Properties of non-structural protein 1 of Semliki Forest virus and its interference with virus replication

Citation for published version:

Digital Object Identifier (DOI):
10.1099/vir.0.2008/000299-0

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published in:
Journal of General Virology

Publisher Rights Statement:
Free via Open Access.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Properties of non-structural protein 1 of Semliki Forest virus and its interference with virus replication

Kaja Kiiver,1,2† Ingrid Tagen,1,2† Eva Žusinaite,1 Nele Tamberg,1 John K. Fazakerley3 and Andres Merits1,2

1Institute of Technology, University of Tartu, Tartu, Estonia
2Estonian Biocentre, Tartu, Estonia
3Centre for Infectious Diseases, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh, UK

Correspondence
Andres Merits
andres.merits@ut.ee

Received 11 January 2008
Accepted 20 February 2008

INTRODUCTION

Semliki Forest virus (SFV) is a member of the genus Alphavirus (family Togaviridae). Along with Sindbis virus (SV), the prototype member of the genus, SFV is one of the most studied alphaviruses. The genome of SFV is a positive-strand RNA of approximately 11.5 kb with a 5′ cap and a 3′ terminal poly(A) tract (Kääriäinen et al., 1987). The 5′ two-thirds of the genome encodes the non-structural (ns) polyprotein P1234. In infected cells, P1234 is autocatalytically cleaved into four individual non-structural proteins designated nsP1, nsP2, nsP3 and nsP4 (Kääriäinen et al., 1987). The SFV-encoded protease, nsP2, is responsible for proteolytic processing of P1234 (Merits et al., 2001; Lulla et al., 2006a). The first cleavage takes place at the nsP3/4 cleavage site and releases mature nsP4, which together with the remaining P123 polyprotein forms the early minus-strand RNA replicase. In contrast, the late replicase complex consists of fully processed non-structural proteins and synthesizes positive-sense genomic and subgenomic (sg) RNA molecules (Lemm et al., 1994; Shirako & Strauss, 1994; Vasiljeva et al., 2003; Kim et al., 2004).

nsP1 participates directly in initiation and elongation of the minus-strand RNA synthesis of alphaviruses (Sawicki et al., 1981; Hahn et al., 1989; Wang et al., 1991). During minus-strand RNA synthesis, nsP1 interacts with nsP4 (Shirako et al., 2000; Fata et al., 2002). The properties of nsP1 of SFV and SV have been studied previously using infected cells, transient and stable expression systems, and purified recombinant proteins. The N-terminal region of nsP1 is a methyltransferase and guanylyltransferase involved in capping the viral positive-strand RNAs (Mi et al., 1989; Mi & Stollar, 1991; Laakkonen et al., 1994; Ahola & Kääriäinen, 1995; Wang et al., 1996; Ahola et al., 1997). nsP1 is also associated with cellular membranes (Peränä et al., 1995) and serves as the sole membrane anchor for the virus replicase complex (Salonen et al., 2003). Two different events are involved in binding of nsP1 to cellular membranes. Firstly, the amphipathic peptide segment, located in the central part of nsP1, binds to cellular membranes (Ahola et al., 1999; Lampio et al.,...
This binding is required for activation of the enzymic activities of nsP1 and for infectivity of the viral genome (Spuul et al., 2007). Secondly, post-translational palmitoylation at cysteine residues 418–420 is responsible for strong membrane association of the protein (Laakkonen et al., 1996). Palmitoylation as such is not required for the enzymic activities of nsP1 or for infectivity of the viral genome (Ahola et al., 2000; Žusinaite et al., 2007). However, the deletion or substitution of cysteines 418–420 in nsP1 of SFV results in a severe defect in virus replication and causes accumulation of compensatory mutations. This effect may result from the incorrect subcellular localization of mutant nsP1 and/or from its inability to interact with nsP4 (Žusinaite et al., 2007). Li et al. (1997) demonstrated that stable transfectants of Hela cells, expressing nsP1 from low-methionine (LM)-adapted SV, SV(LM21), were able to support replication of standard SV in cells maintained in LM medium. The expression of full-length nsP1 had only a minor effect on replication of SV(LM21), but truncated forms of nsP1 acted as dominant-negative mutants.

