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SV40 Reporter Viruses

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Abstract

Three simian virus 40 (SV40) reporter viruses were constructed in this study. One expresses the green fluorescent protein (GFP) as a fusion protein with the first exon of large-T (LT) antigen and is useful for live-cell imaging. A second reporter virus has a FLAG epitope tag at the C-terminus of large-T (LT) antigen (vC-LT^{FLAG}), and a third has the FLAG tag at the N-terminus of LT (vN-LT^{FLAG}). The vC-LT^{FLAG} construct grows to titers near those of wild-type (WT) virus and functions well as a reporter virus for SV40 infection. The vN-LT^{FLAG} construct, while viable, has a defect in the production and spread of infectious particles. All three viruses are useful in detecting superinfecting virus in cells in which nuclear LT is already present, such as persistently infected human mesothelial cells.

Keywords

GFP-tagged SV40; FLAG-tagged SV40; Superinfecting SV40

1. Introduction

Simian virus 40 (SV40) infection has been studied in host cells that are permissive, semi-permissive, and non-permissive for virus infection (Tooze, 1981). Permissive infection denotes a lytic infection of cells, such as African green monkey kidney cells, that results in expression of early and late genes of the virus, DNA replication, virus assembly, and cytopathic effect (CPE). Semi-permissive infection occurs in some human cell lines, e.g. fibroblasts. In these cells, fewer than 5% of the cells are permissive at any given time and these cells undergo viral DNA replication and late gene expression resulting in the death of the cell. Non-permissive infection occurs in rodent cells in which virus entry and early gene expression are unaffected, but viral DNA replication and subsequent late gene expression cannot occur. Most non-permissive infections are abortive but, at a low frequency, viral DNA may integrate into the host genome. This may result in transformation of the cells toward a more tumorigenic phenotype. Primary human mesothelial cells offer a unique system for studying SV40 virus infection as they become infected persistently with SV40 (Bocchetta et al., 2000). During persistent infection, contrary to other SV40 infections, the viral genome is maintained as an episome in the cells, and the cells become transformed by the early gene expression of large-T (LT) and small-t (ST) antigens. Persistently infected cultures contain far fewer genomes of SV40 per cell (50–1000 molecules per cell), 1–2 orders of magnitude lower than the number observed during permissive infection. ST expression is required to maintain this high copy

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number, and cells that lack ST contain only very low levels of episomal viral DNA (Fahrback et al., 2008). Persistently infected cultures produce low but detectable levels of infectious virus continually in the absence of cytopathic effect (CPE).

While studying maintenance of the SV40 genome by mesothelial cells, it was necessary to detect a superinfecting SV40 in cells that already expressed the SV40 early antigens in every cell. This made it impossible to use the standard assay for SV40, detection of nuclear LT by immunofluorescence. Reporter viruses were created that could be monitored following superinfection of LT-expressing cells and distinguished from the original transforming virus. The isolation of three such SV40 reporter viruses, one of which can be used for live-cell imaging are described.

2. Materials and Methods

Cells and viruses

Monkey kidney CV1 cells were used for routine virus growth. COS7 cells (Gluzman, 1981) were used to grow defective viruses that lacked LT antigen. These cells express LT and ST from integrated viral DNA that cannot replicate because of a defective origin of replication. Some experiments were done using 5ADL, a persistently infected human mesothelial cell culture (Fahrback et al., 2008; Yu et al., 2001) that contains episomal DNA of the virus DL-888 (Shenk et al., 1976). DL-888 expresses LT but not ST because of a small deletion across the ST splice donor sequence. All cell lines were grown in Dulbecco's modified Eagle's medium (DME) containing 5% (CV1, COS7) or 10% (5ADL) fetal bovine serum (FBS), 20mM L-glutamine and 100U/mL penicillin-streptomycin.

