



HAL
open science

Antiapoptotic activity of bovine herpesvirus type-1 (BHV-1) UL14 protein

L. de Martino, G. Marfe, M. Irno Consalvo, C. Di Stefano, U. Pagnini, P.
Sinibaldi-Salimei

► **To cite this version:**

L. de Martino, G. Marfe, M. Irno Consalvo, C. Di Stefano, U. Pagnini, et al.. Antiapoptotic activity of bovine herpesvirus type-1 (BHV-1) UL14 protein. *Veterinary Microbiology*, 2007, 123 (1-3), pp.210. 10.1016/j.vetmic.2007.02.026 . hal-00532219

HAL Id: hal-00532219

<https://hal.science/hal-00532219>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Antiapoptotic activity of bovine herpesvirus type-1 (BHV-1) UL14 protein

Authors: L. De Martino, G. Marfe, M. Irno Consalvo, C. Di Stefano, U. Pagnini, P. Sinibaldi-Salimei



PII: S0378-1135(07)00107-1
DOI: doi:10.1016/j.vetmic.2007.02.026
Reference: VETMIC 3605

To appear in: *VETMIC*

Received date: 28-12-2006
Revised date: 14-2-2007
Accepted date: 21-2-2007

Please cite this article as: De Martino, L., Marfe, G., Consalvo, M.I., Di Stefano, C., Pagnini, U., Sinibaldi-Salimei, P., Antiapoptotic activity of bovine herpesvirus type-1 (BHV-1) UL14 protein, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.02.026

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Research**

2 **Title: Antiapoptotic activity of bovine herpesvirus type-1 (BHV-1) UL14 protein.**

3 L. De Martino^{a*}, G. Marfe^b, M Irmo Consalvo^c, C. Di Stefano^b, U. Pagnini^a, P. Sinibaldi-Salimei^b.

4

5 ^aDepartment of Pathology and Animal Health, Infectious Diseases, Medicine Veterinary School,
6 University of Naples “Federico II”, Via F. Delpino, 1 – 80137 Naples, Italy.

7 ^bDepartment of Experimental Medicine and Biochemical Sciences, University of Rome “Tor
8 Vergata”, Via Montpellier 1 – 00133 Rome, Italy.

9 ^cDepartment of Biopatologia e Diagnostica per Immagini, University of Rome “Tor Vergata”, Via
10 Montpellier 1 – 00133 Rome, Italy.

11

12

13

14

15

16 Corresponding author: Luisa De Martino PhD

17 Department of Pathology and Animal Health, Infectious Diseases, Medicine Veterinary School,
18 University of Naples “Federico II”, Via F. Delpino, 1 – 80137 Naples, Italy.

19 Tel.: +39 081 2536178 – FAX: +39 081 2536179

20 email: luisa.demartino@unina.it;

21 luisa_demartino@libero.it

1 **Abstract**

2 Viruses have evolved different strategies to interfere with apoptotic pathways in order to halt
3 cellular responses to infection. One previous study showed that transient transfection of bovine
4 herpesvirus type-1 (BHV-1) UL14 protein is efficient in protecting Madin Darby kidney (MDBK)
5 and human chronic myelogenous leukemia (K562) cells from sorbitol-induced apoptosis. This
6 protein corresponds to a putative protein of BHV-1, which shares aminoacid sequence with a part
7 of the peptide-binding domain conserved in human heat shock protein (HSP70) family. The pBK-
8 CMV-UL14 plasmid transfected MDBK cells treated with sorbitol did not show caspase-3 and
9 caspase-9 activation with respect to non-transfected MDBK cells (UL14 negative). Furthermore,
10 we report that the expression of the full length sequence of BHV-1 UL14 is evident after 7 h of
11 infection of BHV-1 on MDBK cells which were then treated with sorbitol. These results indicate
12 that UL14 gene product has important implications to enhance cell survival in response to apoptotic
13 stimuli.

14
15 **Key words:** BHV-1, UL14, caspase-3, caspase-9

16 17 **1. Introduction**

18 Inhibition of apoptosis is a common strategy of viral pathogenesis that favors virus
19 replication and may contribute to oncogenesis (Thomson, 2001). Bovine herpesvirus 1 (BHV-1)
20 codes for a variety of proteins that cooperate in blocking apoptosis triggered by virus infection, non
21 specific stress agents such as osmotic shock (Geiser and Jones, 2005).

