**INRODUCTION**

Small interfering RNAs (siRNAs) of 21 bp in length with 3′ overhangs of two nucleotides can cause RNA interference (RNAi) by RNA degradation. siRNAs are a powerful tool for sequence-specific, post-transcriptional gene silencing and have a potential therapeutic and prophylactic application against cancer, as well as infectious diseases (reviewed by Pickford & Cogoni, 2003; Shi, 2003; Wall & Shi, 2003). siRNAs have been utilized successfully in cultured mammalian cells to reduce the expression of specific genes (Elbashir et al., 2001). Recent reports have demonstrated that siRNAs can be produced intracellularly from RNA polymerase III (Pol III) promoters, such as H1 (Brummelkamp et al., 2002), U6 (Lee et al., 2002; Miyagishi & Taira, 2002; Paddison et al., 2002; Paul et al., 2002; Sui et al., 2002), as well as polymerase I (Pol I) (McCown et al., 2003) and T7 (Donzé & Picard, 2002) promoters (reviewed by Scherr et al., 2003).

For therapeutic application against virus diseases, siRNA must be able to enter infected cells efficiently and inhibit an ongoing virus infection. As siRNAs are too short to induce an interferon (IFN) response in mammalian cells, siRNA-mediated interference bypasses the non-specific, IFN-mediated antiviral response (Gitlin et al., 2002; Kapadia et al., 2003). After influenza virus infection, viral RNA (vRNA) (minus strand) is transcribed into two classes of plus strand RNAs: namely, the viral mRNA and viral complementary RNA (cRNA). Because of the complementarity of duplex siRNA strands, it could interfere directly with each class of virus-specific RNA in virus-infected cells, including vRNA, viral mRNA and viral cRNA. However, viral mRNAs are the specific target of siRNA-mediated interference (Ge et al., 2003), as influenza vRNAs and cRNAs, bound to nucleoprotein (NP), may be protected from cleavage by RNAi machinery. Furthermore, whilst mRNAs are exported into the cytoplasm, cRNAs remain in the nucleus, thereby making mRNAs the preferred target of siRNA-mediated degradation.

siRNAs have been used recently to interfere with the replication of a number of viruses (reviewed by Ahlquist, 2002; Carmichael, 2002; Lindenbach & Rice, 2002; Saksela, 2003). To investigate whether siRNA can be used to interfere with influenza virus replication, we used siRNAs from the M gene sequence of influenza virus, as its matrix (M1) protein plays a critical role in many aspects of virus replication, including virus assembly and budding (reviewed by Nayak & Hui, 2002). The virus presents a potential target for siRNA-mediated interference and there are a number of advantages in using siRNA against influenza. Firstly, as influenza is restricted primarily to the lungs, a virus vector-based delivery system could be used effectively to reach the lungs via the intranasal route. Secondly, as influenza virus changes annually, siRNA from a conserved M1 sequence may be effective against influenza virus strains arising from either antigenic drift or antigenic shift. In this report, we have used siRNAs targeted against the M gene and show that M1 expression can be inhibited specifically in 293T cells by...
plasmids expressing siRNAs from a Pol III promoter. Furthermore, as lentivirus vectors with an siRNA cassette can efficiently infect and express siRNA in different cells, including stable cell lines (Abbas-Terki et al., 2002), primary cells (Qin et al., 2003; Stewart et al., 2003) and fertilized eggs used in generating transgenic animals (Tiscornia et al., 2003), we have used a lentivirus vector derived from human immunodeficiency virus (HIV)-1 to deliver M gene siRNA into Madin–Darby canine kidney (MDCK) cells, which are commonly used for influenza virus propagation. Our data show that this lentivirus-mediated siRNA delivery method can be used to specifically silence the target M1, but not NP, expression and to inhibit influenza virus replication in MDCK cells.

