JOURNAL OF VIROLOGY, Dec. 1987, p. 4023–4025
0022-538X/87/124023-03$02.00/0
Copyright © 1987, American Society for Microbiology
Vol. 61, No. 12

# Map Location of the Thymidine Kinase Gene of Bovine Herpesvirus 1

LEONARD J. BELLO,* J. CHARLES WHITBECK, AND WILLIAM C. LAWRENCE

*Laboratory of Microbiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6049*

Received 26 June 1987/Accepted 12 August 1987

**Bovine herpesvirus 1 has been reported to contain a thymidine kinase (*tk*) gene which is nonessential for virus replication. We have isolated a thymidine kinase-negative mutant of the virus and localized the mutation by marker rescue experiments to a 1.1-kilobase *Bgl*II-*Sal*I fragment which maps at 0.47 to 0.48 on the bovine herpesvirus 1 genomic map. A thymidine kinase-negative bovine cell line isolated in our laboratory was used in these studies.**

Bovine herpesvirus 1 (BHV-1), a member of the alphaherpesvirus group (13), is an economically important cattle pathogen whose effects range from mild inapparent infections to a wide variety of clinical manifestations. The virus was initially linked with respiratory infections but is now known to also cause conjunctivitis, reproductive tract lesions, abortion, encephalitis, and even fatal systemic infections (5, 10). BHV-1 contains a linear 137-kilobase (kb) double-stranded DNA genome which is divided into long and short unique regions (4, 11). The short unique region is bounded by inverted repeat sequences and can exist in either of two orientations relative to the long unique region. Restriction endonuclease maps of BHV-1 have been published elsewhere (11), and the fragments generated by the restriction endonuclease *Hin*dIII have been cloned (8, 11).

In infected cells, BHV-1 is known to induce a viral thymidine kinase (TK) which is apparently nonessential for virus replication, since TK-negative variants of BHV-1 can be isolated (7, 16). In this report, we describe experiments which have determined the location of the *tk* gene on the physical map of BHV-1. The method used involved isolation of a TK-negative variant of BHV-1 followed by marker rescue experiments in which cloned fragments of wild-type BHV-1 DNA were used to rescue the TK-positive phenotype.

The Colorado 1 (Cooper 1) strain of BHV-1, the Madin-Darby bovine kidney (MDBK) cells, and the bovine turbinate cells used in this study were obtained from the American Type Culture Collection. Propagation of the virus and cells and methods for purification of viral DNA and marker rescue of TK-negative BHV-1 have been described previously (8). Recombinant virus which had regained the wild-type TK-positive phenotype was detected by the plaque autoradiography technique of Tenser et al. (15). TK was assayed as described previously (1).

To facilitate mapping of the BHV-1 *tk* gene, a TK-deficient cell line in which TK-negative and TK-positive strains of BHV-1 could be distinguished from each other was needed. Because TK-deficient cell lines in which BHV-1 can replicate well were not readily available, we developed a TK-deficient variant from the MDBK cell line used to propagate the virus in our laboratory. The strategy employed, essentially that described by Kit et al. (6), was to adapt MDBK cells to growth in gradually increasing concentrations of 5-bromodeoxuridine. Cells resistant to 100 μg of the drug per ml were obtained after about 1 year. The resistant cells, designated MDBK(BU100), were cloned and tested directly for TK activity. The results indicate that extracts prepared from MDBK(BU100) cells contain less than 5% of the TK activity found in the parental MDBK cell line.

To map *tk* by marker rescue, it was first necessary to isolate a BHV-1 variant carrying a mutation in the *tk* gene. Mutants defective in TK were isolated by passing the virus (0.01 PFU per cell) in cultures of MDBK(BU100) cells in the presence of increasing concentrations of arabinosylthymine (50, 100, and 200 μg/ml) until virus resistant to 200 μg of the drug per ml was obtained. The virus was plaque purified once in the presence of 100 μg of 5-bromodeoxyuridine per ml and once again with 200 μg of the drug per ml. The final plaque-purified virus obtained, designated BHV-1(B2), was then tested for its ability to induce TK in MDBK(BU100) cells. The results are shown in Fig. 1. Wild-type BHV-1 induced a 30-fold increase in the specific activity of TK, but BHV-1(B2) infection led to a slight decrease. Despite the absence of TK induction, the yield of virus from BHV-1(B2)-infected cells (182 PFU per cell) was as high as or higher than that from wild-type BHV-1-infected cells (104 PFU per cell). We conclude, therefore, that BHV-1(B2) carries a mutation which renders it TK negative.

