Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 364–368, January 1995
Microbiology

Targeting of a nuclease to murine leukemia virus capsids inhibits viral multiplication

(antivirals/gene therapy/staphylococcal nuclease)

GEORGES NATSOULIS*†, PARTHA SESHAIAH*, MARK J. FEDERSPIEL‡, ALAN REIN‡, STEPHEN H. HUGHES‡, AND JEF D. BOEKE*¶

*Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205; and ‡Advanced BioScience Laboratories–Basic Research Program, National Cancer Institute–Frederick Cancer Research and Development Center, Frederick, MD 21702

Communicated by Norton D. Zinder, The Rockefeller University, New York, NY, October 6, 1994

ABSTRACT **Capsid-targeted viral inactivation is an antiviral strategy in which toxic fusion proteins are targeted to virions, where they inhibit viral multiplication by destroying viral components. These fusion proteins consist of a virion structural protein moiety and an enzymatic moiety such as a nuclease. Such fusion proteins can severely inhibit transposition of yeast retrotransposon Ty1, an element whose transposition mechanistically resembles retroviral multiplication. We demonstrate that expression of a murine retrovirus capsid–staphylococcal nuclease fusion protein inhibits multiplication of the corresponding murine leukemia virus by 30- to 100-fold. Staphylococcal nuclease is apparently inactive intracellularly and hence nontoxic to the host cell, but it is active extracellularly because of its requirement for high concentrations of Ca^{2+} ions. Virions assembled in and shed from cells expressing the fusion protein contain very small amounts of intact viral RNA, as would be predicted for nuclease-mediated inhibition of viral multiplication.**

Although the assembly process of retroviruses is still poorly understood there is good evidence that Gag and Gag-Pol precursor proteins coassemble into a core in which the C termini of these proteins contact the viral RNA inside the virion and the N termini face outward, contacting the viral membrane and possibly the envelope protein (1). Furthermore, many studies have shown that Gag proteins or even portions of Gag proteins are sufficient to mediate virion formation in the absence of any other viral proteins (2). Fusion proteins consisting of an N-terminal Gag moiety and a C-terminal foreign protein moiety can be efficiently packaged into retrovirus and retrotransposon particles. Examples of foreign proteins so incorporated include cytochrome *c*, β-galactosidase, heterologous reverse transcriptases (RTs), and nucleases (3–7). We have taken advantage of this behavior to design and develop an antiviral strategy, capsid-targeted viral inactivation (CTVI), in which virion structural protein–nuclease chimeras are expressed inside sensitive cells. Coassembly of these fusion proteins with normal virion components should yield virions containing the nuclease; such particles should be rendered noninfectious by the action of the nuclease on the viral RNA (7). A nuclease of special interest for use with the CTVI strategy is staphylococcal nuclease (SN), an enzyme that degrades both RNA and DNA and is completely dependent on millimolar concentrations of Ca^{2+} for activity. We have previously shown that this enzyme is nontoxic to yeast cells (7), presumably due to the extremely low intracellular Ca^{2+} concentration. Intracellular concentrations are typically in the nanomolar range and always <1 μM; extracellular concentrations (e.g., in blood or tissue culture medium) are several millimolar. In principle, fusion proteins based on this enzyme would be inactive intracellularly but, when released into the high-Ca^{2+} extracellular environment, could be activated by influx of Ca^{2+} into the virion. Although a perfect, planar lipid bilayer should block such an influx, the viral membrane differs from such a bilayer in its extreme curvature and in its content of viral and possibly cellular proteins. These factors might allow such an ion influx. To attain an intravirion Ca^{2+} concentration sufficient for SN activity, only $\approx$10 Ca^{2+} ions would be required.

Our overall strategy was to deliver and express murine Gag–SN fusion proteins by using the RCAS (replication-competent ALV LTR, splice acceptor) avian retrovirus gene expression system (8, 9) in avian cells. In this Rous sarcoma virus (RSV)-based system, a very high percentage of cells in the culture express the protein of interest. This system allows testing of both the expression and potential toxicity of fusion proteins. The murine Gag–SN fusion protein is not expected to affect the multiplication of the RCAS vectors, because avian and murine retrovirus Gag proteins are very different in primary sequence and do not coassemble. Indeed, we find that the Gag–SN-expressing RCAS vector grows just as well as nonexpressing control vectors. Finally, antiviral efficacy of the murine Gag–SN fusion protein can be assayed in the RCAS-infected cultures by challenging the cultures with an amphotropic murine leukemia virus, Mo4070A, and monitoring its multiplication.