Within the first few hours, alphavirus infection results in exclusion of superinfection by homologous virus (Adams & Brown, 1985) and, in the case of vertebrate cells, in shutdown of host-cell gene expression and induction of apoptotic death. It has been shown or proposed that free nsP2 is required for these effects (Karpi et al., 1997; Garmashova et al., 2006, 2007; Sawicki et al., 2006); however, it has not been demonstrated whether expression of other non-structural proteins causes similar effects. nsP1 expression in infected or transfected cells results in prominent changes, such as induction of filopodia-like structures and rearrangements of actin filaments (Laakkonen et al., 1998). These nsP1-induced effects make it another candidate for superinfection exclusion and/or virus-induced cytotoxic effects.

We studied the properties of nsP1 and its effect on host cells and on SFV infection by combining two powerful approaches, tetracycline-inducible stable cell lines and recombinant SFV genomes carrying marker genes in the replicase or the structural open reading frame (ORF). nsP1 expressed alone in the absence of the other virus replicase proteins was a stable protein with a half-life of about 5 h; stability was not affected by virus infection. Expression of nsP1 induced prominent changes in cell membranes and cell morphology, but had no significant effect on cell viability, virus entry or establishment of virus replication. However, nsP1 expression suppressed genomic RNA synthesis leading to reduced virus yield. The palmitoylation-negative form of nsP1 acted as a dominant-negative mutant and suppressed both genomic and sgRNA synthesis.

METHODS

Plasmids and construction of inducible cell lines. pcDNA4/TO (Invitrogen) plasmids encoding SFV nsP1 and nsP1<sup>421</sup> (harbouring the mutation 419<sup>C</sup>CC<sup>G</sup>420—AAA in the palmitoylation site) were kindly provided by Professor Leevi Kääriäinen (Institute of Biotechnology, University of Helsinki, Finland). Each plasmid (10 µg) was linearized by PvuI and electroporated into HEK293 T-REx cells, and zeocin (30 µg ml<sup>−1</sup>) was added to select transfectants. The expression of recombinant protein was induced by adding tetracycline (1 mg ml<sup>−1</sup>) and verified by Western blotting at 12 h post-induction; the cell lines expressing inducible nsP1 and nsP1<sup>421</sup> were designated T-REx-nsP1 and T-REx-nsP1<sup>421</sup>, respectively, and were grown at 37 °C with 5% CO<sub>2</sub> in Iscove’s modified Dulbecco’s medium (IMDM; Gibco) supplemented with 10% fetal calf serum, bacterialidin (5 µg ml<sup>−1</sup>) and zeocin (30 µg ml<sup>−1</sup>).

**SFV, recombinant viruses and cell infection.** BHK-21 cells, used for multiplication and plaque titration of SFV stocks, were grown as described previously (Žusinaite et al., 2007). SFV4(3H)-Rluc and SFV4-StRluc containing insertion of the Renilla luciferase (Rluc) reporter were constructed as described by Tambeg et al. (2007) and Thomas et al. (2003), respectively. SFV4, SFV4(3H)-Rluc and SFV4-StRluc were generated from corresponding infectious cDNAs, as described previously (Liljestrom & Garoff, 1991), grown and plaque titrated in BHK-21 cells, and used for infection of HEK293 T-REx cells or constructed cell lines.

**Analysis of transcription and translation in stable cell lines.** T-REx-nsP1 cells were grown on 60 mm dishes to 75% confluency and pulse labelled with 50 µCi (1.85 MBq) [<sup>35</sup>S]methionine and [<sup>35</sup>S]cysteine for 30 min. Pulse–chase experiments were carried out as described by Lulla et al. (2006b). The lysates of <sup>35</sup>S- or <sup>38</sup>C- or <sup>32</sup>P-labelled cells were precipitated with 1 ml 10% trichloroacetic acid and the incorporated radioactivity was measured using a 1414 Liquid Scintillation Counter (Wallac).