22 The genome of the Cooper strain of BHV-1, which has been completely sequenced, is
23 135,301 bp in length and contains an estimated 73 genes (GenBank accession number AJ004801).
24 The genome consists of two unique sequences, long or UL (103-kb L segment) and short or US (32-
25 kb S segment), comprising a 10-kb unique region flanked by 11-kb internal and terminal inverted

1 repeats (IR and TR). This arrangement corresponds to the D-type herpesviral genome (McGeoch
2 and Davison, 1999).

3 UL14 is located in a gene cluster also containing genes UL13, UL12, and UL11 which share
4 a common polyadenylation signal downstream of UL11. The product of UL14 gene of herpes
5 simplex virus type 1 (HSV-1) has been described as a minor protein of the tegument of 32 kDa
6 which is expressed late in infection (Cunningham et al., 2000).

7 It has been suggested that HSV-2 and HSV-1 UL14 protein shared some characteristics of
8 heat shock proteins (HSPs) or molecular chaperones, such as nuclear translocation upon heat shock,
9 ATP deprivation and osmotic shock (Yamauchi et al., 2002).

10 Virus-induced cell death plays an important role in the pathogenesis of virus infection.
11 Many viruses have been demonstrated to elicit or inhibit apoptosis either directly or indirectly
12 during their replication cycles (Roulston et al., 1999). Therefore, several viruses have developed
13 specific gene activation programs to inhibit premature death of the infected cells, so as to complete
14 the viral progeny. This extremely complex phenomenon probably involves many genes which need
15 to be characterized. HSV-1 has been extensively studied and several genes with antiapoptotic
16 activity are evidenced (Benetti and Roizman, 2004; Perkins et al., 2003).

17 Apoptosis is a cell suicide mechanism invoked in different situations to remove redundant,
18 damaged, or infected cells. An essential component of the apoptotic machinery, the caspase family,
19 consists of a group of intracellular cysteine proteases with at least 14 members (Weil et al., 1997;
20 Rathmell and Thompson, 2002; Philchenkov, 2004). They can be divided into initiator or upstream
21 caspases (caspases-2, -8, -9, and -10), which go on to activate the downstream or effector caspases
22 (caspases-3, -6 and -7) (Thornberry and Lazebnik, 1998; Slee et al., 1999). Cleavage of a select
23 group of substrates by effector caspases is responsible for dismantling of essential cell components,
24 which results in morphological and biochemical changes that characterize apoptotic cell death:
25 cytoskeletal rearrangement, cell membrane blebbing, nuclear condensation and DNA fragmentation.
26 Using a pBK-CMV-UL14 transfected cell line with inducible expression, we show that UL14

1 alone is sufficient to inhibit apoptosis induced by stress agent, and that it prevents the activation of
2 caspase-3 and caspase-9 in sorbitol-induced apoptosis. These results point to a strategy for viral
3 inhibition of apoptosis based on functional inactivation of a critical component of the cellular death
4 machinery.

5

6 **2. Materials and methods**

7 *2.1. Virus*

8 The reference BHV-1 Cooper strain was used in this study (De Martino et al., 2003).
9 Madin-Darby bovine kidney (MDBK) cells were used for viral propagation. When cytopathic
10 effects were observed, cells were frozen and thawed three times, and the supernatant was used to
11 titre the virus. Virus stocks with titres of 2×10^7 TCID₅₀ ml⁻¹ were stored in liquid nitrogen until use.

12

13 *2.2. Cells and culture conditions*

14 MDBK cells were cultured in DMEM supplemented with 2 mM L-glutamine, 5% pre-
15 screened and heat-inactivated foetal calf serum (FCS) (Eurobio), 100 IU of penicillin, and 100 mg
16 of streptomycin per ml (both antibiotics from Bio-Whittaker), in a 5% CO₂ incubator. This cell line
17 was maintained free of mycoplasma and of bovine viral diarrhoea virus. Monolayers of MDBK
18 cells were either mock infected with medium alone or infected with BHV-1 Cooper at a multiplicity
19 of infection of 10 TICD₅₀/cell in DMEM without serum. Virus inoculum was allowed to proceed
20 for 1 h at room temperature. After removal of the inoculum, the monolayers were overlaid with pre-
21 warmed medium (DMEM containing 2 mM L-glutamine and 2% FCS) and incubated at 37°C in a
22 5% CO₂ incubator. At the end of various incubation times, one group of monolayers was incubated
23 in medium containing 1 M sorbitol for 1 h at 37°C, rinsed once in DMEM, and then reincubated for
24 an additional 2 h in fresh DMEM containing 2 mM L-glutamine and 2% FCS.