Virus stocks were produced by infecting CV1 cells at 0.01 plaque-forming unit per cell, a low multiplicity of infection (MOI), and allowing the infection to proceed until over 90% of the cells showed extensive vacuolation or detached from dishes (4+ CPE). The entire plate (cells and media) was frozen and thawed three times, and cellular debris was removed by centrifugation. To initiate experiments, virus infections were performed by incubating cells with small volumes of virus for 2 hours before replenishing the full volume of culture media. For example, 0.3mL of virus lysate was used for a 3.5 cm dish of cells. Similarly, to determine virus titers, serial dilutions were made of the stocks followed by infection of CV1 cells for 2 hours in a minimum volume of inoculum. After this initial period of infection, cells were overlaid with DME containing 5% FBS and 1% agar and incubated until plaques appeared, usually at 12–14 days.

Plasmids

SV40 early region sequences were manipulated in the plasmid pw2t (Chang et al., 1984; Turk et al., 1993) which consists of 3 Kb plasmid backbone derived from pBR322 and a 2 Kb portion of SV40. This contains the origin/enhancer region of the viral genome (SV40 nucleotide (nt) 346 counterclockwise to the initiating ATG at 5163; the center of the replication origin maps to nt 0/5243) and most of the early coding sequences (5163 to 2533). A HpaI deletion between nt 3733 and 2666 eliminates LT expression from this construct (Phillips and Rundell, 1988). A related plasmid, pw2tBg has a HindIII site at nt 5171 converted to a unique BglII site which is useful for cloning. Viruses containing this alteration are fully viable. The plasmid pmK/SV40 contains full-length SV40 cloned via the unique EcoRI site into the 3.8 Kb plasmid, pMK (Mungre et al., 1994). pMK encodes resistance to kanamycin. To obtain virus stocks, viral genomes were excised from pMK/SV40 plasmids by EcoRI cleavage then recircularized using T4 DNA ligase (New England Biolabs). CV1 or COS7 African green monkey kidney cells were then transfected with religated genomes using polyethylenimine reagent

(Polysciences, Inc.) (Durocher et al., 2002). When 4+ CPE was apparent, virus lysates were prepared as described above.

SV40vGFP cloning

Enhanced GFP was PCR amplified from pEGFP-N1 using the following forward primer that added PflMI restriction enzyme site immediately upstream of the GFP coding sequence (indicated in *italics*): 5'-ATTCCAACCTATGGAAGTGTGAGCAAGGGC. The reverse primer (5'-CCGACTGCGTTAACGATCTAGAGTCGCGCCG) added a HpaI site (indicated in *italics*) 20 nucleotides after the GFP polyadenylation signal. Amplified GFP sequences were gel purified, digested with PflMI and HpaI and cloned into PflMI- and HpaI-digested plasmid pw2t. These restriction sites are located at SV40 nt 4558 (PflMI) and 2666 (HpaI) in pw2t. The PflMI site immediately follows the LT/ST shared splice acceptor site, allowing for efficient splicing of GFP-containing mRNA. After the initial cloning into pw2t, the recombinant early region was used to replace the early region in pMK/SV40, the source for excision and relegation of full-length genomes, using unique SfiI (SV40 nt 5234) and BamHI (SV40 nt 2533) sites. The vGFP recombinant encodes a fusion protein consisting of the first exon (82 amino acids) of LT, then four amino acids of the second exon of LT in frame with the GFP protein. SV40 ST is also encoded by this construct. Although there do not appear to be major size constraints to DNA packaging into SV40 particles in vitro (Kimchi-Sarfaty et al., 2003), an effort was made to keep the GFP recombinant close to the size of WT SV40. The genome of SV40vGFP is only 90 nucleotides smaller than the WT viral DNA.