**Analysis of transcription and translation in SFV4-infected cells.** HEK293 T-REx cells (1 x 10<sup>5</sup>) were infected with SFV4 at an m.o.i. of 20 for 24 h and labelled with either 10 µCi [<sup>35</sup>S]methionine or 50 µCi [<sup>38</sup>C]methionine and [<sup>35</sup>S]cysteine as described above. For the transcription analysis, total RNA from equal amounts of infected and mock-infected cells was isolated using Trizol reagent (Invitrogen) and dissolved in 30 µl DEPC-treated water. Total RNA (10 µl) was subjected to denaturing formaldehyde agarose gel electrophoresis (Sambrook & Russell, 2001). The RNA bands corresponding to cellular pre-mRNAs and mRNAs (the gel area above the SFV4 42S RNA band) were excised from the gel, placed into 3 ml scintillation liquid and the radioactivity quantified by liquid scintillation counting. The translation analysis was carried out as described by Tamm et al. (2008). Inhibition of cellular translation was assessed by measuring the radioactivity incorporated into the protein band corresponding to actin, as described previously (Gorchakov et al., 2004). Immunoprecipitation of [<sup>35</sup>S]methionine- and [<sup>32</sup>P]cysteine-labelled samples was carried out as described previously (Tamm et al., 2008).

**Immunofluorescence and confocal microscopy.** Cells at 75% confluency were induced with tetracycline or infected with SFV4 at an m.o.i. of 20 for 6 h. Uninduced or mock-infected cells were used as a control. Cells were then fixed, permeabilized and treated with specific anti-nsP1 antibodies (Laakkonen et al., 1994) and rhodamine-conjugated concanavalin A (ConA; Sigma) for plasma membrane staining as described previously (Spuul et al., 2007) and analysed with a Bio-Rad MRC-1024 confocal microscope.
**Immunoblotting.** Lysates prepared from equal amounts of induced or uninduced cells were separated by SDS-PAGE (~10^5 cells per cell line). Proteins were transferred to a membrane and detected by antibodies against nsP1 using enhanced chemiluminescence.

**WST-1 assay for cell viability.** T-REx-nsP1 and T-REx-nsP1<sup>16D</sup> cells were seeded in 96-well plates (9 × 10^3 cells per well) and allowed to grow for 18 h. Recombinant protein expression was induced for 24 h; control cells remained uninduced. For comparison, the parental HEK293 T-REx cell line was infected with SFV4 at an m.o.i. of 20 or was mock infected, and cells were incubated for 24 h. Subsequently, 20 μl WST-1 (Roche) was added to each well and the plate was incubated for a further 1 h under the cell culture growing conditions. A<sub>450</sub> was read in a microplate reader. This experiment was repeated at least three times for each cell line.

**Analysis of Rluc activity in infected cells.** HEK293 T-REx, T-REx-nsP1 and T-REx-nsP1<sup>16D</sup> cells grown on 35 mm dishes were induced with tetracycline or mock induced, and infected with SFV4(H3)-Rluc or SFV4-StRluc at an m.o.i. of 0.6. EnduRen Live Cell Substrate (Promega) was added to the medium immediately after infection and the Rluc activity was measured at selected time points using a Glomax 20/20 luminometer (Promega).

**Northern blot analysis.** HEK293 T-REx, T-REx-nsP1 and T-REx-nsP1<sup>16D</sup> cells grown on 35 mm dishes were induced with tetracycline or mock induced, and infected with SFV4(H3)-Rluc at an m.o.i. of 0.6. At 12 h post-infection (p.i.), total RNA from infected cells was purified using Trizol reagent (Invitrogen). Each RNA sample (10 μg) was used for Northern blotting as described previously (Tamm et al., 2008) using an RNA probe complementary to the positive-strand of SFV genomic (nt 10951–11445) and sgRNA.

**Statistical methods and software.** The data from radioactive gels were quantified using Image Quant (Molecular Dynamics) software. Student’s t-test was used for calculation of statistical significance.

**RESULTS**

**Evaluation of the half-life of nsP1 and its subcellular localization**

Stably transfected cell lines inducible for the expression of wild-type (wt) nsP1 or its palmitoylation-negative form (nsP1<sup>16D</sup>) were constructed and analysed. Tetracycline-induced expression of nsP1 and nsP1<sup>16D</sup> was confirmed by immunoblotting (Fig. 1a). The level of wt nsP1 expression reached a plateau at 12 h post-induction (data not shown) and was similar to that of SFV4-infected HEK293 T-REx cells at 6 h p.i. The expression of nsP1<sup>16D</sup> was somewhat lower than that of wt nsP1. Thus, in contrast to previously reported Hela cell lines expressing SV nsP1 (Li et al., 1997), induced T-REx-nsP1 cells did not overexpress SFV nsP1. In our stably transfected inducible T-REx cells, the half-life of nsP1 and nsP1<sup>16D</sup>, as measured by metabolic labelling, was 5 h (Fig. 1b), indicating that nsP1 was highly stable and that this was not changed by mutation of the palmitoylation site. For comparison, the half-life of nsP1 in SFV4-infected HEK293 T-REx cells was determined and found to be approximately 4 h (Fig. 1b).

