Acta virol. **34** : 188—192, 1990

BOVINE HERPES MAMMALITIS VIRUS THYMIDINE KINASE GENE

J. T. MAY, M. AWAD, A. REUM, *M. SHEPPARD

Microbiology Department, La Trobe University, Bundoora, Victoria, and *CSIRO Animal Health Parkville, Victoria, 3083 — Australia

Received November 2, 1988

Summary. — Bovine herpes mammillitis virus (BHMV) codes for a thymidine kinase (Tk), the gene for which is located in a 2.6 kb *Sal*I restriction endonuclease fragment with a map unit of 0.2—0.32. This particular DNA fragment can transfect LTk$^-$ mouse cells and convert them to Tk$^+$. The gene position of BHMV Tk would suggest another homologous gene map position for BHMV and herpes simplex virus (HSV).

Key words: bovine herpesvirus; thymidine kinase; gene location

Introduction

Bovine herpes mammillitis virus (BHMV or bovine herpesvirus 2) has a double-stranded DNA genome of 133 kb (88×10^6 molecular mass) and is similar in structure to herpes simplex virus (HSV). Some proteins are known to be antigenically related between these two viruses (Maragos and May, 1987) and some of these proteins are now known to have homologous gene locations in the genomes of the two viruses. We previously reported identical gene positions of the major DNA-binding proteins of HSV (ICP8) and BHMV (Maragos and May, 1987) which has subsequently been confirmed and extended with the identification of identical glycoprotein B gene regions (Hammershmidt *et al.*, 1988*a*, *b*). Homologous regions of the major capsid protein (VP5) and thymidine kinase (Tk) have been suggested based on hybridization data using both HSV and BHMV DNA (Yeung *et al.*, 1988). The Tk gene of herpesviruses is particularly interesting as it can be manipulated to produce vaccine strains (Kit *et al.*, 1985; Kit *et al.*, 1987) or can be used for genetic engineering of the herpesviruses (Roizman and Jenkins, 1985). A Tk$^-$ BHMV was briefly mentioned (Batterson and Roizman, 1983) but was not described further. We report here the gene location of BHMV Tk based on biological activity of the cloned gene thus identifying the BHMV DNA restriction endonuclease fragment containing the Tk gene and confirming an identical gene position for both of BHMV and HSV Tks.

Materials and Methods

Virus cells and viral DNA preparations. The DNA from virus strain BHM-1 was used for these cloning experiments and was used as described previously using sodium iodide gradients (Maragos and May, 1987) except that the virus DNA was prepared from infected baby hamster

kidney cells (BHK-21). BHMV (both BHM-1 or CSIRO 290 strains; Maragos *et al.*, 1987) can be grown in Tk^- Chinese hamster peritoneal cells (B14I50; American Type Culture Collection, CCL 14.1), but with maximum infection occuring 24 hours after the cells are passaged.

BHMV DNA cloning and restriction endonuclease cleavage. The DNA was cloned in pUC18 plasmids using a PUC cloning kit (Boehringer-Mannheim, West Germany) using the standard methods described by the manufacturer. Restriction endonuclease digestions were as described previously (Maragos and May, 1987; Maragos *et al.*, 1987) using Boehringer-Mannheim enzymes and the standard methods described by the manufacturer. Separation of the DNA fragments was achieved in 0.6% agarose gels in a Trisacetate buffer (Maragos and May, 1987) for 18 hr at 1.5 V/cm.