METHODS

**Plasmid construction.** As the AAGN₄₄UU sequence (N, any nucleotide) has been found to be preferred for siRNA-mediated gene silencing under the control of the Pol III U6 promoter (Elbashir et al., 2002; Lee et al., 2002), we searched for this sequence in the ORF of the M gene. Selected sequences were submitted to a BLAST search against the human genome sequence to ensure that the human genome was not targeted. To construct the hairpin siRNA expression cassette, the following DNA oligonucleotides were synthesized: M-89, 5′-caatgcattgctgcaagaaaaaGCGGAGATCGCAGAGAAGCCATTCGCTGTTATCTCCCTTAAGC-3′; M-331, 5′-caatgcattgctgcaagaaaaaGCTTAAAGGAGGATACAtttacaagAGTGTATCTCCCTTACATAGCTGTTATCTCCCTTAAGC-3′; M-620, 5′-caatgcattgctgcaagaaaaaGCGGAGATCGCAGAGAAGCCATTCGCTGTTATCTCCCTTAAGC-3′. After PCR (60 ℃ annealing temperature, 25 cycles, 50 μl volume) with 10 pmol reverse primer (5′-GGAACACGCTATGACAG-3′) and 10 ng template pBpU6, DNA fragments that contained Ndel–human U6 promoter–siRNA cassette sequence–PstI were ligated into a pCRII vector (Invitrogen). We created three siRNA cassettes from the M gene: pCR-M-89, pCR-M-331 and pCR-M-620 (named on the basis of the nucleotide start site in the M gene) (Fig. 1). To construct a lentivirus vector carrying the siRNA expression cassette, plasmid RRL-U6shLuc-cPPT-PGK-EGFP-SIN (An et al., 2003) was first digested with Ndel and PstI. The resulting vector fragment (7082 bp) was ligated to the fragment that had been isolated from plasmid pCR-M-331 by Ndel and PstI digestion (130 bp). The resulting lentivirus vector containing M1 siRNA was named LYM-V331. The vesicular stomatitis virus (VSV) envelope glycoprotein (G protein) expression plasmid (pCMV-G) and the packaging plasmid for HIV-1-based vectors (pCMVR8.2DVPR), which is used in lentivirus particle production, have been described previously (An et al., 2003). RRL-U6shLuc-cPPT-PGK-EGFP-SIN, an HIV-based vector containing a luciferase siRNA expression cassette, was constructed as described previously (An et al., 2003).

**Cell lines and viruses.** MDCK cells were maintained in DMEM (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Atlanta Biologicals) and antibiotics (100 U penicillin G ml⁻¹ and 100 μg streptomycin ml⁻¹). Human embryonic kidney 293T cells were maintained in Opti-MEM-I medium (Invitrogen) supplemented with 5% FBS and antibiotics. Influenza virus strain A/WSN/33 (H1N1) was used in these experiments and stock viruses were prepared as reported previously (Hui & Nayak, 2001, 2002).

**Transfection of the siRNA expression cassette into 293T cells.** Transfection and protein pulse-labelling were performed as described previously (Hui et al., 2003a, b). Briefly, 10 μg U6 promoter-driven siRNA cassette plasmid (pCR-M-89, pCR-M-331 or pCR-M-620) and 1 μg Pol I–Pol II construct of the M gene (Hoffmann et al., 2000) were mixed with 16 μl TransIT LT1 (Panvera), mixed with Opti-MEM-I, added to 293T cells (1 × 10⁶) on a Becton Dickinson BioCoat poly-d-lysine/laminin six-well culture plate. The DNA transfection mixture was replaced with virus growth medium (VGM) 6 h later. For labelling, 293T cells at 13 h post-transfection (hpt) were starved and pulse-labelled with 100 μCi [³⁵S]protein label (ICN Biomedicals). Cells were then lysed in radioimmunoprecipitation assay buffer, immunoprecipitated with anti-M1 antibodies (Bioside International) and analysed by SDS-PAGE.

**Lentivirus vector particle production.** All virus stocks containing VSV G protein were produced by calcium phosphate-mediated, three-plasmid transfection of 293T cells, as described previously (An et al., 2003). Briefly, 293T cells (1-5 × 10⁶ cells in a 175T flask) were transfected with 5 μg pCHMV-G, 12.5 μg pCMVR8.2DVPR and 12.5 μg LYM-V331 or RRL-U6shLuc-cPPT-PGK-EGFP-SIN and cultured in DMEM supplemented with 10% FBS and antibiotics. Virus supernatants were collected on days 3 and 4 post-transfection, filtered through a 0.22 μm pore-size filter, ultracentrifuged (50 000 g; 1.5 h, 4 ℃) and resuspended in PBS. Virus stocks were titrated by infecting 293T cells (5 × 10⁴ cells in a 12-well plate) with virus dilutions in DMEM supplemented with 10% calf serum and 8 μg hexadimethrine bromide ml⁻¹ and analysed for enhanced green fluorescent protein (EGFP) expression by flow cytometry. Titres of vector virus stocks were routinely 10⁶ infectious units (IU) ml⁻¹.

