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the BICP27 Protein of Bovine Herpesvirus Type 1

ANICK CHALIFOUR, JOHNNY BASSO,[1] NICOLE GAGNON, MICHEL TRUDEL, and CLAIRE SIMARD[2]

Centre de recherche en virologie, Institut Armand-Frappier, Université du Québec, 531 Boulevard des Prairies, Laval des Rapides, Ville de Laval, Québec, Canada, H7V 1B7

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The expression kinetics of an essential *trans*-regulatory protein, ICP27, from herpes simplex virus type 1 correspond to that of an immediate-early gene whose expression increases rapidly upon infection and then decreases as of 7 hr postinfection. In contrast, here we report that the bovine herpesvirus type 1 (BHV-1) homolog BICP27, a 50-kDa protein, is expressed as an early gene. Both the transcript and protein accumulated gradually reaching peak levels at approximately 12 hr postinfection, after which point steady state levels were maintained up to 24 hr. Thus the expression profiles of ICP27 and BICP27 are significantly different, suggesting that they may possess different functions. © 1996 Academic Press, Inc.

Bovine herpesvirus 1 (BHV-1), a member of the *Alphaherpesvirinæ,* is an economically important pathogen of cattle. BHV-1 is primarily associated with two major clinical syndromes of cattle, namely infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV; *1, 2*). The BHV-1 genome, a 136-kbp linear double-stranded DNA molecule, is composed of a long (U_L) and a short (U_S) unique segment, the latter being flanked by inverted repeat sequences (*3*). Two isomeric forms of the genome occur naturally as the U_S region has the ability of inverting its orientation (*4, 5*). The viral DNA is believed to encode at least 70 polypeptides of which 25–33 are structural components of the virion (*6*). It also encodes transcriptional regulatory proteins and enzymes involved in DNA metabolism (*7*).

An international collaboration coordinated by Dr. Schwyzer (Switzerland) was undertaken to complete the sequencing of the BHV-1 genome (*7*). This allowed the identification of numerous open reading frames (ORFs) corresponding to previously identified proteins of herpes simplex virus type 1 (HSV-1). Among these the homolog BICP27 of HSV-1-infected cell polypeptide 27 (ICP27), an essential immediate-early protein (*8, 9*) was identified. We investigated the transcriptional and translational expression kinetics of BICP27 in order to obtain preliminary evidence permitting us to postulate as to whether the BHV-1 and HSV-1 proteins have similar functions. The 1200-bp-long BICP27 ORF was located within the *Hpa*I G restriction fragment of the BHV-1 genome (Fig. 1A), and its corresponding amino acid sequence showed 61 and 69% similarity with that of HSV-1 ICP27 and equine herpesvirus type 1 UL3 counterparts, respectively (*10*). A 1224-bp *Hin*fI–*Hin*cII DNA fragment, representing the complete ORF (Fig. 1A), specifically hybridized to a transcript of 1.7 kb on Northern blots containing total RNA (Fig. 1B) isolated at different times post BHV-1 infection of MDBK cells. Longer exposure of the blots (not shown) revealed the presence of a minor band of 3.0 kb, which most probably represents a 3′ coterminal transcript from the adjacent gene (*10*). The BICP27 1.7-kb-specific transcript was detected at 3 hr postinfection (p.i.; lane 3), its abundance increased sixfold 12 hr p.i. (lane 12), and then slightly decreased at 20 hr p.i. (lane 20). A similar profile was observed with a blot representing poly(A)$^+$ RNA extracted at 6, 12, 18, and 24 hr p.i. (data not shown); this blot permitted us to ascertain that the BICP27 message was still present at 24 hr p.i. The expression profile observed represents that of a gene whose expression is early following BHV-1 infection. Our results are consistent with those of a previous study investigating the spatial and temporal distribution of BHV-1 transcripts and where an early transcript of 1.7 kb originating from the *Hin*dIII fragment N of the viral genome was identified (*11*). Interestingly, the size and kinetic class of the BICP27 transcript are similar to those of the transcript from the EHV-1 homologous gene (UL3; *12*).

To analyze the expression kinetics of the BICP27 protein in BHV-1-infected cells, we set out to generate a

[1] Present address: Eastern Virginia Medical School, Department of Microbiology and Immunology, P.O. Box 1980, Norfolk, Virginia 23501. Fax: (804) 624-2255.

