Antiviral Research 90 (2011) 98–101

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Short communication

Potential of adenovirus and baculovirus vectors for the delivery of shRNA against morbilliviruses

Zaheer Ahmed Nizamani[a], Gunther M. Keil[b], Emmanuel Albina[a], Carine Holz[a], Cécile Minet[a], Olivier Kwiatek[a], Geneviève Libeau[a,*], Renata Servan de Almeida[a]

[a] CIRAD, UMR Contrôle des Maladies, Montpellier F-34398, France
[b] Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

ARTICLE INFO

Article history:
Received 27 July 2010
Received in revised form 17 January 2011
Accepted 18 February 2011
Available online 26 February 2011

Keywords:
RNAi
Morbillivirus
Adenovirus
Baculovirus
PPRV

ABSTRACT

Morbilliviruses are important pathogens of humans, ruminants, carnivores and marine mammals. Although good vaccines inducing long-term immunity are available, recurrent outbreaks of measles, canine distemper and peste des petits ruminants (PPR) are observed. In control strategies, antivirals thus could be useful to confine virus spread and application of interfering RNAs is a promising approach, provided they can be delivered efficiently into the host cells. We have constructed recombinant adenovirus and baculovirus vectors expressing short hairpin RNAs (shRNAs) against the PPR virus (PPRV) and compared them *in vitro*. It was found that both recombinant viruses inhibited PPRV replication with the baculovirus vector, which inhibited generation of infectious progeny by more than 2 $\log_{10}$ and the nucleoprotein expression of PPRV by 73%, being the more efficient. The results show that baculoviral shRNA-expressing vectors have the potential for therapeutic use against morbillivirus infections.

RNA interference (RNAi) is an evolutionarily conserved post transcriptional gene silencing mechanism (Fire et al., 1998; Zhang et al., 2004; Aagaard and Rossi, 2007). RNAi has potential for being used as an effective antiviral therapy if siRNAs or short-hairpin RNAs (shRNAs) can be delivered efficiently into the target cells. Morbilliviruses are important pathogens of humans, ruminants, carnivores and marine mammals. Good vaccines inducing long-term immunity are available against measles, rinderpest, peste des petits ruminants (PPR) and canine distemper. However, the vaccination coverage is often only partial and recurrent outbreaks of measles, canine distemper and PPR are observed. In order to develop an antiviral strategy against these diseases, we previously designed siRNA sequences targeting the nucleoprotein (N) gene of PPR virus (PPRV), rinderpest virus and measles virus. Their efficacy was demonstrated *in vitro* after delivery by liposome-based transfection (Keita et al., 2008; Servan de Almeida et al., 2007). Three sequences directed against the N gene were shown to be most effective. Therapeutic application of siRNA requires correct delivery of these molecules into the cell cytoplasm which poses significant problems *in vivo*. To overcome these problems, several delivery strategies have been developed, including those based on viral vectors. Recombinant replication deficient adenoviruses have been successfully used *in vitro* as shRNA delivery vectors against numerous viruses including measles virus (Uprichard et al., 2005; Otaki et al., 2007; Sakamoto et al., 2008) and also *in vivo* (Kim et al., 2008; Feng et al., 2008; Li et al., 2009). Adenoviruses have advantages over other viral vectors as they can be produced in high titers and can transduce a broad range of cell types (Volpers and Kochanek, 2004). In contrast, baculoviruses like *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), only replicate in insect cells and naturally infect insects belonging to the order *Lepidoptera*. However, they can enter mammalian cells but do not express their genes because the baculovirus promoters are inactive in these cells. One of the interesting consequences of this is the absence of pre-existing antibodies against baculovirus in mammals (Kost and Condreay, 2002; Volkman and Goldsmith, 1983). For gene transfer and expression in mammalian cells, so-called BacMam viruses have been generated by incorporation of mammalian cell-active expression cassettes (Brun et al., 2008). BacMam viruses are capable to transduce a wide variety of cells including non-dividing cells (van Loo et al., 2001) and primary cells (Sarkis et al., 2000). They have also been used as shRNA expression vectors against viral infections *in vitro* (Lu et al., 2006; Suzuki et al., 2008, 2009; Starkey et al., 2009). In this study, a recombinant replication deficient human adenovirus type 5 and a recombinant baculovirus expressing a short hairpin RNA (shRNA) against PPRV were compared for their antiviral efficacy *in vitro*.