In induced T-REx-nsP1 cells, nsP1 localized mostly to the plasma membrane, resulting in the extensive formation of filopodia-like structures. In contrast to SFV4-infected HEK293 T-REx cells, induced nsP1 did not localize to intracellular vesicles (Fig. 2). nsP1<sup>16D</sup> also localized predominately to the plasma membrane, but few filopodia-like structures were induced (Fig. 2). Thus, the half-life and, to some extent, the subcellular localization of nsP1 in induced cell lines were similar to those observed in infected cells.

**Effects of nsP1 expression**

The effect of nsP1 expression on cellular transcription and translation was analysed by metabolic labelling. As
expected, SFV4 infection significantly reduced synthesis of cellular pre-mRNA and mRNA. The reduction was approximately threefold (Fig. 3a). In contrast, there was no statistically significant reduction in cellular transcription following induction of nsP1 expression (Fig. 3a). Similarly, SFV4 infection led to an almost complete shutdown of cellular translation by 24 h p.i. (Fig. 3b), whilst induction of nsP1 expression caused no significant effect.

As nsP1 localizes on the plasma membrane, it may act as an inducer of cell death, for example by activating cell-surface death receptors. A WST-1 cell viability assay revealed that, whilst SFV4 infection considerably reduced viability of HEK293 T-REx cells at 24 h p.i. (Fig. 3b), whilst induction of nsP1 expression caused no significant effect.

As nsP1 localizes on the plasma membrane, it may act as an inducer of cell death, for example by activating cell-surface death receptors. A WST-1 cell viability assay revealed that, whilst SFV4 infection considerably reduced viability of HEK293 T-REx cells at 24 h p.i., induction of nsP1 or nsP16D expression had no effect on cell viability (Fig. 3c). Taken together, these results showed that SFV nsP1 expression causes significant morphological changes in cells but on its own has no effect on transcription, translation or viability.

**nsP1 expression by HEK293 cells does not affect virus entry but interferes with virus replication**

The growth curves of SFV4 in HEK293 T-REx cells, treated or mock treated with tetracycline 12 h prior to infection or at the same time as infection, were essentially identical (Fig. 4a). The same result was obtained for infection of these cells by SFV4 viruses carrying a Renilla luciferase marker gene in the non-structural ORF [SFV4(3H)-Rluc] or in the structural ORF (SFV4-StRluc) (data not shown). As the addition of tetracycline did not have any effect on the replication of SFV in HEK293 T-REx cells and 12 h treatment achieved expression of recombinant protein at levels typically found in SFV-infected cells, these conditions were used in subsequent experiments.

Recombinant nsP1 and nsP16D localized to the plasma membrane of induced cells. To investigate whether this had any effect on the entry of SFV, the T-REx-nsP1, T-REx-nsP16D and HEK293 T-REx cells were infected with virus-like particles (VLPs) containing packaged SFV1–d1EGFP.
replicons, capable of infection and d1EGFP expression, but unable to produce infectious progeny (Žusinaite et al., 2007). The percentage of d1EGFP-positive cells at 12 h p.i. for HEK293 T-REx and uninduced T-REx-nsP1 cells was found to be approximately five times lower than for BHK-21 cells. The susceptibility of uninduced T-REx-nsP16D cells was reduced even further, reaching only 25% that of HEK293 T-REx cells. These relative susceptibilities were reproduced when the full-length EGFP-expressing virus SFV4(3H)–EGFP (Tamberg et al., 2007) was used for infection of these cell lines. Therefore, in all subsequent experiments the number of virions or VLPs used for infection of each cell line was selected according to the individual susceptibilities of the cell lines, e.g. four times more infectious material was used for infection of T-REx-nsP16D cells to achieve the same m.o.i. as for HEK293 T-REx or T-REx-nsP1 cells. Induction of nsP1 or nsP16D expression did not affect infection efficiency in any cell line (Fig. 4b). Thus, expression of nsP1 or nsP16D did not affect the ability of SFV VLPs to enter into cells and establish replication.