[2] To whom correspondence and reprint requests should be addressed. Fax: (514) 686-5626. E-mail: Claire_Simard@IAF.UQUEBEC.CA.

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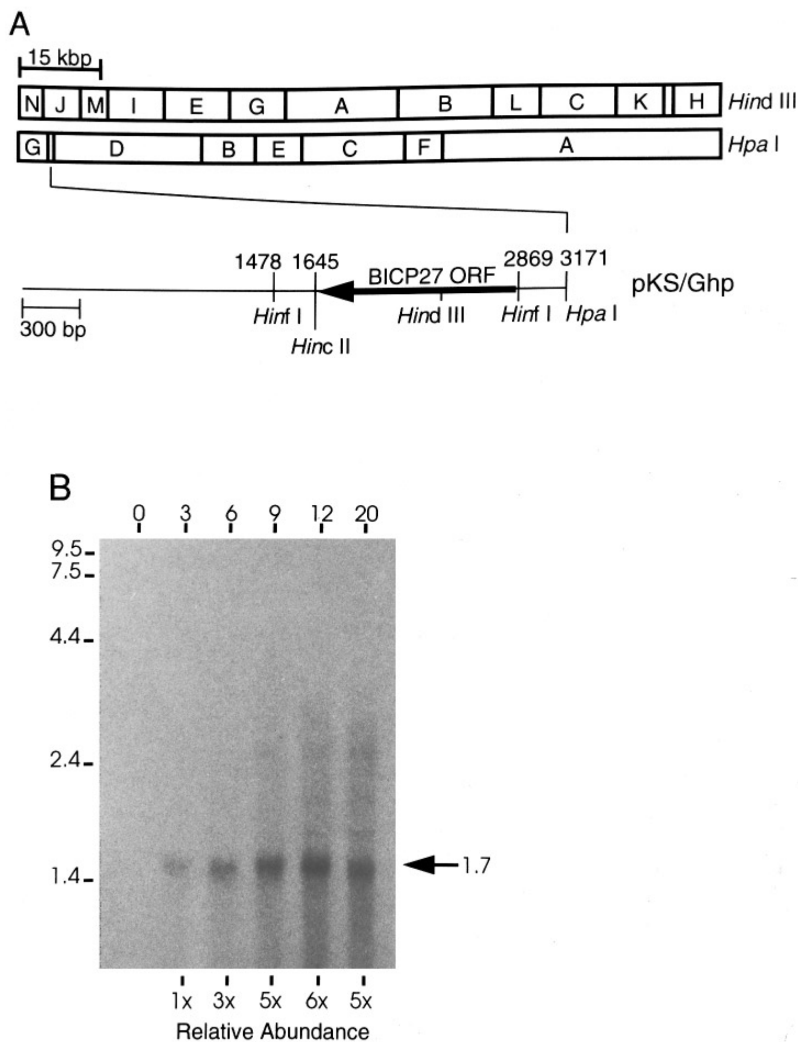

FIG. 1. Transcriptional kinetics of BICP27. (A) Schematic representations of the *Hin*dIII and *Hpa*I restriction maps of the BHV-1 genome illustrating an expanded view of the *Hpa*I G fragment which includes the BICP27 ORF initiating at position 2860 and ending at base 1660. To generate pKS/Ghp, purified BHV-1 DNA (IBR-like isolate 34; *23*) was digested with *Hpa*I, treated with 2 U of S1 nuclease (*24*) and fractionated by agarose gel electrophoresis. The 3.1-kbp fragment was then purified and ligated to the *Eco*RV cloning site of the pKS Bluescript vector (Stratagene) generating pKS/Ghp. Recombinants were identified by hybridization to the *Hin*dIII fragment N of the viral genome. (B) Expression kinetics of the BICP27 transcript. A Northern blot of total RNA extracted from BHV-1-infected cells collected at different hours postinfection (as indicated at the top of the autoradiogram) was performed as previously described (*25*). The blot was hybridized with a 1224-bp *Hin*fI/*Hin*cII-labeled fragment generated from pKS/Ghp and representing the complete BICP27 coding sequence. The numbers to the left correspond to molecular weight sizes in kb. At the bottom of the autoradiogram is indicated the relative abundance of the 1.7-kb transcript. This was evaluated by scanning the autoradiogram using the scanner ScanJet IICX (Hewlett-Packard), and then by analyzing the digitalized data using the Jandel Video Analysis Software (Jadel Scientific, CA).