Recombinant adenovirus expressing shRNA NPPRV1 (rAd_NPPRV1^{shRNA}) was constructed using the commercial kit

* Corresponding author. Tel.: +33 467593850; fax: +33 467593798.
E-mail address: genevieve.libeau@cirad.fr (G. Libeau).

0166-3542/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2011.02.007

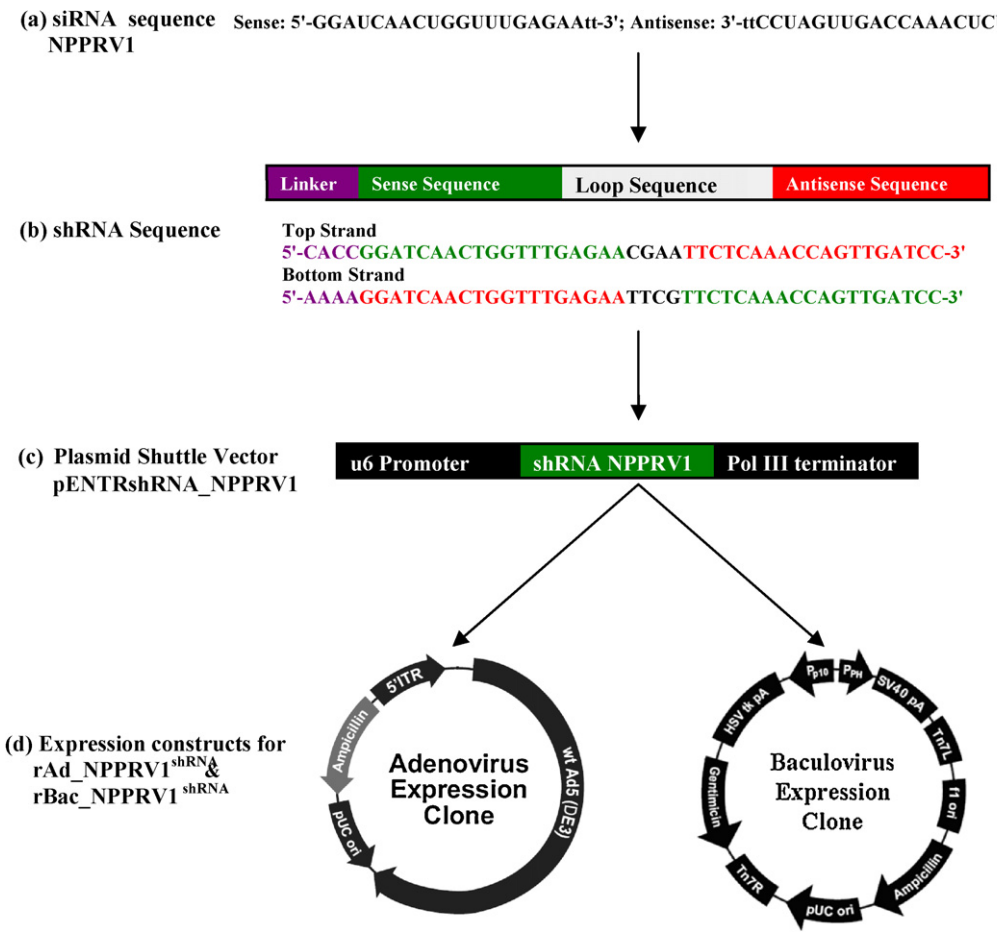

Fig. 1. Schematic presentation of (a) siRNA NPPRV1 sequence, (b) shRNA sequence, (c) plasmid shuttle vector, and (d) expression plasmids for rAd_NPPRV1^{shRNA} and rBac_shRNA_NPPRV1.

BLOCK-iT^{TM} Adenoviral RNAi Expression System according to the supplier's instructions (Invitrogen). For constructing recombinant baculovirus expressing shRNA against PPRV, an insect cell-active GFP-expression cassette was integrated into the commercially available pFastBaDual to yield plasmid pBacPH_GFPpolyA (Keil et al., 2009). This plasmid was used to integrate a blunt ended BsrG1 fragment (400 bp) from pENTR/U6/NPPRV1^{shRNA} (Fig. 1). The resulting plasmid pBacPH_GFP_NPPRV1^{shRNA} was used to generate the recombinant baculovirus (rBac_NPPRV1^{shRNA}), as recommended in the Bac-to-Bac® Baculovirus Expression Systems kit (Invitrogen).

