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STUDIES OF THE MAJOR DNA BINDING PROTEINS OF TWO BOVINE HERPES MAMMILLITIS VIRUS ISOLATES

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Summary. — Antigenic cross-reaction of the DNA-binding protein between herpes simplex virus type 2 (HSV-2) and two bovine herpes mammillitis virus (BHMV) isolates (BHM-1 and BHMV CSIRO 290) was demonstrated by indirect immunofluorescence. Southern blot analysis showed a region of homology between HSV-2 BglII O DNA (coding for the HSV-2 major DNA-binding protein) and BHMV XbaI DNA fragment N, suggesting a similar gene map unit position on both genomes.

Key words: herpes simplex virus type 2; bovine herpes mammillitis virus; DNA-binding protein

Introduction

Bovine herpes mammillitis virus (BHMV or bovine herpesvirus 2) has a double stranded DNA genome of 135 kb (88×10^6 molecular mass), similar in structure (group E genome) to herpes simplex virus (HSV; Buchman and Roizman, 1978*a*, *b*) and has 14 % base sequence homology with HSV-1 (Sterz *et al.*, 1973/4). Antigenically about 4—6 proteins can cross react between BHMV and HSV (Sterz *et al.*, 1973/4; Norrild *et al.*, 1978; Killington *et al.*, 1977) particularly the major DNA-binding protein (Littler *et al.*, 1981; Yeo *et al.*, 1981) of about 130 K for both BHMV and HSV (Halliburton and Freeman, 1985), glycoprotein B (Snowden *et al.*, 1985) and the major capsid protein (Yeo *et al.*, 1981). Tryptic peptide digests of the cross — — reacting DNA-binding proteins of BHMV and HSV (in particular HSV-2) have indicated structural similarities (Littler *et al.*, 1981).

We have previously shown that the HSV-2 major DNA-binding protein, ICP 8 is encoded for in the BglII O restriction endonuclease region (4.5 kb) of HSV-2 DNA (Sheppard *et al.*, 1985). In the current communication, we report on the antigenic cross-reaction of the DNA-binding protein between BHMV and HSV-2 by indirect immunofluorescence and on the localization of the analogous BHMV DNA region homologous to the HSV-2 BglII O region.

Materials and Methods

Viruses, cells and viral DNA preparation. The 2 Australian BHMV isolates used in the study were: BHM-1 isolated from localized infection (cow teat) in Victoria (Turner *et al.*, 1974) and BHMV CSIRO 290 isolated from generalized infection of a cow in the Northern Territory (St George *et al.*, 1980). Both isolates were plaque purified, grown and passaged in bovine turbinate (BT) cells (Commonwealth Serum Laboratories, Melbourne, Australia) and viral DNA was prepared in bovine kidney cells (MDBK) as described previously (Maragos *et al.*, 1986). The Australian strains were used in this study due to import restrictions on this virus.

Restriction endonuclease cleavage of viral DNA and southern blot hybridization. BHM-1 and BHMV CSIRO 290 DNA was cleaved with 20 units of XbaI restriction endonuclease (Boehringer--Mannheim Australia Pty Ltd., St Kilda, Victoria) for a maximum of 5 hr at 37 °C and electrophoresed as described previously (Sheppard *et al.*, 1982; Maragos *et al.*, 1986). The pBO2 DNA (pBR325 containing the HSV-2 BglII O DNA restriction endonuclease fragment in the BamHI site) was cleaved with 10 units of EcoRI restriction endonuclease for a maximum of 5 hr, labelled with [α-^{32}P] (1×10^7 cpm) and nick translated according to the method by Rigby *et al.* (1977). Cleaved BHM-1 and BHMV CSIRO 290 DNA was transferred to nitrocellulose and hybridized to [α-^{32}P]-labelled pBO2 DNA for 20—24 hr at 40 °C or 45 °C in 50 % formamide according to Southern (1985). The nitrocellulose was then exposed to Kodak X-omat X-ray film (Sheppard *et al.*, 1985).