MATERIALS AND METHODS

Vectors and Plasmids. The RCASBP and RCANBP replication-competent avian retroviral vectors used have been described (9). The Gag–SN fusions were first assembled in the adaptor plasmid pCla12Nco (10) between the *Nco* I and *Sal* I sites. The Gag cassettes were constructed by PCR, introducing an *Nco* I site at the ATG *gag* codon and a *Bam*HI site at the 3′ end of *gag* or the 5′ end of *pol* as indicated in Fig. 1. Both Gag cassettes were fully sequenced to ensure lack of mutations. These were joined to the previously described *Bam*HI/*Sal* I SN fragment (7). The two Gag–SN cassettes were then subcloned as *Cla* I fragments into the RCASBP and RCANBP vectors (Fig. 1).

Virus Preparation. Viral pellets were prepared by centrifugation of 30 ml of tissue culture supernatant at low speed for 5 min to remove residual cells followed by centrifugation for 30 min at 35,000 rpm and 4°C in a Beckman SW41 rotor and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CEF, chicken embryo fibroblast; CTVI, capsid-targeted viral inactivation; FIU, focus-inducing unit; MoLV, Moloney murine leukemia virus; SN, staphylococcal nuclease; RSV, Rous sarcoma virus; RT, reverse transcriptase; moi, multiplicity of infection.

†Present address: Avigen, Inc., 1201 Harbor Bay Parkway, No. 1000, Alameda, CA 94501.
¶To whom reprint requests should be addressed.

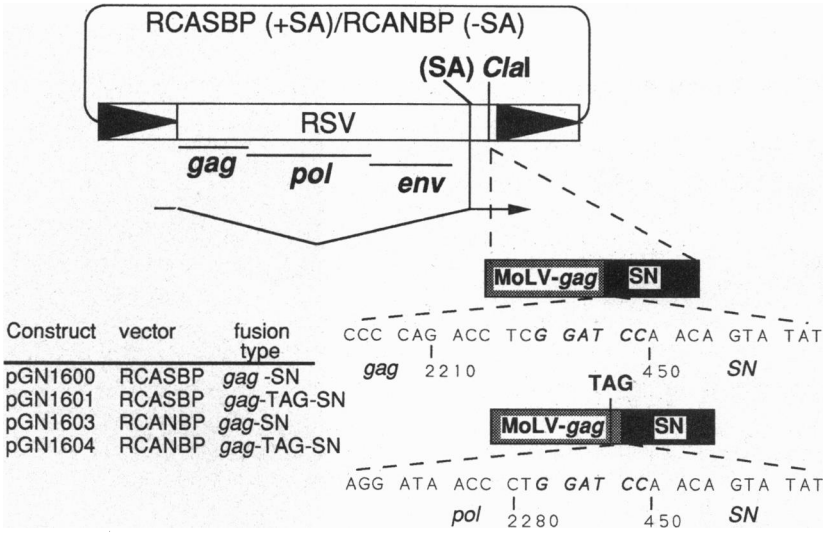

FIG. 1. Gag–SN fusion protein expressed in replication-competent RSV vectors. Structures of the four plasmids (pGN1600–1604) used in this work are indicated. These experiments made use of an experimental plasmid, pGN1600, expressing Gag–SN and three control constructs, pGN1601–1604, that do not express SN due to either a stop codon (pGN1601), lack of a splice acceptor signal (pGN1603), or both (pGN1604). The RCASBP vector has the *src* splice acceptor site, which is absent from RCANBP. Boxed triangles, long terminal repeats; open box, RSV coding regions; hatched box, murine leukemia virus sequences; solid box, SN coding sequences; arrow with V, spliced *src*-like mRNA produced in RCASBP; (SA), splice acceptor (not present in RCANBP). (*Insets*) Fusion junction sequences based on GenBank data base entries for Moloney murine leukemia virus (MoLV) (MLMCG) and SN (STANUCF); italicized bases are *Bam*HI sites introduced at junctions; spaces demarcate codons. See *Materials and Methods* for full descriptions of constructs.

resuspension by Vortex mixing in 500 μl of buffer (50 mM Tris·HCl, pH 6.8/100 mM NaCl), followed by a 15-min centrifugation at 4°C in an Eppendorf microcentrifuge at top speed and final Vortex resuspension in 50 μl of buffer.