BICP27-specific antiserum. For this purpose the complete BICP27 protein was expressed in *Escherichia coli* using the pET21b translation vector (Novagen, Inc.) to serve as an antigen. To generate the recombinant vector pET/BICP27, a 1396-bp *Hin*fI fragment encoding BICP27 was isolated from pKS/Ghp (Fig. 1A) and ligated to a phosphorylated *Bam*HI adaptor (5′-pACTTCTAGACGGATCCGTCTAGA-3′) generating cohesive ends which are compatible with the gene's 5′-end *Hin*fI site and which allowed the in-frame insertion of the coding sequence to that of the T7-Tag coding region of pET21b. The resulting DNA fragment was digested with *Bam*HI and *Hin*cII generating a 1244-bp fragment which was ligated to the filled in *Sal*I and cohesive *Bam*HI ends of pET21b. The resulting recombinant plasmid, pET/BICP27, thus contains the complete BICP27 coding sequence in addition to four codons upstream of the ATG fused in frame to the T7-Tag region. To ascertain proper reading frames, the vector/insert junctions were sequenced, then the recombinant plasmid was used to transform *E. coli* BL21. Lysates of cells induced with IPTG were analyzed by Western blotting using a commercially available anti-T7-Tag monoclonal antibody (MAb). The antibody specifically reacted with a protein of 50 kDa

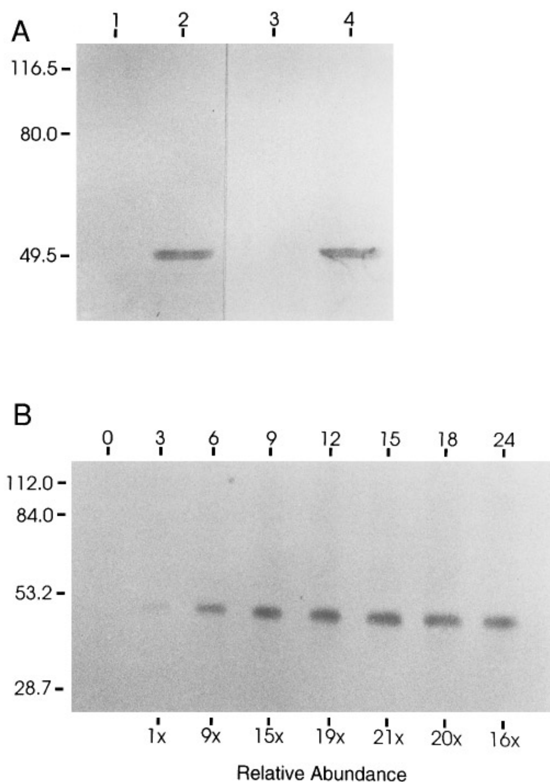

FIG. 2. Expression kinetics of the BICP27 protein. (A) Western blot analyses of *E. coli* extracts derived from cells harboring either pET21b (lanes 1 and 3) or pET/BICP27 (lanes 2 and 4). Recombinant *E. coli* BL21 cells were cultured to an $O.D._{600nm}$ of 0.6 at room temperature without agitation in terrific broth (*24*) containing 50 μg/ml ampicillin and 200 μg/ml arginine and then induced with 0.4 m*M* IPTG for 2 days. Cells were collected, resuspended in 0.1 vol of TEN (10 m*M* Tris–HCl, pH 7.5, 1 m*M* EDTA, 100 m*M* NaCl), containing 1 mg/ml lysozyme, and sonicated. After centrifugation, proteins contained in inclusion bodies were fractionated by SDS–PAGE and then electrotransferred onto a PVDF membrane (Bio-Rad). Western blots were performed as described (*26*) using a commercially available anti-T7-Tag MAb (lanes 1 and 2) or an antiserum raised against the T7-Tag/BICP27 fusion protein expressed in *E. coli* (lanes 3 and 4). The numbers to the left correspond to molecular masses of standards in kDa. (B) Western blot analysis of total proteins from BHV-1-infected cells. Confluent monolayers of MDBK cells (18×10^6 total cells) were infected with BHV-1 at an m.o.i. of 2 for 0, 3, 6, 9, 12, 15, 18, and 24 hr. Cell lysate aliquots of 40 μl, prepared as previously described (*27*), were fractionated by SDS–PAGE. Proteins were electrotransferred onto a PVDF membrane and then reacted with the BICP27-specific antiserum. The numbers to the left correspond to molecular masses of standards in kDa. At the bottom of the blot is indicated the relative abundance of the BICP27 protein. This was evaluated as described in the legend of Fig. 1.