Flag construct cloning

To add the N-terminal FLAG tag, we used a construct provided by Dr. Andrew Koff that has FLAG-LT expressed from the rat probasin promoter in a Bluescript vector (Shaffer et al., 2005). This construct has a plasmid-derived HindIII site immediately upstream of the LT coding region (AAGCTTAGGATCATG, where the ATG is the initiating codon). N-terminal sequences of FLAG-LT were cut out using HindIII and BstXI (SV40 nt 4759) then inserted into HindIII (SV40 nt 5171) and BstXI sites of pw2t (Chang et al., 1984; Porras et al., 1996), a plasmid that contains part of the SV40 early region under its own promoter/enhancer region. The resulting plasmid, pw2t-FLAG, was fully competent for early transcription although it differed slightly from the WT sequence (AAGCTTTGCAAAGATG). To create a construct with the authentic WT sequence, two-stage PCR mutagenesis was used to produce pw2t-FLAG (5'WT). Next, pw2t-FLAG and pw2t-FLAG (5'WT) FLAG-tagged constructs were cloned into pMK/SV40 using unique SfiI (SV40 nt 5234) and BstXI sites.

The QuikChange® Site-Directed Mutagenesis Kit (Stratagene) was used to add the C-terminal FLAG tag to LT by PCR amplification of the plasmid pw2t. The complementary primers that were used placed the FLAG epitope in frame with LT, immediately its stop codon, and mutated an MfeI site at nucleotide 2677 of SV40. The mutated MfeI site was used for screening after cloning (forward primer: 5'-CCTCCCCCTGAACCTGAAACAGATTATAAGGATGATGATAAGTAAATGAA TGgAATTGTTGTTG; where the FLAG tag is in *italics*, the stop codon is underlined, and the mutated nucleotide in the MfeI site is in small caps). Finally, the C-terminally tagged LT was cloned into pMK/SV40.

Western blotting

Cells from confluent dishes were washed once with cold phosphate-buffered saline (PBS), scraped and collected in 1mL cold PBS. Cells were collected by centrifugation at 5000 rpm at 4°C. Cell pellets were lysed in 0.5% nonidet P-40 (NP40) lysis buffer containing 10 µg/mL aprotinin, 10 ng/µL leupeptin, and 10 µg/mL phenylmethylsulfonyl fluoride (PMSF), and supernates were cleared by centrifugation at 14,000 rpm at 4°C. Sample buffer containing 5%

beta-mercaptoethanol was added to the protein extracts and the extracts were boiled for 5 minutes. Samples were electrophoresed on a 12% sodium dodecylsulfate (SDS) polyacrylamide gel (Laemmli, 1970), and protein was transferred to polyvinylidene fluoride (PVDF) membrane (Immobilon). Membranes were incubated with rabbit polyclonal anti-VP1 antibody (kindly provided by Dr. Harumi Kasamatsu, University of California at Los Angeles) diluted 1:40,000. Goat-anti-rabbit secondary antibodies (Cell Signaling) conjugated to horse radish peroxidase (HRP) were used at a 1:1000 dilution. Membranes were incubated with chemiluminescence reagents (Pierce), and exposed to Hyperfilm (Amersham Biosciences).

Sucrose gradient ultracentrifugation

The presence of mature viral particles was detected by sedimentation velocity ultracentrifugation as previously reported (Li et al., 2000). Briefly, nuclei were isolated from infected cells, sonicated and overlaid onto 5–32% sucrose gradients (50 mM N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES) buffer, pH 7.5) in a SW41 tube. The gradient was centrifuged at 37,000 rpm at 4 °C for 80 minutes. Fractions were isolated by timed collection from the top of the tube as 60% sucrose was pumped into the bottom of the tube. The time was set so that 10 total fractions, about 1.0 mL each, were obtained. Equivalent aliquots of each fraction were used for SDS gel electrophoresis and Western blotting using the polyclonal anti-VP1 antibody.