25

26 *2.3. Plasmid construction and establishment of inducible UL14-expressing cell line*

1 The UL14 region of BHV-1 was amplified by polymerase chain reaction (PCR) using the
2 following primer pairs: forward 5'-ATGGCGACGGCGGC-3' and reverse 5'-
3 TGCTGTGGGGCGGC-3'. The amplified fragment was cloned into the FLAG epitope containing
4 pBK-CMV vector (Stratagene). The resulting pBK-CMV-UL14 expresses the UL14 gene as an N-
5 terminal Met-FLAG (MDYKDDDDK) fusion protein under the control of cytomegalovirus
6 immediate-early promoter (Marfe et al., 2006; Cartier et al., 2003).

7

8 *2.4. Extraction of genomic DNA*

9 pBK-CMV-UL14 plasmid transfected MDBK cells (UL14 positive), empty vector
10 transfected MDBK cells, and non-transfected MDBK cells (UL14 negative) were washed twice
11 with phosphate-buffered saline (PBS) and lysed by addition of an extraction buffer containing 5
12 mM ethylene diaminetetra-acetic acid (EDTA), 10 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1%
13 sodium dodecylsulfate (SDS) and 400 µg/ml proteinase K (20 mg/ml) (Sigma Aldrich Co.),
14 incubated at 37°C overnight. Then, DNA samples were digested with 10 mg/ml RNase A for 1 hr at
15 37°C and purified through consecutive TE (10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0)-saturated
16 phenol, phenol-chloroform (1:1 v/v) and chloroform extractions. Finally, DNA samples were
17 precipitated with 0.3 M sodium acetate at pH 5.2 and 2.5 volumes of cold ethanol, centrifuged for
18 30 min at maximum speed at 4°C, washed in 70% cold ethanol and resuspended in sterile water.

19

20 *2.5. DNA fragmentation assay*

21 To analyze the time dependency of apoptotic DNA fragmentation after the treatment with
22 sorbitol, pBK-CMV-UL14 plasmid transfected MDBK cells (UL14 positive), empty vector
23 transfected MDBK cells, and non-transfected/not-treated MDBK cells (UL14 negative) were
24 washed twice with PBS and lysed by addition of a hypotonic solution (50 mM Tris-HCl pH 7.5, 20
25 mM EDTA, 1% NP-40). After centrifugation, supernatants were treated with 1 % SDS and RNase
26 A (final concentration 5 µg/µl) for 2 h at 56°C, followed by digestion with proteinase K (final

1 concentration 2.5 $\mu\text{g}/\mu\text{l}$) at 45°C for at least 6 h. Before hydrolysis a further cleaning of DNA was
2 performed by phenol-chloroform extraction. Pellets were dried for 30 min and resuspended in 200
3 μl Tris-EDTA pH 8.0 (Herrmann et al., 1994). Aliquots of 20 μl containing 10 μg DNA were
4 analyzed by electrophoresis on 1.8% ethidium bromide-containing agarose gels and visualized and
5 quantitated under UV transillumination of apparatus BioRad Gel Doc 1000 (BioRad) with the
6 program Quantity One (Herrmann et al., 1994).

7

8 *2.6. Caspase-3 and 9 assay*

9 To measure caspase-3 and -9 activity, 1×10^6 both transfected cells (UL14 positive) and
10 non-transfected MDBK cells (UL14 negative) were used. These cells were incubated for 1 h at
11 37°C with sorbitol (1 M), and following 2 h in fresh medium without sorbitol. As further control,
12 we used non-transfected MDBK cells (UL14 negative) incubated in fresh medium without sorbitol
13 throughout the experiment. The samples were washed in PBS, pelleted in a microcentrifuge, and
14 resuspended in 50 μl PBS. The appropriate peptide substrate [DEVD-7-amido-4-methylcoumarin
15 (AMC) for caspase-3 and LEHD-AMC for caspase-9] was added according to instructions from the
16 manufacturer (Calbiochem). Fluorescence was measured in a FACSCalibur Becton Dickinson.