**Lentivirus vector transduction and influenza virus superinfection.** MDCK cell monolayers (1 × 10⁶ cells in 35 mm dishes, 10% confluence) were washed with PBS⁺ (PBS plus 0.5 mM MgCl₂ and 1 mM CaCl₂) and incubated with various lentivirus vectors at an m.o.i. of 10 in 300 μl DMEM supplemented with 10% FBS, antibiotics and 8 μg hexadimethrine bromide ml⁻¹ for 2 h at 37 ℃. Unadsorbed viruses were removed by washing with DMEM and cell monolayers were incubated with 2 ml DMEM supplemented with 10% FBS and antibiotics at 37 ℃. After 3 days incubation, cells were analysed for EGFP expression by flow cytometry. Approximately 80% of cells were EGFP-positive. Lentivirus-transduced MDCK cell monolayers were infected with influenza virus (m.o.i., 0-1) in 300 μl virus dilution buffer (Hui et al., 2003b) for 1 h at 37 ℃. Unadsorbed viruses were removed by washing with VGM and the superinfected cell monolayers were then incubated at 33 ℃. Supernatants were harvested at 6, 8 and 10 h post-infection (p.i.) and titrated by plaque assay.

**Flow cytometric analysis.** EGFP expression was analysed with a fluorescence-activated cell sorter (FACS) Calibur flow cytometer (BD Bioscience Immunocytometry Systems). Data were processed with CellQuest software (BD Bioscience Immunocytometry Systems).

**Plaque assay.** Plaque assays were done as described previously (Hui et al., 2003b). Briefly, diluted virus samples were layered on MDCK monolayers (37 ℃, 1 h). Cell monolayers were then overlaid with agar overlay medium, incubated at 33 ℃ for 3 days and plaques were counted. Data are expressed as mean ± SD. The significance of the difference between values was compared by using Student’s t-test; P < 0.001 was considered to be significant.

**EGFP fluorescence and indirect immunofluorescence.** MDCK cells (4 × 10⁴) that had been grown on tissue culture chamber slides (Nunc) were transduced with lentivirus (m.o.i., 10). After 3 days, cells were superinfected with A/WSN/33 (m.o.i., 0-2). At 12 h p.i., cells were washed with PBS⁺, fixed in 4% paraformaldehyde for
30 min at room temperature and then permeabilized in 0.5% Triton X-100 for 20 min at room temperature. Cells were washed with PBS and then incubated with goat anti-M1 antibodies (1:30) for 1 h at 37°C. Cells were washed three times for 10 min each in PBS and incubated with Texas red-conjugated anti-goat IgG (Santa Cruz Biotechnology) (1:400) for 35 min at room temperature. Cells were then washed and mounted. Slides were viewed under an Axioskop 2 fluorescence microscope (Zeiss).

RESULTS

To determine whether M1 gene expression could be inhibited by siRNA, we designed three 21 nt siRNA sequences to DNA from different regions of the M gene, according to the criteria used in earlier studies (Elbashir et al., 2002), and cloned them under the control of the U6 promoter into a pCR 2.1 vector. The resultant plasmids (pCR-M-89, pCR-M-331 and pCR-M-620) synthesize siRNA under the control of the U6 promoter and direct the synthesis of a Pol III-specific RNA transcript, starting with guanosine at position one (Fig. 1), as required for efficient transcription initiation from the U6 promoter (reviewed by Paule & White, 2000). Five thymidines on plasmid DNA that function as a termination signal for Pol III were added at the 3′ end (Bogenhagen et al., 1980). This RNA is predicted to fold back to form a hairpin dsRNA with a 3′ overhang of two uridines and a 9 nt loop and, when cleaved with endonuclease, will generate an siRNA of 21 bp with a guanosine at the 5′ end and two uridines overhanging at the 3′ end (Fig. 1).

To determine whether these M gene-specific siRNAs can suppress the expression of M1 protein, 293T cells were cotransfected with plasmids expressing the M1 protein and siRNA. 293T cells were used because of their high transfection efficiency. Transfected 293T cells were pulse-labelled at 13 hpt and cell lysates were immunoprecipitated with anti-M1 antibodies. The results (Fig. 2) show that M1 protein expression decreased by 52, 70 or 97% when cells were cotransfected with pCR-M-620 (lane 5), pCR-M-89 (lane 3) or pCR-M-331 (lane 4) plasmids, respectively. These results demonstrate that siRNAs from different regions of the M gene had a varying effect in suppressing M1 protein synthesis and that the siRNA targeting the 331–351 nt region of the virus M gene (pCR-M-331) was most effective in inhibiting translation of the M1 protein.