Fluorescence microscopy

GFP was imaged directly in live cells grown in petri dishes using a fluorescence microscopy and digital imaging. For immunofluorescence experiments, cells were grown on coverslips to confluence and infected with the indicated virus. Unless otherwise indicated, cells were fixed after 36 hours with ice-cold methanol:acetone (1:1) for 20'. Coverslips were incubated for 20 minutes with PBS + 20% normal goat serum followed by incubation with the indicated primary antibody for 2 hours. After thorough washing with PBS, coverslips were incubated with the appropriate secondary antibody for 1 hour, washed again and mounted on glass slides with 90% glycerol, 10% PBS, 1% 1,4-diazabicyclo[2.2.2]octane (Dabco) and 4',6-diamidino-2-phenylindole (DAPI). pAB419, a mouse monoclonal that recognizes T antigen, was used at 1:50 (Crawford et al., 1982; Harlow et al., 1981). The FLAG epitope was detected using rabbit polyclonal anti-DYKDDDDK epitope tag antibody at 1:100 (Affinity BioReagents). Secondary antibodies, goat-anti-mouse-fluorescein isothiocyanate (FITC) and goat-anti-rabbit rhodamine isothiocyanate (RITC), were used at 1:1000.

Fluorescent assays for virus

For viruses that could not be quantified by plaque assay because they were nonviable or too slow-growing, titers were determined by quantifying LT or FLAG by immunofluorescence. In this case, serial dilutions of reporter virus or WT controls were used to infect CV1 cells as just described. Titers were expressed as immunofluorescent units (IFU)/ml. To assess the spread of reporter viruses from cell-to-cell, an immunofluorescent plaque assay was performed. CV1 cells were grown to confluence on coverslips in 3.5 cm petri dishes and infected for 2 hours with the virus preparations. The cells were infected at very low MOI, generally 50–500 IFU/3.5 cm dish (approximately 10^6 CV1 cells). After infection, the CV1 cells were overlaid with medium containing 1% agar. On days 6, 7, and 8 following infection, the agar was removed from the petri dishes and the cells were fixed with 1:1 methanol and acetone. LT was detected using pAB419 to visualize “plaques,” clusters of cells where infection had spread.

3. Results

3.1. GFP-expressing SV40

To obtain recombinant SV40 for detection in live cells, the gene for green fluorescence protein (GFP) was inserted into the early region of SV40. The goal was to produce virus that could be detected in cells by fluorescence microscopy or flow cytometry without requiring fixing and staining. An attempt was made initially to replace completely the entire SV40 early coding region so that the GFP open reading frame was immediately after the 5' non-coding region of SV40. However, it was found that actively replicating virus could not be obtained from this construct, possibly because of the need for intronic sequences. To improve GFP expression, we inserted GFP after the splice donor and acceptor sequences of LT. This created a fusion protein consisting of the first exon of LT (82 amino acids) and the first four amino acids of the second exon fused to GFP (Figure 1A). The fusion protein contains the dnaJ domain of LT/ST (Srinivasan et al., 1997) but lacks other critical LT regions, such as the pRb and p53 binding regions or activities required for viral DNA replication (Fanning, 1992; Pipas, 1992). ST would also be expressed from this virus. This recombinant is referred to as SV40vGFP (see Materials and Methods for cloning details).

To produce virus, excised and religated genomes were used to transfect either COS7 cells, which express LT antigen, or CV1 cells, in which case DL888, a ST-defective helper virus (Shenk et al., 1976), was co-transfected along with the SV40vGFP. GFP was observed in these cells after transfection (Figure 1B). SV40vGFP transfected under these conditions was able to replicate, spread from cell to cell, and produce CPE by day 6 post-transfection. Virus lysates were produced by freeze-thawing, cleared of cell debris and used to infect either CV1 or COS7 cells. CV1 and COS7 cells infected with GFPvSV40 expressed GFP as visualized by fluorescent microscopy (Figure 1C and data not shown). The efficiency of infection was also quantified by flow cytometry. In the experiment shown in Figure 1D, 0.5 mL of the lysate grown with the helper virus was used to infect approximately 10^6 CV1 cells in a 3.5 cm dish. Approximately 8% of these cells were expressing GFP 24 hours after infection (Figure 1D). This corresponds to a titer of about 1.6×10^5 infectious viruses per ml. Although this titer is relatively low, it is clear that the GFPvSV40 provides a virus that can be used in live-cell imaging.