In a previous paper, we described the cloning and characterization of restriction endonuclease *Hin*dIII fragments which account for the entire BHV-1 genome (8). These fragments, which were cloned in plasmid pBR322, were used in the experiments described below to determine the map position of the BHV-1(B2) mutation. Bovine turbinate cell cultures were cotransfected with mixtures of infectious DNA prepared from the TK-negative BHV-1(B2) mutant and each of the cloned BHV-1 DNA fragments. Extracellular virus was harvested when the cytopathic effect was 100%, and the virus obtained was plated on the TK-deficient MDBK(BU100) cells. The plaques, when clearly visible, were labeled with [$^{14}$C]thymidine for 4 h. Recombinants in which the TK-positive phenotype had been rescued were detected after autoradiography by their ability to incorporate isotope into DNA and form radioactive plaques (15). The results are shown in Table 1. Infectious BHV-1(B2) DNA transfected into bovine turbinate cells in the absence of wild-type BHV-1 DNA fragments yielded a low number of

* Corresponding author.

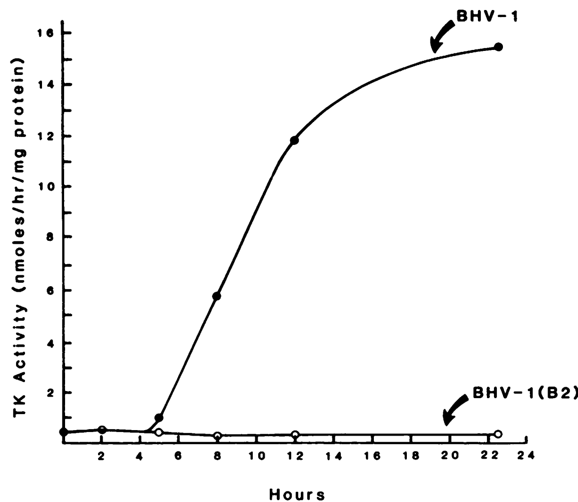


FIG. 1. Induction of TK by BHV-1(B2) and wild-type BHV-1. Confluent monolayers of MDBK(BU100) cells were infected with 5 PFU of BHV-1(B2) or wild-type BHV-1 per cell. At the indicated times, cells were harvested and extracts were prepared and assayed for TK. ●, BHV-1-infected cells; ○, BHV-1(B2)-infected cells.

TK-positive progeny which we assume represented revertants. The addition of *Hin*dIII fragment A resulted in a large increase in the number of TK-positive progeny obtained. None of the other *Hin*dIII fragments tested, which collectively represent the rest of the BHV-1 genome, rescued the TK-positive phenotype. We conclude that the BHV-1(B2) mutation maps somewhere within the 22.1-kb *Hin*dIII A fragment of BHV-1. According to the *Hin*dIII restriction enzyme map of BHV-1 published by Mayfield et al. (11), which is consistent with results from our laboratory, the *Hin*dIII A fragment maps between coordinates 0.38 and 0.54.

To further localize the map position of the BHV-1(B2) lesion, various subfragments of *Hin*dIII fragment A were isolated and tested for their ability to rescue TK-positive

TABLE 1. Marker rescue of BHV-1(B2) with *Hin*dIII restriction enzyme fragments[a]

| Fragment tested | No. of expts | No. of plaques | | % Rescue |
|---|---|---|---|---|
| | | Total | Radioactive[b] | |
| | 10 | 10,875 | 16 | 0.15 |
| A | 8 | 12,220 | 1,416 | 11.6 |
| B | 2 | 2,220 | 0 | <0.05 |
| D | 2 | 1,540 | 0 | <0.06 |
| E | 2 | 5,900 | 0 | <0.02 |
| F | 6 | 9,850 | 10 | 0.10 |
| G | 1 | 2,200 | 0 | <0.05 |
| H | 1 | 1,280 | 0 | <0.08 |
| I | 3 | 8,790 | 1 | 0.01 |
| J | 1 | 500 | 0 | <0.20 |
| K | 1 | 1,100 | 0 | <0.09 |
| L | 1 | 4,000 | 0 | <0.03 |
| M | 2 | 3,080 | 0 | <0.03 |
| N | 2 | 7,300 | 2 | 0.03 |
| O | 2 | 2,980 | 0 | <0.03 |

[a] Intact DNA (5 μg) from the TK-negative BHV-1(B2) mutant was cotransfected into bovine turbinate cells with 15 μg of a recombinant plasmid containing the indicated BHV-1 *Hin*dIII restriction fragment.