Transfections. Mouse LTk^- $Aprt^-$ cells normally maintained in growth medium with 25 μg/ml bromodeoxyuridine were used in this study. pUC18 plasmids containing various BHMV DNA restriction endonuclease fragments were not restriction endonuclease cleaved prior to use in the transfection studies. Transfections were performed essentially as described by Otsuka *et al.*, 1982. For the experiments 3×10^5 LTk^- cells were seeded into 50 mm × 13 mm plastic tissue culture dishes with complete Earles MEM and 10% newborn calf serum (Flow Laboratories, Sydney), 50 μg/ml neomycin but without 5-bromodeoxyuridine. 10—20 μg of plasmid DNA containing BHMV DNA fragments was added to 20 μg of carrier salmon sperm DNA (Sigma Chemical Company, USA) and 2 M $CaCl_2$ added to 250 mol/l. To this mixture an equal volume of 2 × Hepes buffer saline (2 × HBS; 280 mol/l NaCL, 50 mol/l Hepes and 1.5 mol/l Na_2HPO_4 PH7.12) was added and the solution incubated at room temperature for 30 min to allow the DNA to precipitate. The precipitated DNA was added to the cell growth media covering the cell monolayer and incubated at 37 °C for 5 hrs in a humidified 95% air and 5% CO_2 atmosphere. After a 5 hr incubation the media were removed and fresh growth media added and reincubated for a further 20 hr at which time the growth media were supplemented with hypoxathine, aminopterin and thymidine (50 × HAT was purchased from Flow Laboratories, Australia). The HAT media were changed every two days, with the appearance of Tk^+ resistant colonies (up to 15 per plate) after a week. Negative control plates were transfected with salmon sperm DNA alone which always resulted in total cell death and no appearance of HAT resistant colonies.

Results and Discussion

When our strains of BHMV were grown in a TK^- Chinese hamster peritoneal cell line (B14I50) in the presence of 100 μg/ml bromodeoxyuridine, the few surviving viruses could be plaque purified and they were found to be about 5 fold deficient in Tk activity when compared to the untreated virus. This would confirm a Tk activity which was coded for by the virus (see also Batterson and Roizman, 1983).

BHMV DNA was cleaved with restriction endonuclease *Hind*III, the DNA fragments separated by agarose gel electrophoresis (Fig. 1) and the *Hind*III fragment I (11.5 kb) was cloned into a pUC18 plasmid (Fig. 1). This particular DNA fragment is known to have a map unit of about 0.27—0.36 and is the easiest segment to isolate in this area of the BHMV DNA (Buchman and Roizman, 1978). Various segments of the *Hind*III I region were subcloned into pUC18 culminating with the cloning of 2.6 kb *Sal*I fragment (Fig. 2). Several *Sal*I DNA fragments of this size are known to be generated after *Sal*I restriction endonuclease digestion of BHMV DNA (Maragos *et al.*, 1987). All cloned BHMV DNA fragments were tested for their ability to transfect LTk^-. In our hands the smallest fragment we have detected able to transduce LTk^- cells to Tk^+ was the 2.6 kb *Sal*I fragment from approximately 0.3—0.32 map units of BHMV. This *Sal*I fragment (in either orientation in the pUC18 plasmid) was four-fold more efficient at