17

18 *2.7. Southern blot analysis*

19 The DNA samples were separated on a 1,2% agarose gel, and transferred onto a nylon
20 membrane. A 669 kb DNA fragment was used as a template. The ^{32}P -dCTP Prime-it II kit
21 (Stratagene) was used for random priming. Hybridization was carried out at 42°C in UltraHyb
22 hybridization buffer. Probe used were cDNA generated by RT-PCR with the following primer
23 pairs: forward 5'-ATGGCGACGGCGGC-3' and reverse 5'-TGCTGTGGGGCGGC-3'. In order
24 to verify the presence of the UL14 construct in MDBK cells Southern Blot analysis was
25 performed.

26

1 2.8. RNA isolation and Northern blot

2 Total RNAs of control cells, infected MDBK cells (treated with sorbitol) at different times
3 of 1 up to 13 h p.i. were isolated using Tri Reagent (Sigma-Aldrich Chemie GmbH, Taufkirchen,
4 Germany). Aliquots (10 µg) of RNA were electrophoresed on 1% agarose formaldehyde gels and
5 subsequently blotted onto nylon membranes (Hybond N, Amersham, Braunschweig, Germany). The
6 membrane was then UV cross-linked, and hybridized to ³²P-labelled probe.

7 Hybridization was carried out at 42°C in UltraHyb hybridization buffer. We used the probe
8 obtained by PCR with the following primer pairs: forward 5'-ATGGCGACGGCGGC-3' and
9 reverse 5'-TGCTGTGGGGCGGC-3'.

10

11 2.9. Statistical analysis

12 The results are presented as mean ± SD of three independent experiments. One-way
13 ANOVA with Turkey's post test was performed using GraphPad InStat Version 3.00 for Windows
14 95 (GraphPad Software, San Diego, California). An error probability with P<0.05 was selected as
15 significant.

16

17 3. Results

18 3.1. Evaluation of antiapoptotic activity of UL14 gene product

19 In order to investigate the role of UL14 in BHV-1-induced antiapoptotic responses, MDBK
20 cells were transfected with the pBK-CMV-UL14 plasmid that expresses UL14 as an N terminal
21 FLAG fusion protein. Then, we have performed a Southern Blot analysis in order to verify the
22 presence of the UL14 construct in transfected MDBK cells (Fig. 1A)

23 The effect of UL14 on apoptosis induced by exogenous stimuli was investigated in cells
24 treated with sorbitol. UL14 expression rendered transfected MDBK cells resistant to apoptosis

1 induced by sorbitol treatment as measured by the suppression of DNA fragmentation (Fig. 1B).
2 This inhibitory effect was observed neither in non-transfected MDBK cells (UL14 negative) nor in
3 MDBK cells that were transfected with vector only, both treated with sorbitol (Fig. 1B, lane 2 and
4 3, respectively), confirming that UL14 is required for the antiapoptotic activity.

5

6 *3.2. Caspase-9 and 3 activity*

7 Induction of apoptosis results in the activation of caspases. We have tested whether UL14
8 affects the activation of caspase-3 and -9 in sorbitol-treated cells. Caspases activation was evident
9 in non-transfected MDBK cells (UL14 negative) treated with sorbitol, while activation was
10 suppressed when pBK-CMV-UL14 plasmid transfected MDBK cells (UL14-positive) were treated
11 with sorbitol (Fig. 2).

12 As shown in Fig. 2A, we have measured the activity of caspase-3 of the not-transfected
13 MDBK cells (UL14 negative) and pBK-CMV-UL14 plasmid transfected MDBK cells (UL14-
14 positive) after 1 h sorbitol treatment and following 2 h in fresh medium. Caspase-3 activity was
15 significantly increased after 1 h of treatment with sorbitol in non-transfected MDBK cells (UL14
16 negative), while did not alter the caspase-3 activity in transfected MDBK cells (UL14-positive),
17 compared with non-transfected/non-treated MDBK cells (UL14 negative) (Fig. 2A). Because
18 caspase-3 is activated by partially different pathways, it was relevant to investigate the effect of
19 sorbitol on caspase-9 as well. The results presented in Fig. 2B show that also the caspase-9 activity
20 was increased by sorbitol in non-transfected MDBK cells (UL14 negative), but not in transfected
21 MDBK cells (UL14-positive). Thus, expression of UL14 appears to be important for the inhibition
22 of caspase activation.