As MDCK cells, which are used commonly for influenza virus propagation, are not amenable to efficient cDNA transfection, we constructed lentivirus vectors to deliver M gene siRNA to these cells and to determine whether lentivirus expression vectors could be used to cause siRNA-mediated suppression of M1 expression. Transducing lentivirus vectors are typically defective for virus replication, as some of the trans-acting sequences that encode viral proteins have been deleted and used to transfer the exogenous gene into non-dividing target cells (reviewed by Pacchia et al., 2003). As the siRNA encoded by pCR-M-331 was most effective in inhibiting M1 expression (Fig. 2), we cloned the M-331 siRNA cassette into the HIV-1-based lentivirus vector RRL-U6shLuc-cppt-PGK-EGFP-SIN (An et al., 2003), which contained a U6 Pol III promoter. The resultant plasmid, LVV-M-331, was a lentivirus vector carrying the M gene siRNA cassette targeting the 331–349 nt region (Fig. 3a). Defective pseudotype lentiviruses carrying
To further confirm whether M gene siRNA-expressing cells suppressed M1 protein expression, MDCK cells were transduced with LVV-M-331 expressing M1 siRNA and superinfected with influenza virus. These superinfected cells were examined by double-staining immunofluorescence analysis (Fig. 4). EGFP fluorescence represents the expression of lentivirus vector and Texas red represents the signal and intracellular localization of influenza virus M1 protein. In the absence of lentivirus vector transduction, M1 protein was expressed in MDCK cells, as expected (Fig. 4b). However, in cells transduced with LVV-M-331 virus expressing EGFP, M1 protein expression was not observed whereas, in the same field, a cell not expressing EGFP was positive for M1 (Fig. 4c and d). Taken together, these results (Figs 3 and 4) show that LVV-M-331 transduction specifically inhibited the expression of M1 protein.

To analyse the kinetics of influenza virus release from superinfected MDCK cells expressing M1 siRNA, MDCK cells were transduced with lentiviruses with or without M1 siRNA expression and superinfected with influenza virus at an m.o.i. of 0.1. Culture supernatants were collected at 6, 8 and 10 h.p.i. and assayed for p.f.u. Results (Fig. 5) show that, when compared to cells transduced with the lentivirus lacking M gene siRNA, virus production in cells transduced with LVV-M-331 was reduced by approximately 80 % at 6, 8 and 10 h.p.i.; this reduction in virus titre was statistically significant.

As the M1 protein has been shown to be involved in budding (reviewed by Nayak & Hui, 2002), we wanted to determine the effect of LLV-M-331 on the influenza virus budding rate. Accordingly, MDCK cells were transduced with LVV-M-331 and superinfected with influenza virus at an m.o.i. of 0.1. At 10 h.p.i., these cells were washed extensively with medium and incubated for 1 h for virus release, as described previously (Hui & Nayak, 2001, 2002). The influenza virus particles released in the 1 h culture supernatants were assayed for p.f.u. Results (Fig. 6) show that, compared to LVV-transduced cells, virus budding rate was decreased by >40% in LVV-M-331-transduced MDCK cells. These results show that M1 siRNA expression, mediated by a lentivirus vector, suppressed influenza virus replication. Our results compare fairly well with those obtained

VSV G protein on their envelope were produced as described previously (An et al., 2003). This lentivirus carries an EGFP marker protein for quantification by FACS analysis. Vector lentiviruses containing M1 siRNA were titrated into 293T cells and were found to contain 10^8 IU ml^{-1}. We transduced MDCK cells with LVV-M-331 lentivirus (m.o.i., 10) and then incubated them for 3 days to allow lentivirus genome integration and expression in cultured cells. FACS analysis showed that approximately 80 % of MDCK cells had been transduced by the vector lentivirus. At 72 hpt, these transduced MDCK cells were superinfected with A/WSN/33 influenza virus (m.o.i., 0.1); they were pulse-labelled at 10 h.p.i. for 30 min. Cell lysates were prepared and subjected to immunoprecipitation using anti-M1 antibodies. LVV containing an unrelated siRNA (luciferase siRNA) was used as a control. RRL-U6shLuc-cPPT-PGK-EGFP-SIN has previously been shown to cause inhibition of luciferase (An et al., 2003). Results