Immunofluorescence. Indirect immunofluorescence was performed essentially as described by Yeo *et al.* (1981), except using BHMV infected BT cells. The cross reacting DNA-binding protein was determined by using monospecific rabbit antiserum to the HSV-2 major DNA-binding protein (ICSP 11/12 equivalent to ICP 8; gift of Dr. K. L. Powell).

Results and Discussion

By immunofluorescence, antibody to ICSP 11/12 stained the nucleus of BHM-1 and BHMV CSIRO 290 infected BT cells brightly 19 hr post-infection (Fig. 1). This is similar to immunofluorescence data previously published by Yeo *et al.* (1981), indicating that the DNA-binding proteins of HSV-2 and the 2 strains of BHMV have group specific antigenic determinants.

On the basis of this antigenic cross-reaction of the DNA-binding protein between HSV-2 and BHMV, we were interested in locating the possible BHMV DNA region analogous to the HSV-2 major DNA-binding protein coding region (BglII O of HSV-2; Sheppard *et al.*, 1985). XbaI DNA restriction endonuclease cleavage profiles of BHM-1 and BHMV CSIRO 290 were identical (Maragos *et al.*, 1987) and consistent with the BHMV, XbaI DNA cleavage profile previously published for another BHMV strain by Buchman and Roizman (1978*a*). Cloned HSV-2 Bgl II O DNA restriction endonuclease fragment hybridized strongly to XbaI restriction endonuclease fragment N of BHM-1 and BHMV CSIRO 290 isolates (Fig. 2-C and D) under stringent hybridization conditions of 45 °C. This region corresponds to a calculated region of approximately 0.385—0.415 map units on the BHMV genome (based on a DNA length of 0—1 for HSV-2 and BHMV). Although, BHMV has a smaller overall genome to HSV-2 (Buchman and Roizman, 1978*b*) this calculated location is similar to the HSV-2 DNA-binding protein coding region (Sheppard *et al.*, 1985).

Under less stringent hybridization conditions of 40 °C, the HSV-2 BglII O also partially hybridized to the XbaI G DNA restriction endonuclease fragment of BHMV (Fig. 2-B) suggesting that the complete DNA coding

region of BHMV major DNA-binding protein may not be located solely in the XbaI N fragment of BHMV but a small portion of the gene coding region may be located to the right of the XbaI N fragment. Whether this homologous BHMV DNA region does indeed code for the BHMV major DNA-binding protein remains to be confirmed by gene expression studies (Sheppard *et al.*, 1985). However, no other BHMV gene locations have been reported to date.

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Explanation to Figures (Plates XXXII—XXXIII):

Fig. 1. Immunofluorescence with antiserum to the HSV-2 major DNA-binding protein, ICSP 11/12 indicating cross-reacting BHMV DNA- binding protein in BHM-1 infected BT cells. Similar immunofluorescence was obtained for BHMV CSIRO 290 infected BT cells.

Fig. 2. Southern blot hybridization of nick translated [α-^{32}P] dCTP — labelled pBO2 DNA with XbaI cleaved BHMV DNA. XbaI DNA restriction endonuclease cleavage profile of BHM-1

showing DNA fragments (A) to the right. XbaI cleaved BHM-1 (B and C) and BHMV CSIRO 290 DNA (D) was transferred to nitrocellulose by the method of Southern (1975) and then hybridized to [^{32}P]-labelled pBO2 DNA (nick translated as described by Rigby *et al.* 1977) at 40 °C (B) or 45 °C (C and D) showing strong binding to the N fragment (B, C and D) and partial binding to the G fragment (B). λ HindIII DNA markers in kilobases (kb) are indicated. The plasmid pBR325 used as a negative control, did not hybridize with either XbaI N and G DNA fragments of BHM-1 and BHMV CSIRO 290 under low-stringent or stringent hybridization conditions of 40 °C or 45 °C, respectively.