3.2. N-terminally FLAG-tagged construct

In an effort to construct a viable epitope-tagged reporter virus, the FLAG epitope (DYKDDDDK) was introduced into the early protein, LT. A FLAG-tag consists of only eight amino acids and was expected to disrupt only minimally the viral genome. Constructs were designed to introduce the FLAG tag into LT antigen without deletion of any LT sequences because LT is required for SV40 replication. In the first construct made, we added a FLAG-tag to the amino terminus of LT (vN-LT^{FLAG}; see Materials and Methods for cloning details). This inserts the N-terminal FLAG tag into ST as well, but it has been shown previously that this makes ST completely unstable (Shaffer et al., 2005). Therefore, studies of vN-LT^{FLAG} were always done in comparison to DL-888 [Shenk, 1976 #196], a ST-negative SV40 that lacks the ST splice acceptor but makes normal levels of LT.

It was apparent, following transfection of recircularized vN-LT^{FLAG} into CV1 cells, that the virus had greatly reduced viability. For example, transfection of DNA from the ST mutant virus, DL-888, led to CPE in CV1 cells in fewer than 10 days. Significant CPE was not detected until nearly 20 days when vN-LT^{FLAG} was used. Similarly, when lysates were then analyzed by plaque assay, the DL-888 virus showed plaques by day 14 while vN-LT^{FLAG} plaques were minute and barely detectable even at day 21. Because it was difficult to visualize these plaques,

titers were estimated following immunofluorescent staining for T antigen. The vN-LT^{FLAG} virus grew to titers between 10⁵ and 10⁶ IFU/ml.

To elucidate where the defect is in vN-LT^{FLAG}, several experiments were performed. No defects were observed in the rate of appearance of LT or in viral DNA synthesis in the first few days post-infection (data not shown). Also, as shown in Fig. 2A, there is late gene expression by this recombinant virus, indicated by expression of the capsid protein VP1. However, late gene expression appeared to be delayed by approximately one day post-transfection of CV1 cells with viral genomes relative to DL-888 virus. Similar delays in the appearance of VP1 were observed after infection as well as transfection.

These data indicate that vN-LT^{FLAG} SV40 may have a defect in late events in virus assembly and packaging causing it to be slow in cell-cell spread (plaque formation) and overall virus production (titer). It was possible that even with successful genome replication and capsid protein expression that the insertion of the FLAG sequence at this locus disrupted a packaging signal for virus assembly and maturation. Sucrose gradient ultracentrifugation of infected cell lysates followed by Western blotting for VP1, however, indicated that particles with the density of virions were present, albeit at lower levels than in the control infections (Figure 2B).

Another possible explanation for the delay in CPE following vN-LT^{FLAG} genome transfection or virus infection was a defect in the cell-to-cell spread of the virus. A fluorescent plaque assay was performed to look at individual plaque sizes representing the event of initial infection by the mutant virus of one cell at early time points. Confluent monolayers of CV1 cells plated on coverslips were infected with 50–500 IFU per culture so that individual events could be observed. Following infection by either vN-LT^{FLAG} or DL-888, the cells were overlaid with medium containing agar so that only cells adjacent to the initial site of infection would become infected as that initial cell produced virus. The agar was removed from the cells on days 6, 7, and 8 following infection and the cells were fixed onto the coverslips. The fixed cells were stained with PAb419 for T antigen expression as a marker for the presence of SV40. Even at these early times, vN-LT^{FLAG} showed smaller plaques as indicated by fewer T antigen positive cells relative to the control DL-888 virus (Figure 2C). Therefore, vN-LT^{FLAG} appears to produce lower amounts of VP1 and assembled virus, but is even more delayed in its ability to spread and infect adjacent cells. The slow rate of plaque development and the inability to maintain a cell monolayer contribute to the great difficulty in monitoring vN-LT^{FLAG} by plaque assay.