Infection. Mo4070A supernatants containing ≈2 × 10^6 focus-inducing units (FIU)/ml were produced by growth on NIH 3T3 cells for 5 days; accumulation of virus was monitored by RT assay or by the S^+L^- focus assay (11). Chicken embryo fibroblast (CEF) cells (a 10-cm dish of 80% confluent cells) were infected at high multiplicity of infection (moi) (≈2 FIU per cell) as follows. CEF cells were pretreated with 20 μg of DEAE-dextran per ml at 37°C for 1 h and washed with phosphate-buffered saline. Then 5 ml of Mo4070A supernatant plus 5 ml of fresh medium was added to the cells. Low moi (≈0.1 FIU per cell) was done the same way except 250 μl of supernatant plus 9.75 ml of fresh medium were used.

Enzymatic Assays. RT activity was assayed as described (12) except that virions were pelleted before assay. The RT produced by RCASBP is essentially undetectable under these conditions, because the avian virus RT incorporates very little radioactivity in the presence of Mn^{2+}. Assays on crude supernatants were unreliable due to interference by SN in strains producing Gag–SN.

SN was assayed as follows: samples to be assayed for activity were mixed with 0.5 μg of phage λ DNA (predigested with *Hin*dIII) for 2 h at 37°C in 20 μl of SN assay buffer (100 mM Tris·HCl, pH 8.8/10 mM $CaCl_2$).

RESULTS

Murine Gag–SN Fusion Gene in a Replication-Competent Avian Retroviral Vector Delivery/Expression System. We constructed two fusion genes in which the SN gene was joined in frame to (*i*) the portion of MoLV *gag* encoding the C terminus or (*ii*) MoLV *pol*, just 46 nucleotides downstream of the *gag* termination codon. A fusion protein of ≈78 kDa was expected to be expressed from the former, whereas the latter control fusion should express only Gag protein. This is because the MoLV *gag-pol* readthrough signal is incomplete in this construct due to the position of insertion of SN DNA and, consequently, readthrough of the *gag* termination codon into SN is not expected (13, 14).

These *gag*–SN fusion genes were separately cloned into two replication-competent avian retrovirus vectors based on RSV, RCASBP and RCANBP (Fig. 1). In RCASBP, protein encoded by the insert is expressed via the *src* splicing signals; in RCANBP, the *src* splice acceptor has been deleted and thus expression of inserted protein coding regions is not expected (9, 10, 15). The four recombinant vectors were introduced into CEF cells by transfection. Spread of the recombinant viruses was monitored by immunoblotting with an anti-RSV capsid (CA) antibody and by RT assays; the four constructs produced these RSV proteins with similar kinetics to RCASBP itself (Fig. 2*A*; data not shown).

Gag–SN Fusion Proteins Are Secreted and Enzymatically Active. The cultures were assayed for production of fusion protein by immunoblotting with anti-MoLV CA and anti-SN antibodies and also by direct enzyme assays. Because retroviral Gag proteins contain all necessary signals for particle formation, and many Gag fusion proteins have been shown to be competent for particle formation, we assayed particulate fractions of the culture supernatants for fusion proteins. Fig. 2*B* shows that the RCASBP constructs pGN1600 and pGN1601 produce proteins of the expected molecular masses for a Gag–SN fusion protein (78 kDa) and native unprocessed Gag protein (65 kDa), respectively, that are detected with anti-MoLV Gag antibody. Fig. 2*C* shows that only the pGN1600 protein is reactive with anti-SN antibody. This blot was deliberately overexposed to show that no Gag–SN readthrough protein is detectable in the pGN1601 control construct. We conclude that both the MoLV Gag precursor and the Gag–SN fusion protein are expressed in chicken cells from RCASBP, assembled into virus-like particles, and released into the supernatant.

To determine whether Gag–SN fusion proteins retained enzymatic activity, the viral pellets were assayed for Ca^{2+}-dependent nuclease activity against bacteriophage λ DNA together with a standard curve of purified SN. Fig. 3 shows that these particles indeed contain a Ca^{2+}-dependent nuclease activity.