Transductions were performed on Vero cells after 24 h of culture in 24-well plates. The rAd_NPPRV1^{shRNA} and an adenovirus expressing scrambled shRNA (Vector Biolabs) rAd5_SCR^{shRNA} were added at MOI 100, 200, 300, and 400 and the plates were further incubated overnight at 37 °C, 5% CO_2. Inocula were removed and 1 ml of Eagle's Minimum Essential Medium (EMEM) with 5% of fetal bovine serum was added to each well. For baculovirus transductions, the culture medium was removed and the cells were washed twice with Dulbecco's phosphate-buffered saline containing calcium and magnesium (Sigma–Aldrich). Then, rBac_NPPRV1^{shRNA} and, as a control, rBac_eGFP^{shRNA} were added to the wells at MOIs of 100, 200, 300 and 400. The plates were incubated for 30 min at 27 °C under agitation (100 rpm) and then centrifuged at 27 °C for 1 h at 600 × *g*. Inocula were discarded and 1 ml EMEM was added to each well. Cells were finally infected with PPRV (MOI 0.1), 24–72 h post-transduction. To assess the antiviral effect, cytopathic effects (CPE) were scored from 0 to 100%, at 72 and 96 h post-infection. In addition, cell supernatants were collected 48, 72 and 96 h post-infection. Since CPE were consistently seen from 72 h post-infection, they were scored from 0 to 100% at 72 and 96 h post-infection. In contrast, virus titers could be determined as early as 48 h post-infection. The supernatants were titrated using 10-fold serial dilutions according to Reed and Muench (1938). Viral titers were expressed in terms of 50% cell culture infectious unit dose per ml ($CCID_{50}$/ml). The relative expression of PPRV nucleoprotein was also measured 96 h post-infection by flow cytometry as described by Servan de Almeida et al. (2007).

The maximum antiviral effect of rAd_NPPRV1^{shRNA}, observed at an MOI of 200 and 72 h after PPRV infection, reached CPE reductions of 25% and a decrease in viral progeny production of 0.5 $\log_{10}$, respectively (Table 1 and Fig. 2). Transduction with

Table 1
Effect of rBac_NPPRV1^{shRNA} and rAd_NPPRV1^{shRNA} upon percentage of CPE induction by PPRV at 72 h and 96 h post-infection.

	72 h	96 h
Virus control	75	100
rBac_NPPRV1^{shRNA} MOI 100	25	50
rBac_NPPRV1^{shRNA} MOI 200	25	25
rBac_NPPRV1^{shRNA} MOI 300	25	25
rBac_NPPRV1^{shRNA} MOI 400	12.5	25
Virus control	100	100
rAd_NPPRV1^{shRNA} MOI 100	75	100
rAd_NPPRV1^{shRNA} MOI 200	75	100
rAd_NPPRV1^{shRNA} MOI 300	75	100