3.3. C-terminally-FLAG-tagged construct

Mutations at the C-terminus of LT antigen have little effect on the viability of SV40 in CV1 cells (Pipas, 1985; Poulin and DeCaprio, 2006). Therefore, it seemed likely that the introduction of FLAG sequences would have relatively little deleterious effect on virus replication and assembly in these cells. Accordingly, the FLAG epitope was introduced at the C-terminus of LT antigen, without deleting any LT C-terminal sequences, as described in Materials and Methods. The growth of the vC-LT^{FLAG} virus was more like that of the WT control, and CPE was noted at about the same time post-transfection. A second round infection with virus produced from transfected cultures showed similar kinetics. Plaque formation by a vC-LT^{FLAG} was slightly slower than that of the WT virus, with plaque sizes being approximately half that of the WT at various times post-infection.

Because the vC-LT^{FLAG} SV40 grew nearly as well as WT SV40, we employed this virus in the remaining experiments. First it was confirmed that the FLAG epitope could be detected in cells infected with vC-LT^{FLAG} SV40. CV1 cells plated on coverslips were infected with serial dilutions of vC-LT^{FLAG} for 15 hours, fixed and stained with antibody recognizing the FLAG tag (Affinity BioReagents). Figure 3 shows that infected cells could be detected by

immunofluorescence and that mock-infected cells showed only background levels of fluorescence.

The tagged viruses made in this study were of particular use for monitoring superinfection of persistently infected cells lines in which all cells already express LT. To show that superinfecting virus can be differentiated from virus already present in SV40 transformed mesothelial cells, serial dilutions of vC-LT^{FLAG} SV40 were used to infect 5ADL cells, an immortalized human mesothelial cell line that expresses LT in every cell because of persistent infection with DL-888 [Fahrbach, 2008 #1006]. At 36 hours post-infection, cells were assayed for the expression of the FLAG tag (vC-LT^{FLAG}-infected), as well as for the resident DL-888 virus using PAB419 to detect LT (Crawford et al., 1982; Harlow et al., 1981). In the example shown in Figure 4, all of the 5ADL nuclei were positively stained with PAB419 (Figure 4D), and a fraction of the nuclei stained with FLAG antibody (compare Figure 4A, total nuclei, with 4B). These data indicate that the superinfecting virus can be distinguished from a resident virus in persistently infected human mesothelial cells.

4. Discussion

The data present three new recombinant forms of SV40 that can be used as reporter viruses when using an antibody against SV40 antigens is otherwise inappropriate. SV40vGFP is of particular interest because it can be used for live-cell imaging. The cloning of GFP after the splicing signals and the shared 82-residue N-terminal sequences of LT/ST proved to be important in this construction, because we were unable to generate much virus when the first exon was not included. While we have not yet grown SV40vGFP virus to very high titers, mechanical methods of concentrating virus, e.g. ultracentrifugation, could be used as needed. It may also be possible to increase yields of SV40vGFP using different complementing viruses or cell lines. In these approaches, stocks would need to be monitored carefully for WT recombinants emerging in the populations. While these would not be detected in live-cell imaging, such recombinants might reduce the yield of the GFP virus, so monitoring their presence would be informative. After two rounds of passaging SV40vGFP in COS7 cells, we detected only minimal WT virus by plaque assay in CV1 cells (less than 50 total pfu in a lysate).

SV40 vGFP expresses ST from an alternatively spliced mRNA, just as occurs in the WT virus infection. An interesting use of this virus would be to study effects of ST expression on GFP + cells identified or sorted by flow cytometry. This would be possible for virus grown in COS7 cells in which helper virus is not required.