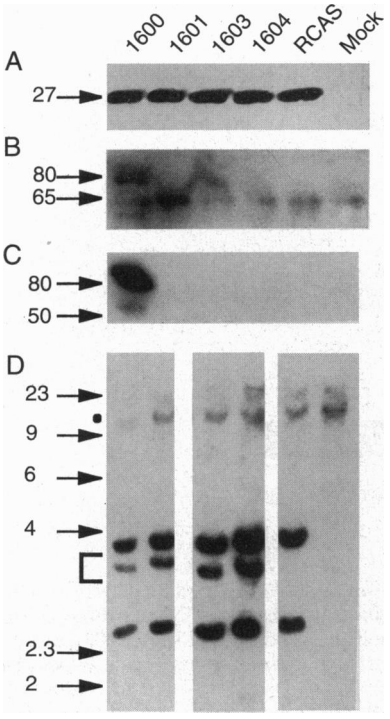

FIG. 2. Replication of recombinant vectors and expression of Gag–SN fusion proteins. Protein and DNA samples were harvested six passages after transfection with 5 μg of plasmid DNA. (*A–D*) All derive from the same infection experiment. (*A*) RSV CA proteins were detected by immunoblotting viral pellet fractions prepared as described with an anti-RSV CA antibody (kindly provided by L. Stewart and V. Vogt). (*B*) MoLV Gag (65 kDa) and Gag–SN (78 kDa) were detected by immunoblotting viral pellet fractions with an anti-MoLV CA antibody (16). There is an additional lower abundance cross-reacting protein of 65 kDa (of unknown identity) present in the chicken cells. (*C*) Gag–SN was detected by immunoblotting viral pellet fractions with anti-SN antibody (7). Size markers in *A–C* are in kDa. (*D*) Recombinant provirus structure analyzed by genomic DNA blotting. Total cellular DNA (10 μg) was digested with *Eco*RI and blotted with a RSV *Sal* I probe that detects viral DNA and c-*src* (9, 10). Bracket indicates DNA fragments containing the Gag–SN DNAs; dot is the c-*src* band, which serves as a loading control. Size markers in *D* are in kbp.

Evaluation of Cellular Toxicity of Gag–SN Fusion Protein. The presence of nuclease activity raises the question of whether expression of Gag–SN might be toxic to host cells. When genes encoding toxic proteins (such as retroviral *env* genes) are cloned into the RCASBP/RCANBP vectors in parallel and the resulting viruses are passaged in CEFs, the toxic gene is selectively lost only from the RCASBP vector, which expresses the toxic product. We have examined the stability of the Gag–SN inserts in these viruses by genomic DNA blot analysis of the provirus populations in these cultures. Virus was propagated for six passages on CEF cells following initial transfection, and the genomic DNA was extracted from the infected cells, digested with *Eco*RI, and analyzed by DNA blotting using a RSV probe (Fig. 2*D*). From this analysis, we conclude that the internal structure of the provirus populations derived from all four recombinants are similar in stability to each other and to that of the RCASBP vector and also that the amount of proviral DNA (relative to the internal c-*src* control) is similar. In separate experiments, we have isolated stable cell lines expressing Gag–SN constructs and shown that these express extracellular SN activity for at least a year (data not shown). These results suggest that the Gag–SN protein is not toxic to CEF cells.