(Fig. 3b) show that mock-transduced (Fig. 3b, lane 1), LVV vector (lacking the siRNA)-transduced (Fig. 3b, lane 2) and LVV carrying luciferase siRNA-transduced (Fig. 3b, lane 4) MDCK cells expressed M1 protein at an essentially similar level. However, in cells transduced with LVV-M-331 lentivirus, which expresses the M gene siRNA targeting nt 331–349, expression of M1 was reduced by 70 % (Fig. 3b, lane 3). Furthermore, lack of inhibition of NP expression (Fig. 3b) in these virus-infected MDCK cells demonstrated the specificity of M1 siRNA in causing the inhibition of M1 protein synthesis. These results indicate that M1 siRNA caused inhibition of M1 synthesis, whereas lentivirus vector alone or with luciferase siRNA did not inhibit M1 expression.

Fig. 2. Expression of the M1 protein in 293T cells transfected with U6-driven siRNA expression cassettes. pCR-M series plasmids (10 μg) and Pol–Pol II constructs of the M gene (1 μg) were cotransfected into 293T cells. (a) At 16 hpt, 293T cells were pulse-labelled with [35S]protein label for 2 h, lysed, immunoprecipitated with anti-M1 antibodies and analysed by SDS-PAGE (12 %). A non-specific host protein band is present at the 42 kDa position. (b) The amount of M1 protein was quantified and normalized to the non-specific, coimmunoprecipitated protein for loading difference. Values represent means of three independent experiments with <10 % variation.
previously by lentivirus-mediated delivery of siRNA in other systems (An et al., 2003; Qin et al., 2003).

**DISCUSSION**

siRNAs have been shown to interfere with the replication of a number of animal viruses, including Rous sarcoma virus (Bitko & Barik, 2001; Hu et al., 2002), HIV-1 (reviewed by Pomerantz, 2002; Akkina et al., 2003; Stephenson, 2003), flock house virus (Li et al., 2002), dengue virus type 2 (Adelman et al., 2002), poliovirus (Gitlin et al., 2002), hepatitis C virus (Kapadia et al., 2003; Sen et al., 2003), hepatitis B virus (Chen et al., 2003; Hamasaki et al., 2003), human papillomavirus (Hall & Alexander, 2003), West Nile virus (reviewed by Nussenzweig & Nussenzweig, 2002), and influenza virus (Kapadia et al., 2003; Sen et al., 2003).

**Fig. 3.** (a) Construction of the lentivirus vector with siRNA LVV-M-331 (LVV-M-331). Abbreviations: cppt, central polypurine tract; PGK promoter, internal human phosphoglycerate kinase RNA Pol II promoter; U6 promoter, human U6 Pol III promoter; LTR, long terminal repeat; PBS, primer binding repeat; RRE, Rev-responsive element; RRL, enhancer and promoter from U3 region of Rous sarcoma virus joined to the R region of the HIV-1 LTR; ψ, retrovirus packaging signal. (b) Expression of M1 protein in MDCK cells transduced with lentivirus vector carrying the siRNA expression cassette. Vector lentivirus particles containing VSV G protein with or without M-331 siRNA or with luciferase siRNA were produced by cotransfection in 293T cells, as indicated in Methods. MDCK cell monolayers (10% confluence) were transduced with lentivirus particles (m.o.i., 10) and, at 3 days post-transfection, were 80% positive for EGFP expression (as measured by flow cytometry). These transduced MDCK cells were superinfected with influenza virus (m.o.i., 0-1). At 10 h.p.i., superinfected cells were pulse-labelled for 30 min, lysed, immunoprecipitated with anti-M1 and anti-NP antibodies, analysed in an SDS gel (upper panel) and quantified by densitometry (lower panel). Values in the lower panel represent means of two independent experiments with <10% variation. The NP protein band is present at the 53 kDa position.

