Rev Argent Microbiol. 2017;49(3):301–302

asociación argentina de microbiología

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

MICROBIOLOGICAL IMAGE

Recombinant *Bovine herpervirus 5* expressing enhanced green fluorescent protein

Recombinante de herpesvirus bovino 5 expresando proteina verde fluorescente

Carolina A. Silvestro*, **Ana C. Bratanich**

Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Cátedra de Virología, Argentina

Received 30 December 2016; accepted 30 March 2017
Available online 12 July 2017

Bovine alpha herpesvirus 5 (BoHV-5) induces neurological disease in cattle, including tremors, nystagmus, teeth grinding, circling, ataxia, recumbence, paddling, and death[2,6,7,9]. Nonsuppurative meningoencephalitis is a hallmark of this infection. Like the majority of the alpha herpes viruses, it generates latency in trigeminal ganglia[5]. The latency-related (LR) gene is the main responsible for the maintenance of latency[1,3,4,8]. To evaluate the LR function within the viral infection, a recombinant virus was constructed deleting the LR promoter to avoid its expression without affecting overlapping genes in the antisense strand (the immediate early *bicp0* gene).

The enhanced green fluorescent protein (EGFP) was used as a reporter to facilitate recombinant virus detection in the cell culture plates. For that purpose, EGFP was PCR-amplified from pEGFP-C1 (Clontech™) and subsequently cloned into a plasmid surrounded by selected 5′ and 3′ recombination arms of BoHV-5. The recombinant plasmid was linearized with *ScaI* and cotransfected with full-length BoHV-5 DNA previously extracted with DNazol® (Invitrogen™) into CRFK (Crandell Feline Kidney) cells plated in a 24-well plate dish at 90% of cell density. Twenty-four hours later cells were reseeded into a 60 mm-plate and covered with 1% methylcellulose medium overlaid after their attachment. EGFP positive plaques were identified under fluorescent microscope, isolated and reseeded in a fresh monolayer. Viral clones were purified six times before being aliquoted and frozen at −80 °C. The correct site of recombination and removal of the promoter target was confirmed by sequencing of the recombination ends toward the reporter gene. The recombinant virus was denominated BoHV-5 ΔLR +GFP. To our knowledge, this is the first bovine herpesvirus in which the LR gene has been replaced by EGFP, making this recombinant a powerful tool for *in vitro* and *in vivo* studies.

In this section we showed the recombinant BoHV-5 ΔLR +GFP-infecting CRFK cells. Figure 1 shows typical viral plaque morphology. In Figure 2 we used DAPI to contrast the nuclei and to facilitate visualization of cytopathic effects.

* Corresponding author.
E-mail address: carolina@silvestro.com.ar (C.A. Silvestro).

http://dx.doi.org/10.1016/j.ram.2017.03.005

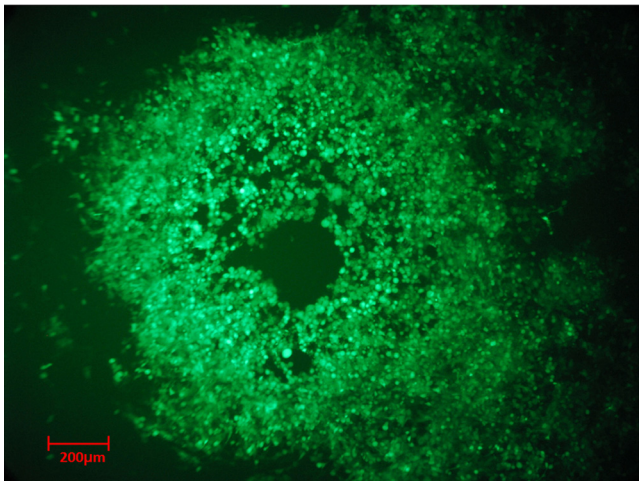

Figure 1 CRFK cells were infected with the BoHV-5 ΔLR +GFP at a MOI of 0.1 and later overlaid with 1% methylcellulose medium. Forty-eight hours later, when the cytopathic effect was evident, the plates were visualized under a fluorescent microscope to detect GFP expression.