found within *E. coli* extracts harboring the recombinant plasmid (Fig. 2A, lane 2) but not in that derived from cells harboring only the vector (lane 1). The observed molecular mass of the polypeptide was within the size range expected (45.4 kDa) for the T7-Tag/BICP27 construct, confirming its identity. The T7-Tag/BICP27 fusion protein was purified twice by electroelution following preparative SDS–PAGE and then used to immunize mice for the production of a BICP27-specific antiserum. In this manner an antiserum which specifically recognized a 50-kDa protein within *E. coli* extracts harboring pET/BICP27 (Fig. 2A, lane 4) but not pET21b (lane 3) was obtained. This antiserum also specifically reacted with a protein of 49 kDa within extracts of COS cells transfected with a eukaryotic expression vector (pcDNA3/BICP27) containing the BICP27 coding sequence but not in that from cells transfected with only the vector (results not shown). The difference in the molecular masses of the protein observed following expression in *E. coli* as compared to that in COS cells is ascribed to the absence of the T7-Tag protein domain in the latter case.

The expression kinetics of the BICP27 protein in BHV-1-infected cells were investigated by Western blot analyses of total proteins extracted at different times postinfection (Fig. 2B). The BICP27-specific antiserum did not react with any protein before 3 hr p.i. (results not shown). As of 3 hr p.i., a 49-kDa protein whose abundance increased 21-fold 15 hr p.i. was detected. After this time point a progressive decrease in the levels of this protein was observed up to 24 hr following infection. The observed molecular mass of the BICP27 protein correlates well with that reported (50 kDa; *13*) using an antiserum directed against a synthetic peptide derived from the deduced amino acid sequence of the BICP27 ORF. The expression profile observed is consistent with that obtained at the transcript level and correlates with that of a protein expressed early following BHV-1 infection. Worthwhile to mention, the BICP27 antiserum did not react with any protein from purified BHV-1 (not shown), indicating that BICP27 does not constitute a structural component of the virion.

Thus in contrast to HSV-1, the transcription and protein expression profiles of BICP27 correlate with that of an early (β) rather than that of an immediate-early (α) gene. Furthermore, the expression profiles of the BICP27 protein and of its specific transcript fluctuate in a manner which is distinct from that reported for ICP27 (*14–16*). In contrast to the expression profiles which are observed with ICP27, those of BICP27 extend over a longer period of time. At the transcriptional level, ICP27's peak abundance is reached approximately 5 hr postinfection (*16*), whereas that of BICP27 is attained 12 hr following infection. Subsequent to peak expression, a detectable decrease in the levels of both the ICP27- and BICP27-specific transcripts is observed 7 and 20 hr, respectively, following viral infection. The expression profiles of the proteins essentially follow that of their transcripts (this study, *14, 15*). The difference in the broadness of the two profiles suggests differences in the function of the two proteins.

The expression profile of BICP27 suggests that it is needed until fairly late in the infectious cycle. Interestingly, from 3 to 12 hr p.i. the expression profile at the level of the protein parallels that of the transcript but the protein's accumulation was three times that of its

transcript. For instance at 12 hr p.i., the BICP27 transcript level was sixfold higher than that observed at 3 hr, whereas the protein level was 19-fold. This observation indicates that BICP27 is subject not only to transcriptional control but also to either translational or posttranslational control. The decreased expression level of ICP27 and BICP27, at the transcript and protein level, respectively, may reflect a reduced requirement as well as autogenous regulation of the protein's expression once peak levels are attained. This mode of regulation is supported by the observation that the transcript level of a temperature-sensitive ICP27 structural mutant of HSV-1 accumulates to a much higher level and for a longer duration than that of the wild type (*16*).

The present study will enable us to address the question as to whether the BICP27 protein possesses regulatory activities similar to its counterparts in other alphaherpesviruses. In HSV-1 for instance, ICP27 influences viral gene expression (*17, 18*), mRNA processing (*19, 20*), and intracellular localization of other regulatory proteins (*21, 22*).