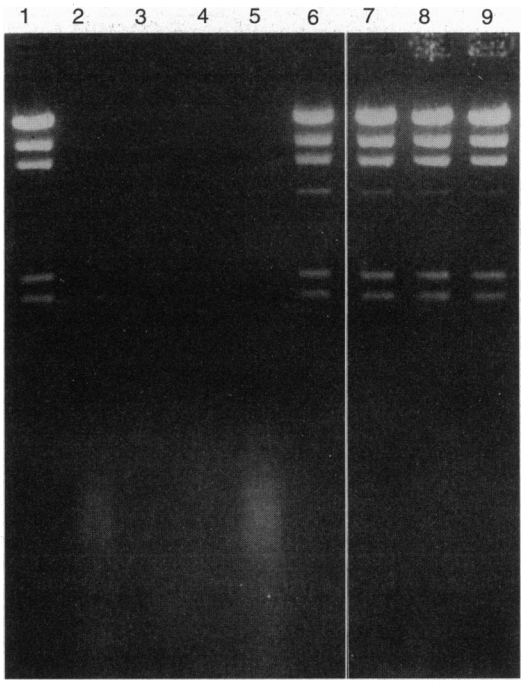

FIG. 3. Gag–SN from cell supernatants has Ca^{2+}-dependent nuclease activity. If enzymatically active Gag–SN fusion protein is encapsidated into virions, it should be possible to detect the Ca^{2+}-dependent nuclease activity of SN in viral pellet fractions. Protein from viral pellets harvested six passages after transfection (or similarly prepared fractions from mock-transfected CEF cells) was resuspended in buffer (50 mM Tris·HCl, pH 6.8/100 mM NaCl/10 mM $MgCl_2$) and boiled for 1 min. (Boiling increases the total amount of SN activity recovered and eliminates the activity of residual cellular nucleases.) Viral pellet fractions (1 μl), prepared as described, or various amounts of purified native SN were assayed as described. Lanes: 1 and 7, CEF supernatant; 2 and 8, CEF/pGN1600 supernatant. Lanes 7–9 also contained EGTA at 10 mM instead of $CaCl_2$. Lanes 3–5 and 9 had purified SN added (lanes 3 and 9, 10 ng; lane 4, 1 ng; lane 5, 0.1 ng).

Multiplication of Mo4070A Retrovirus Is Inhibited by Gag–SN Fusion Protein. To examine antiretroviral effects of the Gag–SN fusion protein, we challenged CEFs expressing this protein with a murine retrovirus. We then assayed the ability of the murine virus to replicate, both within the CEF cultures themselves and upon subculturing progeny murine viruses in murine cell lines. CEF cultures infected with the Gag–SN-expressing and control constructs (and uninfected CEFs) were challenged with a murine retrovirus, Mo4070A, that has a MoLV-derived *gag* gene but an *env* gene derived from the amphotropic murine leukemia virus 4070A (17) (Fig. 4*A*). This envelope gives the virus a wide host range that includes CEFs. In low moi, the Mo4070A virus was unable to multiply efficiently in the cells expressing Gag–SN but replicated normally in the control cells. This multiplication defect was demonstrated in three ways. (*i*) The appearance of Mo4070A-specific RT activity was reduced relative to control lines (Fig. 4*B*). (*ii*) The extracellular supernatants from the infected CEF cells were reduced in infectivity. The amount of infectious Mo4070A in these supernatants was measured indirectly by the kinetics of appearance of RT after infection of murine 3T3 cells with the CEF supernatants; the appearance of RT activity in the 3T3 supernatants was delayed in the Gag–SN-expressing supernatants relative to the controls (Fig. 4*C*). (*iii*) The above data correlate well with the lack of Mo4070A viral RNA in viral pellets from the Gag–SN-expressing line (Fig. 4*D*).

A second experiment to directly and quantitatively assess the inhibition of Mo4070A multiplication was subsequently car-