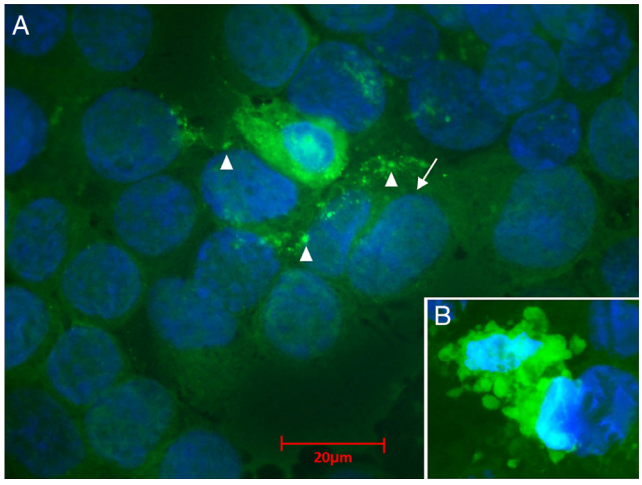

Figure 2 CRFK cells were infected with the BoHV-5 recombinant (ΔLR +GFP) at a MOI of 0.1. Twenty-four hours later they were fixed and nuclear DNA was stained with 4′,6,-diaminodino-2-phenylindole (DAPI) (Invitrogen™). DAPI and GFP expression were monitored under a fluorescent microscope; the obtained images were merged into the same picture using the ImageJ program. (A) Observed expression of GFP in all the cytoplasms of infected cells with the presence of multinucleated cells (arrow), and cytoplasmic inclusion bodies (triangle). (B) Advanced infections showed evidence of apoptosis with cell shrinkage, condensed and pyknotic nuclei and apoptotic bodies.

Ethical responsibilities

Protection of human and animal subjects. The authors state that for this investigation no experiments have been performed on humans or animals.

Confidentiality of data. The authors state that in this article there are no patient data.

Right to privacy and informed consent. The authors state that in this article there are no patient data.

Funding

This research was supported by the Agencia Nacional de Promocion Cientifica y Tecnologica (PICT 2010-0925). CS is recipient of a Fellowship for the conclusion of the PhD degree from the Conicet (Consejo Nacional de Investigaciones Cientificas y Tecnicas).

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

We thank Centro de Virología Animal, Instituto de Ciencia y Tecnología Dr. Cesar Milstein, CONICET, Ciudad de Buenos Aires, Argentina for the use of the fluorescence microscope and Carlos Palacios for his assistance.

References

1. Carrillo BJ, Ambrogi A, Schudel AA, Vazquez M, Dahme E, Pospischil A. Meningoencephalitis caused by IBR virus in calves in Argentina. Zentralbl Veterinarmed B. 1983;30:327–32.
2. Del Medico Zajac MP, Ladelfa MF, Kotsias F, Muylkens B, Thiry J, Thiry E, Romera SA. Biology of bovine herpes virus 5. Vet J. 2010;184:138–45.
3. Jones C, da Silva LF, Sinani D. Regulation of the latency-reactivation cycle by products encoded by the *Bovine herpesvirus 1* (BHV-1) latency-related gene. J Neurovirol. 2011;17:535–45.
4. Jones C, Geiser V, Henderson G, Jiang Y, Meyer F, Perez S, Zhang Y. Functional analysis of *Bovine herpesvirus 1* (BHV-1) genes expressed during latency. Vet Microbiol. 2006;113(3-4):199–210.
5. Jones C. *Herpes simplex virus* type 1 and *Bovine herpesvirus 1* latency. Clin Microbiol Rev. 2003;16:79–95.
6. Ladelfa MF, Del Medico Zajac MP, Kotsias F, Delgado F, Muylkens B, Thiry J, Thiry E, Romera SA. Comparative study on the *in vitro* and *in vivo* properties of two *Bovine herpesvirus-5* reference strains. Acta Vet Scand. 2011;53:37.
7. Salvador SC, Lemos RAA, Riet-Correa F, Roeche PM, Osório ALR. Meningoencefalite em bovinos causada por herpesvirus bovino-5 no Mato Grosso do Sul e São Paulo. Pesq Vet Brasil. 1998;18:76–83.
8. Silvestro C, Bratanich A. The latency-related gene of *Bovine herpesvirus* types 1 and 5 and its modulation of cellular processes. Arch Virol. 2016;161:3299–308.
9. Studdert MJ. Bovine encephalitis herpesvirus. Vet Rec. 1989;125:584.