23

24 *3.3. Expression of sorbitol-induced apoptosis-related UL14 gene in BHV-1-infected MDBK cells*

25 To examine the role of the UL14 gene in BHV-1-infected MDBK cells and treated with
26 sorbitol at different time of post infection (1 up to 13 h p.i.), we investigated of mRNA expression

1 of this gene by a semi-quantitative RT-PCR method. Extraction of RNAs from MDBK monolayers
2 was performed after 1 h of incubation with 1 M sorbitol and following 2 h of incubation in fresh
3 medium. As shown in Fig. 3, UL14 gene expression appeared at 7 h p.i. and β -actin DNA band
4 was distributed at similar levels in all samples.

5 In order to confirm this result a Northern Blot was performed. Using this technique the
6 detection of UL14 gene expression was observed after 7 h p.i. and further remarkably increased at
7 11 h p.i. (Fig. 4).

8 This result could suggest that UL14-BHV-1 protein in a late phase of infection inhibits the
9 activity of key molecules acting at the execution phase of apoptosis in order to prevent cell death.

10

11 **4. Discussion**

12 Several BHV-1-encoded proteins that are dispensable for virus replication and growth in
13 tissue cultures play important roles in the interaction between the virus and the infected cell.

14 Our previous study indicated that BHV-1 is able to suppresses sorbitol-induced apoptosis (De
15 Martino et al., 2003). More recently, we have identify a novel gene of BHV-1, using the
16 CODEHOP (COnsensus DEgenerate Hybrid Oligonucleotide Primers) strategy, whose sequence
17 was well overlapped to the DNA sequence of BHV-1 Cooper strain in UL14 (Marfe et al., 2006).

18 In this study, we have investigated the mechanism by which UL14 alters apoptotic responses
19 using a transfectant with inducible UL14 expression. We have demonstrated that expression of
20 UL14 is sufficient for blocking DNA fragmentation and caspases activation induced by sorbitol.
21 UL14 expression rendered cell line resistant to apoptosis induced by sorbitol treatment as measured
22 by the suppression of the DNA fragmentation. This inhibitory effect was observed in neither
23 non-transfected MDBK cells (UL14 negative) nor empty vector transfected MDBK cells,
24 confirming that UL14 is required for the apoptotic activity.

1 In this study, we used an UL14-inducible expression system that allowed to directly
2 determine the involvement of caspase-9 and 3 in the induction of apoptosis. The results demonstrate
3 that UL14 gene is able to inhibit caspase-9 and 3 activation during the treatment with sorbitol.

4 Caspases, found in mammalian cells as inactive protease precursors, are grouped into
5 upstream initiator caspases and downstream effector caspases. Inactive initiator caspases (caspases-
6 8 and -9) are first activated in response to apoptotic stimuli and are responsible for processing and
7 activation of effector caspases, such as caspase-3, -6, or -7. Activated effector caspases,
8 subsequently, execute apoptosis by cleaving various cellular substrates that are vital for cell survival
9 (Shi, 2002).

10 In our experiment the inhibition of caspase-3 was seen after 1 h treatment with sorbitol in
11 transfected cells. As well as the mitochondria-dependent caspase-9 were inhibited after 1 h in
12 transfected cells, but not when the non-transfected MDBK cells (UL14 negative) were treated with
13 sorbitol.

14 The BHV-1 UL14 protein, such as herpes simplex virus UL14 protein (Yamauchi et al.,
15 2003), is able to blocks apoptosis in the late phase of infection to maintain nuclear integrity needed
16 for efficient egress. The blockage of apoptosis may also be important in the late phase when egress
17 take place. Since we have also found that transient transfections with pBK-CMV-UL14 plasmid
18 rendered the MDBK cells resistant to sorbitol-induced apoptosis, in the current study we show that
19 the full length sequence of BHV-1 UL14 at 7 h of p.i. was mainly detected with respect to early
20 times of p.i..

21 The final interesting finding of the present work is the identification of UL-14 gene, which
22 appears to inhibit apoptosis by mimicking or hijacking a cellular antiapoptotic response. On the
23 basis of such results, further work needs to analyze the antiapoptotic properties and subcellular
24 localization of the BHV-1 UL14 protein during the infection in order to clarify the precise role of
25 this gene.

