, D. E., and V. Misra.** 1991. The most abundant protein in bovine herpes 1 virions is a homologue of herpes simplex virus type 1 UL47. J. Gen. Virol. **72:**3077–3084.
4. **Carpenter, D. E., and V. Misra.** 1992. Sequences of the bovine herpesvirus 1 homologue of herpes simplex virus type-1 alpha-trans-inducing factor (UL48). Gene **119:**259–263.
5. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67:**1759–1816.
6. **Elliott, G. D., and D. M. Meredith.** 1992. The herpes simplex virus type 1 tegument protein VP22 is encoded by gene UL49. J. Gen. Virol. **73:**723–726.
7. **Fawl, R. L., and B. Roizman.** 1994. The molecular basis of herpes simplex virus pathogenicity. Semin. Virol. **5:**267–271.
8. **Gibbs, E. P. J., and M. M. Rweyemamu.** 1977. Bovine herpesviruses. I. Bovine herpesvirus-1. Vet. Bull. **47:**317–343.
9. **Goding, C. R., and P. O'Hare.** 1989. Herpes simplex virus Vmw65-octamer binding protein interaction: a paradigm for combinatorial control of transcription. Virology **173:**363–367.
10. **Hall, L. M., K. G. Draper, R. J. Frink, R. H. Costa, and E. K. Wagner.** 1982. Herpes simplex virus mRNA species mapping in *Eco*RI fragment I. J. Virol. **43:**594–607.
11. **Hay, J., and W. T. Ruyechan.** 1992. Regulation of herpes simplex virus type 1 gene expression. Curr. Top. Microbiol. Immunol. **179:**1–14.
12. **Kowalski, J., S. A. Gilbert, S. van Drunen Littel-van den Hurk, J. van den Hurk, L. A. Babiuk, and T. J. Zamb.** 1993. Heat-shock promoter-driven synthesis of secreted bovine herpesvirus glycoproteins in transfected cells. Vaccine **11:**1100–1107.
13. **LaBoissiere, S., M. Trudel, and C. Simard.** 1992. Characterization and transcript mapping of a bovine herpesvirus type 1 gene encoding a polypeptide homologous to the herpes simplex virus type 1 major tegument proteins VP13/14. J. Gen. Virol. **73:**2941–2947.

13a. **Liang, X., et al.** Unpublished data.

14. **Liang, X., L. A. Babiuk, S. van Drunen Littel-van den Hurk, D. R. Fitzpatrick, and T. J. Zamb.** 1991. Bovine herpesvirus 1 attachment to permissive cells is mediated by its major glycoproteins gI, gIII, and gIV. J. Virol. **65:**1124–1132.
15. **Liang, X., L. A. Babiuk, and T. J. Zamb.** 1992. An in vivo study of a glycoprotein gIII-negative bovine herpesvirus 1 (BHV-1) mutant expressing beta-galactosidase: evaluation of the role of gIII in virus infectivity and its use as a vector for mucosal immunization. Virology **189:**629–639.
16. **Liang, X., M. Tang, B. Manns, L. A. Babiuk, and T. J. Zamb.** 1993. Identification and deletion mutagenesis of the bovine herpesvirus 1 dUTPase gene and a gene homologous to herpes simplex virus UL49.5. Virology **195:**42–50.
17. **Mayfield, J. E., P. J. Good, H. J. VanOort, A. R. Campbell, and D. E. Reed.** 1983. Cloning and cleavage site mapping of DNA from bovine herpesvirus 1 (Cooper strain). J. Virol. **47:**259–264.
18. **McLauchlan, J., K. Liefkens, and N. D. Stow.** 1994. The herpes simplex virus type 1 UL37 gene product is a component of virus particles. J. Gen. Virol. **75:**2047–2052.
19. **McLean, G., F. Rixon, N. Langeland, L. Haarr, and H. Marsden.** 1990. Identification and characterization of the virion protein products of herpes simplex virus type 1 gene UL47. J. Gen. Virol. **71:**2953–2960.
20. **McNabb, D. S., and R. J. Courtney.** 1992. Characterization of the large tegument protein (ICP1/2) of herpes simplex virus type 1. Virology **190:**221–232.
21. **Meredith, D. M., J. A. Lindsay, I. W. Halliburton, and G. R. Whittaker.** 1991. Posttranslational modification of the tegument proteins (VP13 and VP14) of herpes simplex virus type 1 by glycosylation and phosphorylation. J. Gen. Virol. **72:**2771–2775.
22. **Misra, V., A. C. Bratanich, D. Carpenter, and P. O'Hare.** 1994. Protein and DNA elements involved in transactivation of the promoter of the bovine herpesvirus (BHV) 1 IE-1 transcription unit by the BHV α gene *trans*-inducing factor. J. Virol. **68:**4898–4909.
23. **Misra, V., R. M. Bumenthal, and L. A. Babiuk.** 1981. Proteins specified by bovine herpesvirus 1 (infectious bovine rhinotracheitis virus). J. Virol. **40:**367–378.
24. **Pastoret, P. P., G. Burtonboy, A. Aguilar-Setien, M. Godart, M. E. Lamy, and F. Schoenaers.** 1980. Comparison between strains of infectious bovine rhinotracheitis virus (bovine herpesvirus I) from respiratory and genital origins, using polyacrylamide gel electrophoresis of structural proteins. Vet. Microbiol. **5:**187–192.
25. **Pinard, M. F., R. Simard, and V. Bibor-Hardy.** 1987. DNA-binding proteins of herpes simplex virus type 1-infected BHK cell nuclear matrices. J. Gen. Virol. **68:**727–735.
26. **Powers, M. A., and D. J. Forbes.** 1994. Cytosolic factors in nuclear transport: what's importin? Cell **79:**931–934.
27. **Preston, C. M., and E. L. Notarianni.** 1983. Poly(ADP-ribosyl)ation of a herpes simplex virus immediate early polypeptide. Virology **131:**492–501.
28. **Pyles, R. B., N. M. Sawtell, and R. L. Thompson.** 1992. Herpes simplex virus type 1 dUTPase mutants are attenuated for neurovirulence, neuroinvasiveness, and reactivation from latency. J. Virol. **66:**6706–6713.
29. **Riggio, M. P., and D. E. Onions.** 1993. DNA sequence of a gene cluster in the equine herpesvirus-4 genome which contains a newly identified herpesvirus gene encoding a membrane protein. Arch. Virol. **133:**171–178.
30. **Roizman, B., and A. E. Sears.** 1990. Herpes simplex viruses and their replication, p. 1795–1841. *In* B. N. Fields, D. M. Knipe, et al. (ed.), Virology, 2nd ed. Raven Press, Ltd., New York.
31. **Whittaker, G. R., M. P. Riggio, I. W. Halliburton, R. A. Killington, G. P. Allen, and D. M. Meredith.** 1991. Antigenic and protein sequence homology between VP13/14, a herpes simplex virus type 1 tegument protein, and gp10, a glycoprotein of equine herpesvirus 1 and 4. J. Virol. **65:**2320–2326.
32. **Yanagida, N., S. Yoshida, K. Nazerian, and L. F. Lee.** 1993. Nucleotide and predicted amino acid sequences of Marek's disease virus homologues of herpes simplex virus major tegument proteins. J. Gen. Virol. **74:**1837–1845.
33. **Zhang, Y., and J. L. C. McKnight.** 1993. Herpes simplex virus type 1 UL46 and UL47 deletion mutants lack VP11 and VP12 or VP13 and VP14, respectively, and exhibit altered viral thymidine kinase expression. J. Virol. **67:**1482–1492.