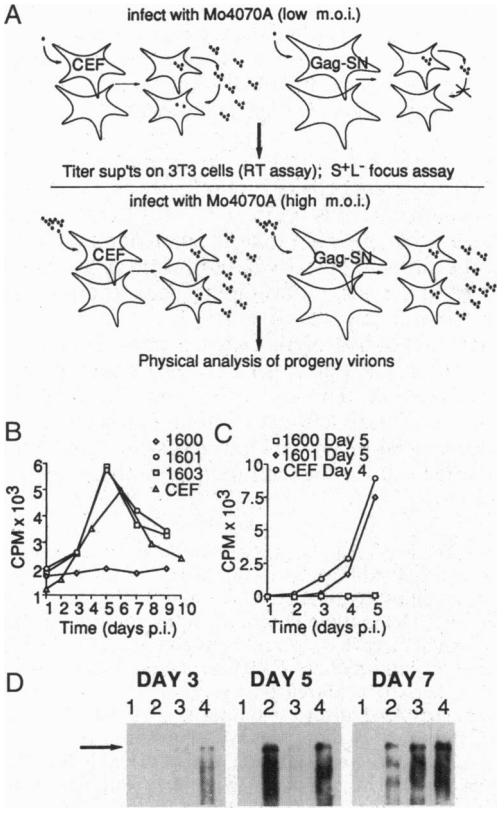

FIG. 4. Reduction of infectious virus in supernatants from cells expressing Gag–SN. (*A*) Experimental design. Both low and high moi of CEF cells with Mo4070A were carried out. The low moi experiments were performed to quantify effects on viral multiplication (see *B–D* and Fig. 5) and the high moi progeny virions were analyzed to determine the mechanism of the antiviral effect (see Fig. 6). (*B*) Mo4070A replicates more slowly in Gag–SN-expressing cells. Replication of Mo4070A was assayed by monitoring the appearance of Mo4070A RT activity on the indicated day postinfection (p.i.). In *B* and *C*, raw cpm incorporated per 5 μl of supernatant are indicated. After background subtraction, Gag–SN expression resulted in a 10-fold reduction in RT activity in this experiment; in an independent experiment, Mo4070A RT activity was reduced only 4-fold in Gag–SN-expressing cells relative to controls. (*C*) Supernatants from cells expressing Gag–SN have reduced virus titers. Supernatant (1 ml) from CEF cells expressing the indicated construct and challenged with Mo4070A was added to a 3-cm dish of NIH 3T3 cells. RT activity in the NIH 3T3 cells was assayed on the indicated day. (*D*) Reduced Mo4070A viral RNA in viral pellets from superinfected Gag–SN-expressing cell supernatants, harvested at the times indicated. RNA blot of viral pellets was hybridized to a MoLV *pol* probe. Lanes: 1, pGN1600; 2, pGN1601; 3, pGN1603; 4, CEF. Full-length RNA is indicated (arrow).

ried out. A low moi experiment identical to that described above was carried out, except that multiplication of Mo4070A was assayed directly and quantitatively by the S^+L^- focus-formation assay (11). The results of these experiments are presented in Fig. 5. It can readily be seen that in the Gag–SN-expressing CEF line, viral titers are reduced by at least 30- to 120-fold relative to three control CEF lines that do not express Gag–SN.

Mo4070A Virions Assembled in the Presence of Gag–SN Fusion Protein Contain Reduced Amounts of Virion RNA and a Processed Form of Gag–SN Fusion Protein. The above results do not indicate the mechanism by which Mo4070A multiplication is inhibited. To accomplish this, a high moi Mo4070A infection was carried out. At a high moi, normal amounts of Mo4070A virions should be assembled, allowing biochemical amounts of virions assembled in the presence or

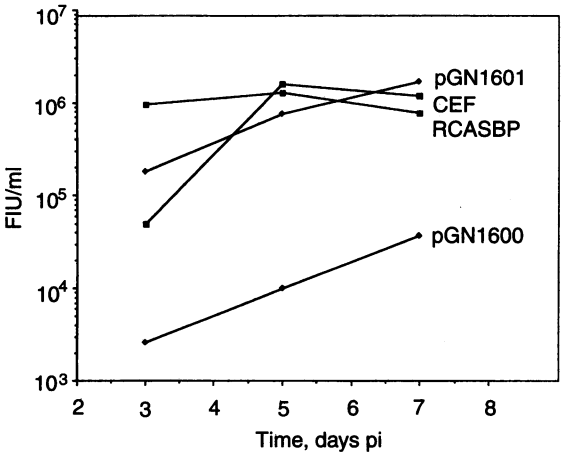

FIG. 5. Quantitative assay of antiviral effect of Gag–SN fusion protein. CEF cells were transfected with RCASBP, pGN1600 (expressing Gag–SN), or pGN1601 or mock-infected and passaged six times. Cell cultures were infected with Mo4070A at low moi and, after the indicated number of days postinfection (pi), the supernatants (30 ml) were harvested; 5 ml was set aside for the S^+L^- focus-formation assay and 25 ml was pelleted and assayed for RT and SN activity as described in Figs. 3 and 4, with similar results (data not shown).

absence of Gag–SN fusion proteins to be produced and analyzed. In this way, we could investigate whether the newly produced virions were defective in nucleic acid content or in some other way.