1 **References:**

- 2 Benetti, L., Roizman, B., 2004. Herpes simplex virus protein kinase US3 activates and functionally
3 overlaps protein kinase A to block apoptosis. *Proc. Natl. Acad. Sci. USA* 101, 9411-9416.
- 4 Cartier, A., Broberg, E., Komai, T., Henriksson, M., Masucci, M., G., 2003. The herpes simplex
5 virus-1 Us3 protein kinase blocks CD8T cell lysis by preventing the cleavage of Bid by
6 granzyme B. *Cell Death Differ.* 10, 1320–1328.
- 7 Cunningham, C., Davison, A.J., MacLean, A.R., Taus, N.S., Baines, J.D., 2000. Herpes simplex
8 virus type 1 gene UL14: phenotype of a null mutant and identification of the encoded protein. *J.*
9 *Viro.* 74, 33-41.
- 10 De Martino, L., Marfe, G., Di Stefano, C., Pagnini, U., Florio, S., Crispino, L., Iovane, G.,
11 Macaluso, M., Giordano, A., 2003. Interference of bovine herpesvirus 1 (BHV-1) in sorbitol-
12 induced apoptosis. *J. Cell. Biochem.* 89, 373-380.
- 13 Geiser V., Jones C., 2005. Localization of sequences within the latency-related gene of bovine
14 herpesvirus 1 that inhibit mammalian cell growth. *J. Neurovirol.* 11, 563-570.
- 15 Herrmann, M., Lorenz, H.M., Voll, R., Grunke, M., Woith, W., Kalden, J.R., 1994. A rapid and
16 simple method for the isolation of apoptotic DNA fragments. *Nucleic Acids Res.* 22, 5506-
17 5507.
- 18 Marfe, G., De Martino, L., Filomeni, G., Di Stefano, C., Giganti, M.G., Pagnini, U., Napoletano, F.,
19 Iovane, G., Ciriolo, M.R., Sinibaldi Salimei, P., 2006. Degenerate PCR method for
20 identification of an antiapoptotic gene in BHV-1. *J. Cell. Biochem.* 97, 813-823.
- 21 McGeoch, D.J., Davison, A.J., 1999. The molecular evolutionary history of the herpesviruses,
22 p.441. *In* E. Domingo , R. Webster and J. Holland (ed.), *Origin and evolution of viruses.*
23 Academic Press, London, England.
- 24 Perkins, D., Pereira, E.F., Aurelian, L., 2003. The herpes simplex virus type 2 R1 protein kinase
25 (ICP10 PK) functions as a dominant regulator of apoptosis in hippocampal neurons involving

- 1 activation of the ERK survival pathway and upregulation of the antiapoptotic protein Bag-1. J.
2 Virol. 77, 1292-1305.
- 3 Philchenkov, A., 2004. Caspases: potential targets for regulating cell death. J. Cell. Mol. Med. 8,
4 432-444.
- 5 Rathmell, J.C., Thompson, C.B., 2002. Pathways of apoptosis in lymphocyte development,
6 homeostasis, and disease. Cell. 109, 97-107.
- 7 Roulston, A., Marcellus, R.C., Branton, P. E., 1999. Virus and apoptosis. Annu. Rev. Microbiol.
8 53, 577-628.
- 9 Shi Y., 2002. Mechanisms of caspase activation and inhibition during apoptosis. Mol. Cell 9, 459-
10 470.
- 11 Slee, E.A., Harte, M.T., Kluck, R.M., Wolf, B.B., Casiano, C.A., Newmeyer, D.D., Wang, H.G.,
12 Reed, J.C., Nicholson, D.W., Alnemri, E.S., Green, D.R., Martin, S.J., 1999. Ordering the
13 cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -
14 10 in a caspase-9-dependent manner. J. Cell. Biol. 144, 281-292 .
- 15 Thomson, B.J., 2001. Viruses and apoptosis, Int. J. Exp. Pathol. 82 , 65-76.
- 16 Thornberry, N.A., Lazebnik, Y., 1998. Caspases: enemies within. Science 281, 1312-1316.
- 17 Weil, M., Jacobson, M.D., Raff, M.C., 1997. Is programmed cell death required for neural tube
18 closure? Curr. Biol. 1, 281-284.
- 19 Yamauchi, Y., Daikoku, T., Goshima, F., Nishiyama, Y., 2003. Herpes Simplex virus UL14
20 protein blocks apoptosis. Microbiol. Immunol. 47, 685-689.
- 21 Yamauchi, Y., Wada, K., Goshima, F., Daikoku, T., Ohtsuka, K., Nishiyama, Y., 2002. Herpes
22 simplex virus type 2 UL14 gene product has heat shock protein (HSP)-like functions. J. Cell
23 Science 115, 2517-2527.
- 24