**Fig. 4.** Inhibition of M1 protein expression by expression of LVV-M1-331. MDCK cells were either mock-infected (panels a and b) or infected with LVV-M1-331 (m.o.i., 10) (panels c and d) and superinfected with A/WSN/33 influenza virus (m.o.i., 0-1). Cells were then fixed, permeabilized and stained with anti-M1 antibody. Green represents signal from EGFP in the lentivirus vector. Red (Texas red) represents staining with anti-M1 antibody. Arrows mark the same cells in a field. Images were taken by using a fluorescence microscope under a 630× oil objective lens. Note: some cells expressing EGFP were negative for M1 (open arrow) and a cell not expressing EGFP was positive for M1 (solid arrow).
virus (McCown et al., 2003), severe acute respiratory syndrome-associated coronavirus (Zhang et al., 2003) and cytomegalovirus (Wiebusch et al., 2004). As siRNA-mediated inhibition of virus replication is independent of antigenic variation, immune modulation and IFN-mediated interference, it has a potential application in the therapy and prophylaxis of virus diseases. For therapy, it is necessary to be able to efficiently deliver and express siRNAs in target cells and effectively inhibit an ongoing virus infection. In this report, we have shown that M1 siRNAs can be delivered by using lentivirus vectors and expressed under the control of a Pol III promoter in MDCK cells. We also show that M1 protein expression and influenza virus replication can be suppressed significantly by lentivirus-mediated delivery of M1 siRNA.

In two recent reports (Ge et al., 2003; McCown et al., 2003), the role of siRNA in influenza virus replication has been investigated. In both reports, siRNAs against the M gene targeted to M1 mRNA had only a partial effect against influenza virus replication. Ge et al. (2003) observed that siRNAs against M1 caused, at most, 50% reduction of M1 mRNA. However, NP and PA gene targets were most effective at virus inhibition, implying the role of segment-specific siRNA in virus interference. On the other hand, McCown et al. (2003) reported that, within the M gene, two siRNAs against M1 caused only partial inhibition, whereas one against M2 interfered most effectively against M2 protein expression and virus replication. Our results with M gene siRNAs differ significantly from these published reports. We found that one M gene-specific siRNA (M-331) was a potent inhibitor of both M1 protein synthesis and virus replication, whereas two other M gene siRNAs caused only partial inhibition of M1 protein expression. In addition to the different delivery systems and host cells used in these reports, our results point towards the importance of the specific sequence within the M gene that is targeted by the siRNA in gene silencing and virus interference. Poor inhibition of M1 protein expression by 5′ M1 siRNA and 3′ M1 siRNA (McCown et al., 2003) was probably due to targeting of siRNAs to the M1–M2 splice sites. Similarly, partial inhibition by M-39 (nt 39–57) and M-731 (nt 731–749) [reported here (Fig. 2)] could be due to the proximity of these sequences to the 5′ M1–M2 splice donor site (nt 51 and 52) and 3′ splice acceptor site (nt 739 and 740). Complex regulatory proteins that are involved in splicing may bind to these regions and, thereby, may interfere with the siRNA interaction with mRNA (Elbashir et al., 2002). Moreover, some sequences on the viral mRNA might be buried within secondary structures or highly folded regions of the target mRNAs and may not be accessible for interaction with siRNA (Elbashir et al., 2002). In addition, the level of production and stability of siRNAs may also affect their interfering ability.

We have also shown here that the siRNA can be delivered effectively by a lentivirus vector system and used to suppress
protein expression and virus replication. In fact, siRNA has been delivered and expressed successfully by retroviruses (Devroe & Silver, 2002; Yang et al., 2003), lentiviruses (Abbas-Terki et al., 2002; An et al., 2003; Matta et al., 2003; Qin et al., 2003; Stewart et al., 2003; Tiscornia et al., 2003) and adenoviruses (Xia et al., 2002; Shen et al., 2003) to suppress specific host protein synthesis and interfere with virus replication. The levels of inhibition of protein expression and virus replication observed in our report were similar to those reported in other studies using lentivirus vectors (An et al., 2003; Qin et al., 2003). Furthermore, we show that the suppression of M1 protein synthesis is specific for M1 siRNA, as NP protein expression was not inhibited by M1 siRNA and M1 protein expression was not suppressed by luciferase siRNA.

In conclusion, we have shown that sequence-specific M1 siRNA and lentivirus-mediated siRNA expression could be used to interfere with viral protein synthesis and to inhibit virus replication and, therefore, has a potential application in therapy against influenza. Results from this and other studies suggest that lentivirus vector expression of siRNA could be a useful tool in addressing the complex interaction of viral and cellular regulatory proteins that are involved in the virus life cycle. This system can also be used to determine the role of specific host proteins in virus budding. However, as the level of virus inhibition was only moderate (~80%), further optimization of conditions for choosing the targeting siRNA sequence and delivering the siRNA in virus-infected cells is needed.

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