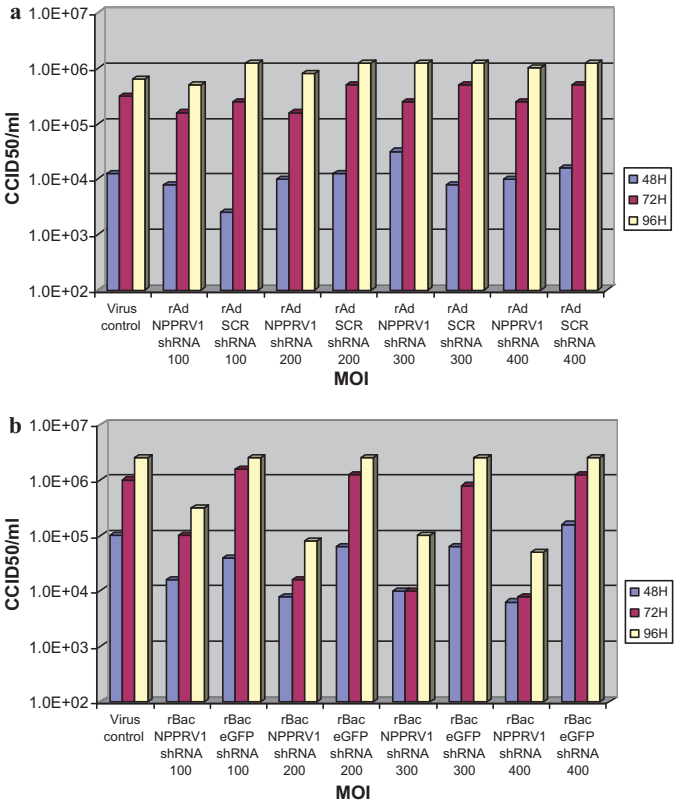

Fig. 2. Inhibition of PPRV progeny virus production by (a) rAd_NPPRV1^{shRNA} and (b) rBac_NPPRV1^{shRNA} 48, 72 and 96 h post-infection with PPRV MOI of 0.1. Viral titers are expressed in terms of 50% cell culture infectious unit dose per ml ($CCID_{50}$/ml).

higher MOIs did not improve inhibition of PPRV replication but had a deleterious effect on the cell culture which could be seen in the form of rounding and detachment of the cells. In contrast, rBac_NPPRV1^{shRNA} inhibited the PPRV CPE up to 75% and reduced PPRV progeny virus titers by more than 2 $\log_{10}$ at 96 h post-infection (Table 1 and Fig. 3). No toxic effect was observed in cells transduced with rBac_NPPRV1^{shRNA} even using the higher MOI of 400. This inhibitory effect on PPRV replication was reflected by the up to 73% reduction of PPRV nucleoprotein expression by rBac_NPPRV1^{shRNA} transduction (Fig. 3). No difference in CPE expression by PPRV was found between virus control and cells transduced with the two recombinant viruses expressing irrelevant or scrambled shRNA.

This study provides evidence that recombinant adenoviruses or baculoviruses expressing active shRNAs can interfere with the replication of PPRV. However, the baculovirus proved to be more efficient than adenovirus when used at same MOIs. The low efficiency of the adenovirus against PPRV replication differs from studies in which other pathogens were targeted. *In vitro*, shRNA-expressing adenoviruses achieved titer reductions of 3 $\log_{10}$ and 1.39 $\log_{10}$ for porcine picornavirus and circovirus when used at MOIs of 80 and 1000, respectively (Kim et al., 2008; Feng et al., 2008). In contrast, rAd_NPPRV1^{shRNA} did not reduce PPRV titers more than 0.5 $\log_{10}$. In contrast, our recombinant baculovirus showed a reduction of 2 $\log_{10}$ on PPRV infectious replication. In comparable studies, Lu et al. (2006) and Suzuki et al. (2009) reported a decrease in titer for a porcine arterivirus and human influenza viruses to a maximum of 0.6 and 0.9 $\log_{10}$, respectively, which is at least 1 $\log_{10}$ less than in the present study. In addition, the results obtained with rBac_NPPRV1^{shRNA} are in accordance to those previously reported by our group when siRNA delivered to cell cultures by Lipofectamine^{TM} reduced the PPRV infectious progeny titer by 3–4 $\log_{10}$ and PPRV nucleoprotein expression by 90% (Keita et al., 2008).

Compared to liposomes, recombinant baculoviruses are not aggregated and degraded by serum proteins (Li et al., 1999) and they are easier and cheaper to produce in large quantities. However, they are susceptible to complement inactivation (Hofmann and Strauss, 1998). Chemical or genetic modification, can overcome this problem (Kaname et al., 2010; Yang et al., 2009). Furthermore, it seems that baculoviruses do not have deleterious effects on mammalian cells even when used at very high MOIs (Andersson et al., 2007). In agreement with this report and in contrast to rAd_NPPRV1^{shRNA} transduction, we did not find adverse effects in Vero cells even when transduced at an MOI of 400. In conclusion, a recombinant replication deficient adenovirus and a baculovirus expressing shRNA against nucleoprotein of PPRV were constructed and tested *in vitro*. This study shows that both recombinants can inhibit PPRV replication *in vitro*, however, the baculovirus vector was more efficient whether rBac_NPPRV1^{shRNA} is suitable for inhibition of PPRV replication also *in vivo* needs to be elucidated.