1 **Legends**

2 Figure 1.

3 A. Southern Blot analysis of genomic DNAs extracted by pBK-CMV-UL14 plasmid transfected
4 MDBK cells (UL14 positive) (Lane 5), empty vector transfected MDBK cells (Lane 3), and
5 non-transfected MDBK cells (UL14 negative) (Lane 2). Ladder (Lane 1).

6 .B. Time course study of sorbitol-induced fragmentation of cellular DNA in empty vector
7 transfected and pBK-CMV-UL14 transfected MDBK cells. Extracted DNAs were
8 electrophoresed on 2% agarose gel. Lane 1 and 2 indicate DNAs extracted from non-
9 transfected MDBK cells (UL14 negative), not treated with sorbitol, and sorbitol treated not-
10 transfected MDBK cells (UL14 negative), respectively. Lanes 3 and 4 indicate DNAs extracted
11 from empty vector transfected and pBK-CMV-UL14 transfected MDBK cells both treated with
12 sorbitol.

13

14 Figure 2.

15 Effect of sorbitol on caspases activity. The activity of caspases 3, and 9 was measured in non-
16 transfected MDBK cells, not treated with sorbitol (UL14 neg), pBK-CMV-UL14 transfected
17 MDBK cells treated with sorbitol (UL14 p+s) and non-transfected MDBK cells treated with
18 sorbitol (UL14 n-s), using caspase-specific fluorogenic substrates. The results are normalised to
19 the activity levels in not treated controls. A) histogram displaying the caspase-3 activity in our
20 experimental conditions. B) histogram displaying the caspase-9 activity in our experimental
21 conditions. One representative experiment out of three is shown.

22

23 Figure 3.

24 Southern Blot of PCR amplification. Effect of sorbitol on the steady state levels of UL14 cDNAs in
25 MDBK cells at different times of BHV-1 post infection. Cultures were incubated either with

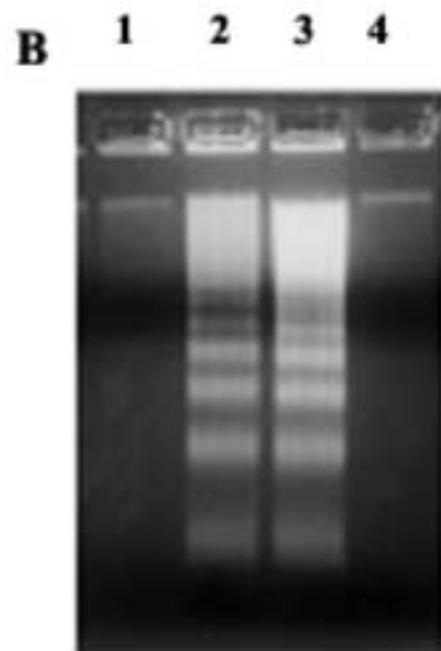
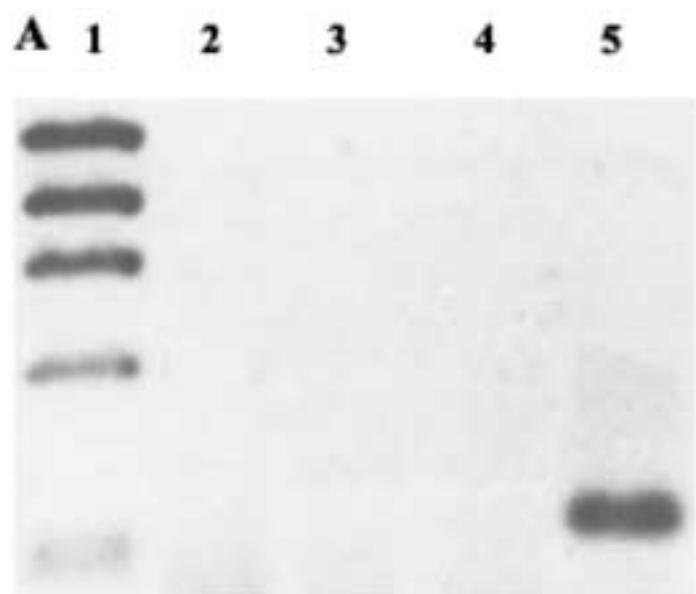
1 culture medium (control) or treated with sorbitol at the indicated times of BHV-1 post infection
2 (1, 2, 3, 4, 5, 6, 7, 9, 11 and 13 h p.i.).