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REFERENCES

1. Kit, S., *Adv. Exp. Med. Biol.* **251,** 219–236 (1989).
2. Wyler, R., Engels, M., and Schwyzer, M., *In* "Herpesvirus Diseases of Cattle, Horses, and Pigs" (G. Wittmann, Ed.), pp. 1–72. Kluwer Academic Publishers, Boston/Dordrecht/London, 1989.
3. Mayfield, J. E., Good, P. J., Vanoort, V. H., Campbell, A. R., and Reed, D. E., *J. Virol.* **47,** 259–264 (1983).
4. Farley, I., Skare, B., and Skare, J., *In* "The Human Herpes Viruses: An Interdisciplinary Perspective" (A. J. Nahmias, W. R. Dowdle, and R. F. Schinazi, Eds.), p. 590. Elsevier/North-Holland Publishing Co., New York, 1981.
5. Engels, M., Giuliani, C., Wild, P., Beck, T. M., Loepfe, E., and Wyler, R., *Virus Res.* **6,** 57–73 (1986).
6. Misra, V., Blumenthal, R. M., and Babiuk, L. A., *J. Virol.* **40,** 367–378 (1981).
7. Schwyzer, M., *In* "Immunobiology of Viral Infections" (M. Schwyzer, *et al.,* Eds.), pp. 108–113. Proc. 3rd Congress Europ. Soc. Vet. Virol., 1995.
8. Sacks, W. R., Greene, C. C., Aschman, D. P., and Schaffer, P. A., *J. Virol.* **55,** 796–805 (1985).
9. McCarthy, A. M., McMahan, L., and Schaffer, P. A., *J. Virol.* **63,** 18–27 (1989).
10. Schwyzer, M., Styger, D., Vogt, B., Lowery, D. E., Simard, C., LaBoissière, S., Misra, V., Vlcek, C., and Paces, V., Submitted.
11. Wirth, U. V., Gunkel, K., Engels, M., and Schwyzer, M., *J. Virol.* **63,** 4882–4889 (1989).
12. Zhao, Y., Holden, V. R., Harty, R. N., and O'Callaghan, D. J., *J. Virol.* **66,** 5363–5372 (1992).
13. Köppel, R., Singh, M., Fraefel, C., Ackermann, M., and Schwyzer, M., *In* "Immunobiology of Viral Infections" (M. Schwyzer *et al.,* Eds.), pp. 126–131. Proc. 3rd Congress Europ. Soc. Vet. Virol., 1995.
14. Honess, R. W., and Roizman, B., *J. Virol.* **14,** 8–19 (1974).
15. Honess, R. W., and Roizman, B., *Proc. Natl. Acad. Sci. USA* **72,** 1276–1280 (1975).
16. Sandri-Goldin, R. M., *In* "Herpesvirus Transcription and Its Regulation" (E. K. Wagner, Ed.), pp. 77–103. CRC Press, 1991.
17. Sekulovich, R. E., Leary, K., and Sandri-Goldin, R. M., *J. Virol.* **62,** 4510–4522 (1988).
18. Samaniego, L. A., Webb, A. L., and Deluca, N. A., *J. Virol.* **69,** 5705–5715 (1995).
19. McLauchlan, J., Phelan, A., Loney, C., Sandri-Goldin, R. M., and Clements, J. B., *J. Virol.* **66,** 6939–6945 (1992).
20. Hardwicke, M. A., and Sandri-Goldin, R. M., *J. Virol.* **68,** 4797–4810 (1994).
21. Zhu, Z. M., and Schaffer, P. A., *J. Virol.* **69,** 49–59 (1995).
22. Zhu, Z. M., Cai, W. Z., and Schaffer, P. A., *J. Virol.* **68,** 3027–3040 (1994).
23. Simard, C., LaBoissière, S., Séguin, C., and Trudel, M., *Intervirology* **32,** 117–126 (1991).
24. Sambrook, J., Fritsch, E. F., and Maniatis, T., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
25. Simard, C., Bastien, N., and Trudel, M., *Virology* **190,** 689–701 (1992).
26. Trudel, M., Nadon, F., Séguin, C., Boulay, G., and Lussier, G., *Vaccine* **5,** 239–243 (1987).
27. Simard, C., Langlois, I., Styger, D., Vogt, B., Vlcek, C., Chalifour, A., Trudel, M., and Schwyzer, M., *Virology* **212,** 734–740 (1995).