The vC-LT^{FLAG} SV40 grows quite well and should be useful for monitoring virus infection of cells that already express LT and cannot be monitored by PAb419 antibody staining. In our studies, this virus has been useful for detecting superinfection of persistently infected mesothelial cells, a strategy that was used most recently in evaluating the role of the ST antigen in maintaining high copy numbers of the SV40 genome (Fahrbach et al., 2008). The construction of reporter viruses was undertaken initially because different clones and sublines of persistently infected human mesothelial cells appeared to respond differently to a superinfecting virus. Reporter viruses were useful in determining the infectibility of such sublines. Reporter viruses might be similarly useful in cells that express LT from stably integrated expression plasmids. For example, LT has been used to immortalize a variety of cell lines, and SV40 infection of these could not be probed by monitoring LT expression.

The C-terminus of LT antigen shows a great deal of strain variability (Forsman et al., 2004) and deletions in this region affect viral host range (Pipas, 1985; Poulin and DeCaprio, 2006). Deletions at the C-terminal affect viral capsid synthesis (Tornow et al., 1985) and production of infectious virus progeny (Spence and Pipas, 1994) in some African green monkey cells.

Although it is possible that vC-LT^{FLAG} will not grow well in some cell lines, it should be noted that the FLAG epitope was added as a C-terminal extension and that no C-terminal sequences of LT were deleted. Thus, the virus is likely to have the same host range as the non-tagged parent and, if necessary, similar constructions could be done using viruses with other C-terminal sequences.

Finally, a potential new regulatory region for SV40 virus maturation was identified by this investigation of vN-LT^{FLAG} SV40. While the exact defect elicited by the FLAG insertion in this region was not identified, there are fewer viruses packaged during a productive infection, and the cell-cell spread of this virus is delayed. This delay seems greater than might be expected by the slightly slower rate of VP1 expression by this virus. It will be interesting in future studies to determine whether N-terminal sequences of LT promote the assembly and release of SV40 virions, even though LT itself is not found in virus particles.

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Figure 1.

SV40vGFP recombinant virus. (A) Map showing the site where the GFP sequences were substituted in the early region of the SV40 circular genome immediately after the LT/ST splice acceptor site. (B) Live-cell imaging of GFP expression in COS7 cells 18 hours post-transfection with religated SV40vGFP genomes. (C) Live-cell imaging of GFP expression 15 hours post-infection with SV40vGFP virus stocks obtained from previously transfected COS7 cells. (D) Flow cytometry data representing 15,000 mock or virus infected CV1 cells with GFP expression indicated by the x-axis. Forward scatter (FS) is shown on the y-axis.

Figure 2. vN-LT^{FLAG} SV40 produces virions, but with delayed late gene expression and cell-cell spreading. (A) Western blotting for SV40 capsid protein VP1 of CV1 cells transfected with either DL-888 or vN-LT^{FLAG} SV40 on days 1–4 post-transfection. (B) Western blotting for VP1 in fractions obtained by sucrose gradient ultracentrifugation of nuclear extracts from cells transfected with either DL-888 or vN-LT^{FLAG} SV40. (C) Fluorescent plaque assay showing the spread of DL-888 or vN-LT^{FLAG} virus in a monolayer of cells on days 6–8 post-infection. PAB419 was used to detect LT expression.

Figure 3.

Immunofluorescent staining of FLAG epitope in vC-LT^{FLAG} SV40. Cells plated on coverslips were either mock infected (A) or infected with vC-LT^{FLAG} (B) and then stained for the presence of the FLAG epitope (pink) using a FLAG specific polyclonal antibody followed by an anti-rabbit-IgG-RITC antibody. Nuclei were counter-stained with DAPI (blue).

5ADL x vC-LT^{FLAG}

Figure 4. vC-LT^{FLAG} allows for detection of a superinfecting SV40 virus in persistently infected mesothelial cells. The mesothelial cell line 5ADL was superinfected with vC-LT^{FLAG} SV40 and the cells were probed for FLAG-tagged LT (B). The total LT was detected by immunofluorescence using PAb419 (D). Panels A and C show the DAPI-stained nuclei in panels B and D, respectively.