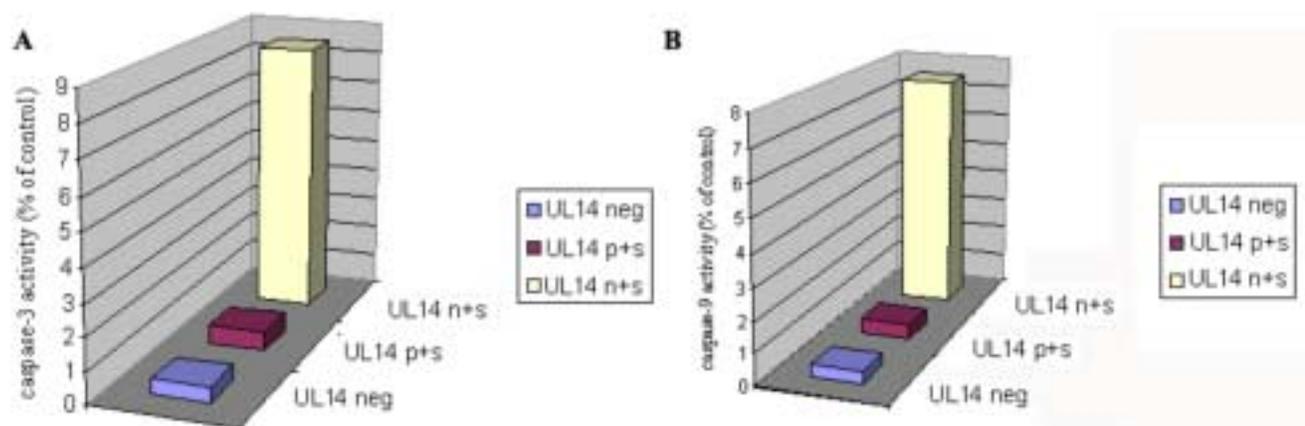
3 Figure 4.

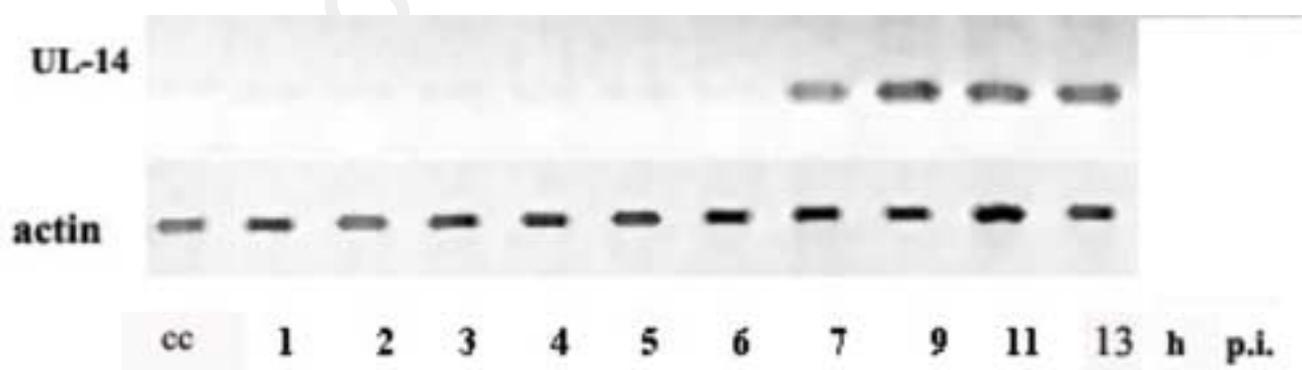
4 Northern Blotting. Effect of sorbitol on the steady state levels of UL14 mRNA in MDBK cells at
5 different times of BHV-1 post infection. Cultures were incubated either with culture medium
6 (control) or treated with sorbitol at the indicated intervals of BHV-1 post infection (1, 3, 5, 7, 9,
7 11 and 13 h p.i.).

8

Accepted Manuscript







Accepted Manuscript