[b] A radioactive plaque indicated that the infected cells in a plaque incorporated [$^{14}$C]thymidine and, therefore, had regained a wild-type TK-positive phenotype.

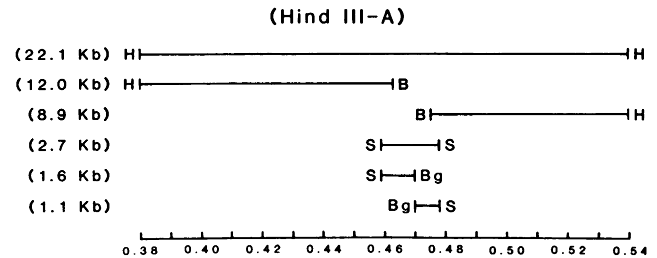


FIG. 2. Location of subfragments which lie within the BHV-1 *Hin*dIII-A region. *Hin*dIII fragment A is located on the BHV-1 physical map between coordinates 0.38 and 0.54 (11). The locations and sizes of subfragments listed in Table 2 are shown at the top in relationship to *Hin*dIII-A. The size of each fragment in kilobases is given in parentheses on the left. Restriction enzyme symbols: B, *Bam*HI; H, *Hin*dIII; S, *Sal*I; Bg, *Bgl*II.

virus. Figure 2 shows the relationship of the fragments tested to the *Hin*dIII A fragment, and Table 2 shows the marker rescue experimental results. Neither the 12.0-kb *Hin*dIII-*Bam*HI fragment nor the 8.9-kb *Bam*HI-*Hin*dIII fragment, which collectively represent most of the *Hin*dIII A fragment, rescued the TK-positive phenotype. However, a 2.7-kb *Sal*I-*Sal*I fragment, which overlaps both the above fragments and includes all the untested portion of A, yielded a large number of TK-positive recombinants. Thus, the BHV-1(B2) lesion lies somewhere within this fragment. The 2.7-kb *Sal*I-*Sal*I fragment contains a single *Bgl*II site which divides the fragment into 1.6- and 1.1-kb subfragments. When these subfragments were tested, only the 1.1-kb *Bgl*II-*Sal*I fragment rescued. This fragment, which maps between 0.47 and 0.48 on the BHV-1 genomic map, therefore contains the BHV-1(B2) mutation site and is the probable locus of a portion of the BHV-1 *tk* gene.

*tk* now is the second BHV-1 gene to be mapped. We previously mapped the position of the BHV-1 gene for glycoprotein g130, a homolog of herpes simplex virus type 1 glycoprotein gB (8). Recently, Liu and Manning reported the cloning of a 2-kb BHV-1 DNA fragment which they suggested may contain the entire BHV-1 *tk* gene (9). The fragment which they cloned hybridized to BHV-1 restriction enzyme fragments which centered the *tk* gene at about map coordinate 0.14, far from our map position of 0.47 to 0.48. It is possible that the BHV-1 strain studied by Liu and Manning contains a rearrangement which has altered the position of *tk* in their strain. We believe that the map position which we are reporting for *tk* in this communication is more typical

TABLE 2. Marker rescue of BHV-1(B2) with subfragments of the BHV-1 *Hin*dIII A fragment[a]

| Fragment tested[b] | Size (kb) | No. of expts | No. of plaques | | % Rescue |
|---|---|---|---|---|---|
| | | | Total | Radio-active | |
| *Hin*dIII-*Bam*HI[c] | 12.0 | 6 | 9,420 | 5 | 0.05 |
| *Bam*HI-*Hin*dIII[c] | 8.9 | 9 | 9,770 | 10 | 0.10 |
| *Sal*I-*Sal*I[c] | 2.7 | 11 | 24,390 | 1,376 | 5.6 |
| *Sal*I-*Bgl*II[d] | 1.6 | 5 | 9,031 | 1 | 0.01 |
| *Bgl*II-*Sal*I[d] | 1.1 | 5 | 11,160 | 789 | 7.1 |

[a] Marker rescue of wild-type TK-positive BHV-1 from the TK-negative BHV-1(B2) mutant was carried out as described in Table 1, footnote *a*.