We first showed that similar amounts of Mo4070A virions were produced in parallel cultures expressing or not expressing Gag–SN fusions by monitoring RT activity (Fig. 6*A*). The amount of virions produced in this experiment (as assayed by RT activity) in the presence of Gag–SN was reduced by no more than 2-fold. Culture supernatants were harvested 1 and 3 days postinfection, the virions were pelleted, and the RNA was extracted and analyzed by gel electrophoresis and blotting with a MoLV probe (Fig. 6*B*). It is readily apparent from this

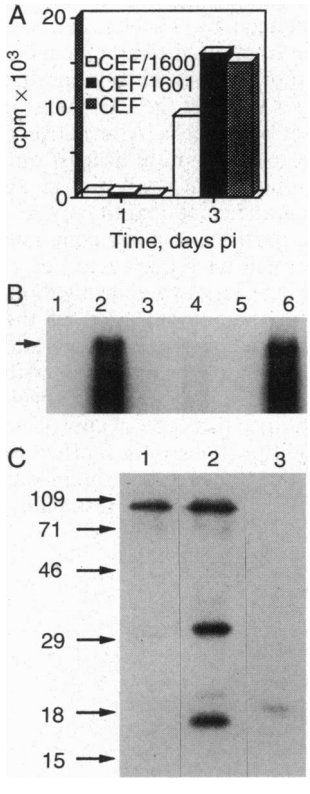

FIG. 6. Analysis of virions produced in high moi. (*A*) Mo4070A RT activity is produced in similar amounts in Gag–SN-producing and control cultures (raw cpm incorporated are presented). pi, Postinfection. (*B*) Full-length virion RNA (arrow) is greatly reduced in viruses produced by cells expressing Gag–SN. RNA blot of viral pellets was hybridized to a MoLV *pol* probe. Strains were as follows: lanes 1 and 2, pGN1601; lanes 3 and 4, pGN1600; lanes 5 and 6, CEFs. Supernatants were assayed on day 1 (lanes 1, 3, and 5) and day 3 (lanes 2, 4, and 6). (*C*) Immunoblot with anti-SN antibody on viral pellets. Gag–SN is processed upon infection by Mo4070A. Lane 1, uninfected; lane 2, Mo4070A-infected for 3 days; lane 3, purified SN (1 ng). Size markers are in kDa.

experiment that the amount of full-length Mo4070A virion RNA recovered is much lower in the case of the Gag–SN-expressing host than in the controls. In a control blot done on the same samples, there was no difference in the amount of RSV virion RNA levels among the viral pellets examined, so the lack of Mo4070A RNA is not due to general degradation of the RNA in this sample (data not shown). Thus, we can account for the loss of infectivity of the Mo4070A virions produced by the pGN1600 cells by a specific deficit in encapsidated Mo4070A genomic RNA.

A further prediction of the CTVI strategy is that Gag–SN fusion proteins should be coassembled into virions with native Mo4070A viral proteins and hence become susceptible to the Mo4070A protease. If processing occurs at the normal retroviral sites, the 78-kDa precursor Gag–SN protein observed in the uninfected cells should be processed to a nucleocapsid-SN (NC-SN) protein of ≈30 kDa when the cell is infected with Mo4070A; this superinfection-dependent molecular mass shift was observed by immunoblotting (Fig. 6*C*). In addition, the Mo4070A-infected cells contain an ≈17-kDa SN-immunoreactive species, suggesting an additional protease cleavage site near the N terminus of SN.

DISCUSSION

We have demonstrated that a murine leukemia virus Gag–SN fusion protein can be expressed and that this expression is readily tolerated by tissue culture cells. The Gag–SN fusion protein can assemble into virus-like particles that are shed by these cells, and the fusion proteins within these particles have a Ca^{2+}-dependent nuclease activity that is easily detected *in vitro*. When these expressing cells are infected with the corresponding amphotropic murine leukemia virus, the Gag–SN fusion protein coassembles with native Gag and Gag-Pol proteins, forming viral particles that resemble normal virions, except that they contain Gag–SN protein and their infectivity is greatly reduced. Biochemical analysis of the virions produced in the presence of these Gag–SN fusion proteins shows that they contain greatly reduced amounts of virion RNA.

In this study, we used a quantitative focus assay to directly assay the number of infectious virus particles. The titers of infectious virus appear to be reduced by 97–99% in these experiments. Nevertheless, a small fraction of virus appears to avoid inactivation. This might represent expression of the Gag–SN fusion protein in slightly <100% of the CEF cells or low levels of expression in a subset of the cells. Alternatively, if a small fraction of virions fails to incorporate at least one molecule of the fusion protein, that fraction will fail to be inactivated. Finally, it may be that a sufficient quantity of Ca^{2+} ions is not allowed into all of the particles to allow complete inactivation. Further experiments will be required to determine the mechanism by which some virions avoid inactivation.