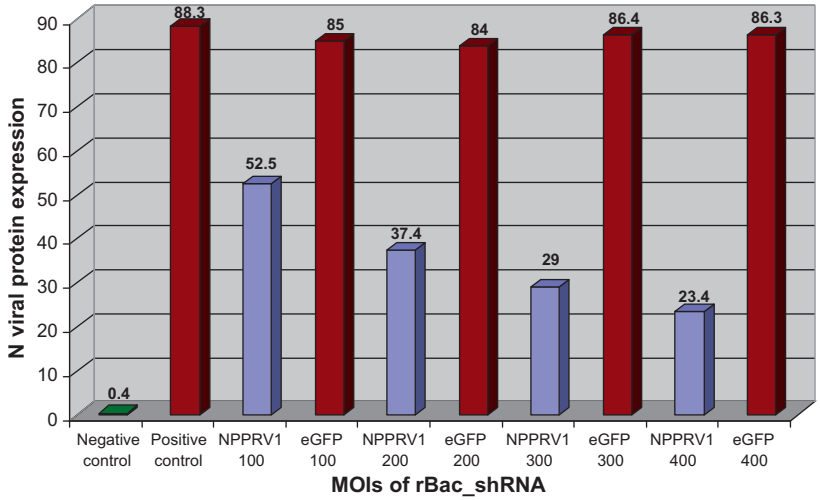

Fig. 3. Inhibition of nucleoprotein expression of PPRV by rBac_NPPRV1^{shRNA} measured by flow cytometry.

Acknowledgements

This work was supported by EPIZONE, and a PhD scholarship from Higher Education Commission (HEC), Pakistan.

References

Aagaard, L., Rossi, J.J., 2007. RNAi therapeutics: principles, prospects and challenges. Adv. Drug Deliv. Rev. 59, 75–86.

Andersson, M., Warolen, M., Nilsson, J., Selander, M., Sterky, C., Bergdahl, K., Sorving, C., James, S.R., Doverskog, M., 2007. Baculovirus-mediated gene transfer and recombinant protein expression do not interfere with insulin dependent phosphorylation of PKB/Akt in human SHSY-5Y and C3A cells. BMC Cell Biol. 8, 6.

Brun, A., Albina, E., Barret, T., Chapman, D.A., Czub, M., Dixon, L.K., Keil, G.M., Klonjkowski, B., Le Potier, M.F., Libeau, G., Ortego, J., Richardson, J., Takamatsu, H.H., 2008. Antigen delivery systems for veterinary vaccine development. Viral-vector based delivery systems. Vaccine 26, 6508–6528.

Feng, Z., Jiang, P., Wang, X., Li, Y., Jiang, W., 2008. Adenovirus-mediated shRNA interference against porcine circovirus type 2 replication both in vitro and in vivo. Antivir. Res. 77, 186–194.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., Mello, C.C., 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 806–811.

Hofmann, C., Strauss, M., 1998. Baculovirus-mediated gene transfer in the presence of human serum or blood facilitated by inhibition of the complement system. Gene Ther. 5, 531–536.

Kaname, Y., Tani, H., Kataoka, C., Shiokawa, M., Taguwa, S., Abe, T., Moriishi, K., Kinoshita, T., Matsuura, Y., 2010. Acquisition of complement resistance through incorporation of CD55/decay-accelerating factor into viral particles bearing baculovirus GP64. J. Virol. 84, 3210–3219.

Keil, G.M., Klopfleisch, C., Giesow, K., Blohm, U., 2009. Novel vectors for simultaneous high-level dual protein expression in vertebrate and insect cells by recombinant baculoviruses. J. Virol. Methods 160, 132–137.

Keita, D., Servan de Almeida, R., Libeau, G., Albina, E., 2008. Identification and mapping of a region on the mRNA of Morbillivirus nucleoprotein susceptible to RNA interference. Antivir. Res. 80, 158–167.

Kim, S.M., Lee, K.N., Park, J.Y., Ko, Y.J., Joo, Y.S., Kim, H.S., Park, J.H., 2008. Therapeutic application of RNA interference against foot-and-mouth disease virus in vitro and in vivo. Antivir. Res. 80, 178–184.

Kost, T.A., Condreay, J.P., 2002. Recombinant baculoviruses as mammalian cell gene-delivery vectors. Trends Biotechnol. 20 4, 173–180.

Li, G., Jiang, P., Li, Y., Wang, X., Huang, J., Du, Y., Zeshan, B., 2009. Effective suppression of replication of porcine reproductive and respiratory syndrome virus by adenovirus-mediated small interfering RNAs targeting ORF1b, 5 and 7 genes. J. Virol. Methods 157, 40–46.