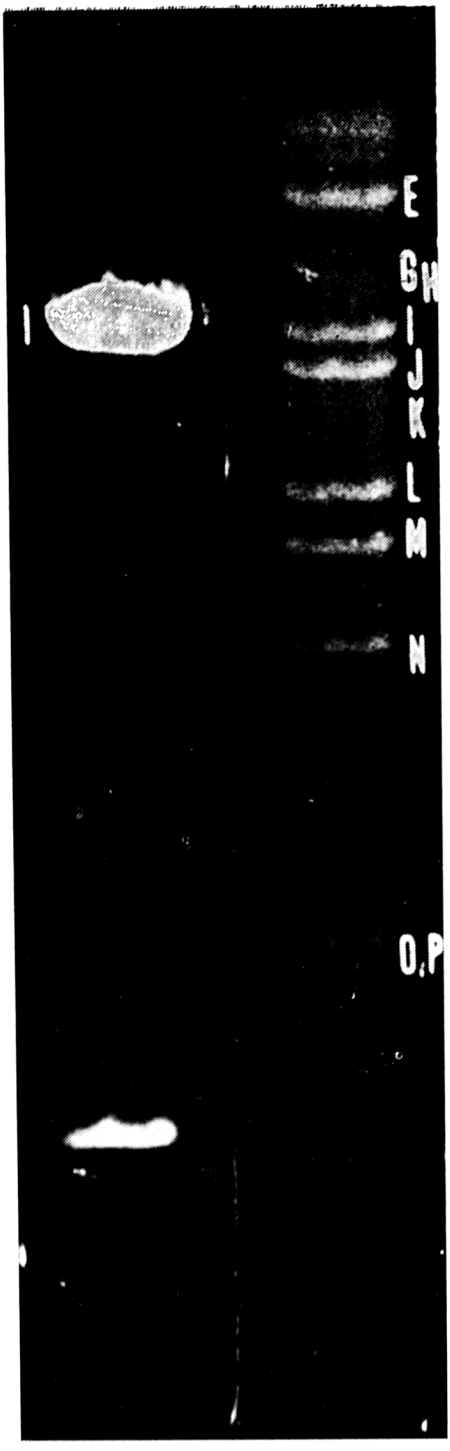

Fig. 1. *Hind*III restriction endonuclease digestion of both BHMV DNA (right track) and a pUC18 plasmid containing the *Hind*III I fragment of BHMV DNA (left track)
Separation of the DNA fragments was by agarose gel electrophoresis (see methods).

transforming LTK⁻ cells to Tk⁺ than the original *Hind*III I fragment. While the *Sal*I fragment is about twice size of the expected Tk gene (Kit, 1985) it has detectable Tk biological activity. However, no cloned DNA fragment that was cleaved at the indicated *Xba*1 site (Fig. 2) was found to have Tk transfecting ability possibly indicating this site is within the actual

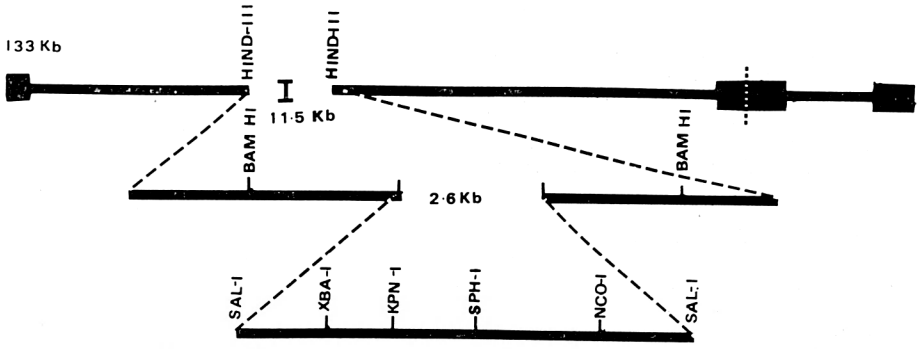

Fig. 2

Map of BHMV DNA indicating the *Sal*I 2.6 kb region coding for the Tk activity *HindIII I* can be subcloned in several ways using the *Bam*H1 sites and the *Xba*1 site into PUC18. The *Sal*I region has the restriction endonuclease sites occurring only once as indicated, based on gel electrophoresis DNA profiles.

gene itself. The presence in the *Sal*I fragment of restriction endonuclease sites only occuring once is indicated in Fig. 2, as detected by gel electrophoresis profiles. These sites should allow the manipulation (by nucleotide base deletions or insertions) of the Tk gene based on the loss of biological activity.

Yeung *et al.* (1988) reported in hybridization studies using HSV-1 Tk probes that these hybridized weakly to the *Xba*1 H and strongly to the *Xba*1 I/J fragments of BHMV DNA. The main region of the 2.6 kb *Sal*I fragment comes from the *Xba*1 J region with the *Xba*1 site within the *Sal*I fragment representing the junction between *Xba*1 H and J (see also Buchman and Roizman, 1978; Maragos and May, 1987). We have not been able to demonstrate any significant hybridization between BHMV DNA and HSV-1 Tk probes nor with small nucleotide sequences (12 bases) based on the very limited Tk regions of homology (Kit, 1985). Nevertheless, the biological activity detected here indicates the position of BHMV Tk gene is in the position suggested by the hybridization data of Yeung *et al.*, 1988.

BHMV is a potential bovine-specific vector virus as some strains produce no disease and are not transmitted to other animals or people in field situations (Maragos *et al.*, 1987). The virus is also naturally temperature sensitive (Letchworth and Carmichael, 1984) further restricting its infection.

Clearly BHMV has a Tk gene region for DNA manipulative purposes and furthermore with the removal of the Tk activity herpesviruses become less pathogenic (Kit *et al.*, 1985).

Acknowledgements. We acknowledge the support of the Australian Research Grant Committee for the support of this project.

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