Our previous study (7) showed that mobilization of the retrotransposon Ty1, which lacks a membrane envelope and whose life cycle is wholly intracellular, could be interfered with by this general approach. This study demonstrates that enveloped retroviruses are also susceptible to this general approach. In the study of Ty1, Gag–SN fusion proteins were ineffective against Ty1, in spite of the fact that the fusion protein was efficiently assembled into Ty1 virus-like particles. Presumably, the lack of effect on Ty1 transposition reflects the fact that Ty1 has no extracellular phase and hence is never exposed to a high enough Ca^{2+} concentration to activate SN. In contrast, an enzyme that did not require Ca^{2+} ions, barnase, was able to block Ty1 transposition very effectively when fused in a similar manner.

We previously showed that SN fusion proteins had no toxicity against yeast cells (7). This study and others we have done in mammalian cells (G.N., P.S., and J.D.B., unpublished data), suggest that SN is not toxic in avian or mammalian tissue culture cells either. Thus, SN-based fusion proteins represent excellent candidates for developing gene therapy strategies against retroviral diseases.

Incorporation of hydrolytic enzymes into virions in the form of fusion proteins is a promising general strategy for limiting viral multiplication. The use of enzymes like SN that are inactive intracellularly but active in an extracellular environment holds special promise. The successful demonstration of antiviral effects in tissue culture paves the way for CTVI studies *in vivo*.

We thank David Shortle for advice on SN and for supplying initial samples of anti-SN antibody and Jane Mirro for technical assistance. The Johns Hopkins University, G.N., and J.D.B. have a financial interest in the CTVI method described in this publication. The terms of this arrangement have been reviewed and approved by the School of Medicine Committee on Conflict of Interest. This work was supported in part by a Merck postdoctoral fellowship (G.N.), U.S. Department of Agriculture Competitive Grant 92-37204-8041 (M.J.F.), Contract NO1-CO-74101 with Advanced BioScience Laboratories (M.J.F., A.R., and S.H.H.), Public Health Service Grants P01-CA16519 and U01-AI35282 from the National Institutes of Health, and American Cancer Society Faculty Research Award FRA-366 (J.D.B.).

1. Bolognesi, D. P., Montelaro, R. C., Frank, H. & Schafer, W. (1978) *Science* **199,** 183–186.
2. Wills, J. W. & Craven, R. C. (1991) *AIDS* **5,** 639–654.
3. Hansen, M., Jelinek, L., Whiting, S. & Barklis, E. (1990) *J. Virol.* **64,** 5306–5316.
4. Weldon, R. A., Erdie, C. R., Oliver, M. G. & Wills, J. W. (1990) *J. Virol.* **64,** 4169–4179.
5. Gabriel, A. & Boeke, J. D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9794–9798.
6. Mathias, S. L., Scott, A. F., Kazazian, H. H., Jr., Boeke, J. D. & Gabriel, A. (1991) *Science* **254,** 1808–1810.
7. Natsoulis, G. & Boeke, J. D. (1991) *Nature (London)* **352,** 632–635.
8. Hughes, S. H. & Kosic, E. (1984) *Virology* **136,** 89–99.
9. Petropoulos, C. J. & Hughes, S. H. (1991) *J. Virol.* **65,** 3728–3737.
10. Hughes, S. H., Greenhouse, J. J., Petropoulos, C. J. & Sutrave, P. (1987) *J. Virol.* **61,** 3004–3012.
11. Bassin, R. H., Tuttle, N. & Fischinger, J. R. (1971) *Nature (London)* **229,** 564–566.
12. Goff, S., Traktman, P. & Baltimore, D. (1981) *J. Virol.* **38,** 239–248.
13. Wills, N. M., Gesteland, R. F. & Atkins, J. F. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6991–6995.
14. Feng, Y.-X., Yuan, H., Rein, A. & Levin, J. G. (1992) *J. Virol.* **66,** 5127–5132.
15. Greenhouse, J. J., Petropoulos, C. J., Crittenden, L. B. & Hughes, S. H. (1988) *J. Virol.* **62,** 4809–4812.
16. Versteegen, R. J. & Oroszlan, S. (1980) *J. Virol.* **33,** 983–992.
17. Ott, D. E., Keller, J., Sill, K. & Rein, A. (1992) *J. Virol.* **66,** 6107–6116.