Li, S., Tseng, W.C., Stolz, D.B., Wu, S.P., Watkins, S.C., Huang, L., 1999. Dynamic changes in the characteristics of cationic lipidic vectors after exposure to mouse serum: implications for intravenous lipofection. Gene Ther. 6, 585–594.

Lu, L., Ho, Y., Kwang, J., 2006. Suppression of porcine arterivirus replication by baculovirus-delivered shRNA targeting nucleoprotein. Biochem. Biophys. Res. Commun. 340, 1178–1183.

Otaki, M., Jiang, D.P., Sasayama, M., Nagano-Fujii, M., Hotta, H., 2007. Generation of recombinant adenovirus expressing siRNA against the L mRNA of measles virus and subacute sclerosing panencephalitis virus. Microbiol. Immunol. 51, 985–991.

Reed, L.J., Muench, H., 1938. A simple method of estimating fifty per cent endpoints. Am. J. Epidemiol. 27, 493–497.

Sakamoto, N., Tanabe, Y., Yokota, T., Satoh, K., Sekine-Osajima, Y., Nakagawa, M., Itsui, Y., Tasaka, M., Sakurai, Y., Cheng-Hsin, C., Yano, M., Ohkoshi, S., Aoyagi, Y., Maekawa, S., Enomoto, N., Kohara, M., Watanabe, M., 2008. Inhibition of hepatitis C virus infection and expression in vitro and in vivo by recombinant adenovirus expressing short hairpin RNA. J. Gastroenterol. Hepatol. 23, 1437–1447.

Sarkis, C., Serguera, C., Petres, S., Buchet, D., Ridet, J.L., Edelman, L., Mallet, J., 2000. Efficient transduction of neural cells in vitro and in vivo by a baculovirus-derived vector. Proc. Natl. Acad. Sci. U.S.A. 97, 14638–14643.

Servan de Almeida, R., Keita, D., Libeau, G., Albina, E., 2007. Control of ruminant morbillivirus replication by small interfering RNA. J. Gen. Virol. 88, 2307–2311.

Starkey, J.L., Chiari, E.F., Isom, H.C., 2009. Hepatitis B virus (HBV)-specific short hairpin RNA is capable of reducing the formation of HBV covalently closed circular (CCC) DNA but has no effect on established CCC DNA in vitro. J. Gen. Virol. 90, 115–126.

Suzuki, H., Saitoh, H., Suzuki, T., Takaku, H., 2009. Inhibition of influenza virus by baculovirus-mediated shRNA. Nucl. Acids Symp. Ser. (Oxf.), 287–288.

Suzuki, H., Tamai, N., Shimotohno, K., Matsuura, Y., Takaku, H., 2008. Suppression of HCV RNA replication by baculovirus-mediated shRNA expression vectors. Antivir. Res. 78, A49–A49.

Uprichard, S.L., Boyd, B., Althage, A., Chisari, F.V., 2005. Clearance of hepatitis B virus from the liver of transgenic mice by short hairpin RNAs. Proc. Natl. Acad. Sci. U.S.A. 102, 773–778.

van Loo, N.D., Fortunati, E., Ehlert, E., Rabelink, M., Grosveld, F., Scholte, B.J., 2001. Baculovirus infection of nondividing mammalian cells: mechanisms of entry and nuclear transport of capsids. J. Virol. 75, 961–970.

Volkman, L.E., Goldsmith, P.A., 1983. In Vitro Survey of Autographa californica Nuclear Polyhedrosis Virus Interaction with Nontarget Vertebrate Host Cells. Appl Environ Microbiol. 45 3, 1085–1093.

Volpers, C., Kochanek, S., 2004. Adenoviral vectors for gene transfer and therapy. J. Gene Med. 6 (Suppl. 1), S164–171.

Yang, Y., Lo, S.L., Yang, J., Goh, S.S., Wu, C., Feng, S.S., Wang, S., 2009. Polyethylenimine coating to produce serum-resistant baculoviral vectors for in vivo gene delivery. Biomaterials 30, 5767–5774.

Zhang, H., Kolb, F.A., Jaskiewicz, L., Westhof, E., Filipowicz, W., 2004. Single processing center models for human Dicer and bacterial RNase III. Cell 118, 57–68.