[b] Map positions of the restriction enzyme DNA fragments are shown in Fig. 2.

[c] Fragment contained in recombinant plasmid.

[d] Fragment obtained from preparative agarose gel.

of BHV-1, since it is similar to that reported for varicella-zoster virus (3, 14), pseudorabies virus (2), equine herpesvirus type 1 (G. R. Robertson, G. C. Hudson, N. A. Scott, and J. M. Whalley, personal communication), and the $I_L$ isomer of herpes simplex virus (12).

This study was supported by grants 86-CRCR-1-2221 and 83-CRSR-2-2237 and Section 1433 Formula Funds from the U.S. Department of Agriculture and by Commonwealth of Pennsylvania Department of Agriculture grant ME-4606.

## LITERATURE CITED

1. **Bello, L. J.** 1974. Regulation of thymidine kinase synthesis in human cells. Exp. Cell Res. **89**:263–274.
2. **Ben-Porat, T., R. A. Veach, and S. Ihara.** 1983. Localization of the regions of homology between the genomes of herpes simplex virus, type 1, and pseudorabies virus. Virology **127**:194–204.
3. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67**:1759–1816.
4. **Farley, J. E., I. B. Skare, and J. Skare.** 1981. Inverted repeat sequences in infectious bovine rhinotracheitis virus DNA, p. 590. *In* A. J. Nahmias, W. R. Dowdle, and R. F. Schinazi (ed.), The human herpesviruses. Elsevier/North-Holland Publishing Co., New York.
5. **Kahrs, R. F.** 1977. Infectious bovine rhinotracheitis: a review and update. J. Am. Vet. Med. Assoc. **171**:1055–1064.
6. **Kit, S., D. R. Dubbs, and P. M. Frearson.** 1966. HeLa cells resistant to bromodeoxyuridine and deficient in thymidine kinase activity. Int. J. Cancer **1**:19–30.
7. **Kit, S., and H. Qavi.** 1983. Thymidine kinase (TK) induction after infection of TK-deficient rabbit cell mutants with bovine herpesvirus type 1 (BHV-1): isolation of $TK^-$ BHV-1 mutants. Virology **130**:381–389.
8. **Lawrence, W. C., R. C. D'Urso, C. A. Kundel, J. C. Whitbeck, and L. J. Bello.** 1986. Map location of the gene for a 130,000-dalton glycoprotein of bovine herpesvirus 1. J. Virol. **60**: 405–414.
9. **Liu, K. Y. C., and J. S. Manning.** 1986. Identification of the thymidine kinase gene of infectious bovine rhinotracheitis virus and its function in *Escherichia coli* hosts. Gene **44**:279–285.
10. **Ludwig, H.** 1983. Bovine herpesviruses, p. 135–214. *In* B. Roizman (ed.), The herpesviruses, vol. 2. Plenum Publishing Corp., New York.
11. **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.
12. **Reyes, G. R., K.-T. Jeang, and G. S. Hayward.** 1982. Transfection with the isolated herpes simplex virus thymidine kinase genes. I. Minimal size of the active fragments from HSV-1 and HSV-2. J. Gen. Virol. **62**:191–206.
13. **Roizman, B., L. E. Carmichael, F. Deinhardt, G. de The, A. J. Nahmias, W. Plowright, F. Rapp, P. Sheldrich, M. Takahashi, and K. Wolf.** 1982. Herpesviridae: definition, provisional nomenclature and taxonomy. Intervirology **16**:201–217.
14. **Sawyer, M. H., J. M. Ostrove, J. M. Felser, and S. E. Straus.** 1986. Mapping of the varicella zoster virus deoxypyrimidine kinase gene and preliminary identification of its transcript. Virology **149**:1–9.
15. **Tenser, R. B., J. C. Jones, S. J. Ressel, and F. A. Fralish.** 1983. Thymidine plaque autoradiography of thymidine kinase-positive and thymidine kinase-negative herpesviruses. J. Clin. Microbiol. **17**:122–127.
16. **Weinmaster, G. A., V. Misra, R. McGuire, L. A. Babiuk, and E. Declercq.** 1982. Bovid herpesvirus type-1 (infectious bovine rhinotracheitis virus)-induced thymidine kinase. Virology **118**: 191–201.