To determine whether expression of nsP1 or nsP16D interferes with SFV infection, induced or mock-induced HEK293 T-REx, T-REx-nsP1 and T-REx-nsP16D cell lines were infected with SFV4 and the virus titres at 8 and 12 h p.i. were compared. As expected, addition of tetracycline 12 h prior to infection did not have a significant effect on the multiplication of SFV4 in HEK293 T-REx cells (Fig. 4c). In contrast, induction of the expression of nsP1 or nsP16D significantly decreased SFV4 titres (Fig. 4c). At 8 h p.i., the decrease in titre was greater in induced T-REx-nsP1 cells than in induced T-REx-nsP16D cells. At 12 h p.i., the situation was reversed: the titre from induced T-REx-nsP1 cells was only decreased 2.5-fold compared with almost a 5-fold decrease observed in induced T-REx-nsP16D cells (Fig. 4c). Thus, the expression of wt nsP1 of SFV or its palmitoylation-negative mutant inhibited extracellular accumulation of virus.

Expression of nsP1 or nsP16D interferes with different steps in the SFV infection process

Given that nsP1 did not affect virus entry (Fig. 4b), the reduced extracellular virus titres observed following nsP1 or nsP16D expression could reflect defects in production of viral transcripts, virus structural proteins, or assembly and release of virions. To determine whether expression of nsP1 or nsP16D had any effect on virus protein synthesis, the reporter viruses SFV4(3H)-RLuc and SFV4-StRLuc were used to monitor the translation of genomic and sgRNAs. Translation of genomic RNA is proportional to its copy number only at early stages of infection; late in infection, translation of genomic RNAs is inhibited but RNA synthesis remains active. Expression of RLuc in cells

http://vir.sgmjournals.org 1461
infected with SFV4(3H)-Rluc was therefore measured only during the first 6 h of infection. Rluc activity produced by SFV4-StRluc should be proportional to the amount of expressed viral structural proteins throughout the course of infection and this was monitored up to 12 h p.i. Treatment of HEK293 T-REx cells with tetracycline prior to infection with marker viruses resulted in increased translation of the replicase ORF (Fig. 5a) but had no effect on translation of the structural protein ORF (Fig. 5b). In contrast, tetracycline induction of the T-REx-nsP1 cell line resulted in up to a 4-fold reduction in replicase ORF-derived Rluc activity (Fig. 5c). Marker expression, mediated by the sgRNA, was strongly suppressed only in the early stages of infection; by 12 h p.i., no difference between induced and uninduced cells was detectable (Fig. 5d). Induction of the T-REx-nsP16D cell line resulted in less than 2-fold reduction in replicase ORF-derived Rluc activity (Fig. 5e), but caused strong and persistent inhibition of structural protein ORF-derived RLuc activity (Fig. 5f), indicating that the mechanism of action of the mutant protein differed from that of wt nsP1.

Rluc activity produced by SFV4(3H)-Rluc or SFV4-StRluc depends on copy number of viral RNAs, availability of these for translation, rates of translation, extent and timing of shut-off of host cell and replicase translation, and stability of the marker protein. To determine whether nsP1 or nsP16D expression affected levels of viral RNA, total RNA was extracted from induced and mock-induced HEK293 T-REx, T-REx-nsP1 and T-REx-nsP16D cells 12 h after infection with SFV4(3H)-Rluc and analysed by Northern blotting. The amounts of viral RNAs in mock-induced HEK293 T-REx and T-REx-nsP1 cells were similar to each other, whilst the viral RNA level in uninduced T-REx-nsP16D cells was slightly lower (Fig. 6). Treatment of HEK293 T-REx cells with tetracycline had no effect on viral RNA levels. Induction of nsP1 expression decreased the amount of genomic RNA and, to some extent, the amount of sgRNA (Fig. 6). Thus, nsP1 expression specifically

**Fig. 4.** Effect of tetracycline treatment on the replication of SFV4 in HEK293 T-REx cells (a) and the effects of tetracycline-induced expression of nsP1 or nsP16D on the extent of infection with SFV VLPs (b) or production of infectious SFV4 (c). (a) HEK293 T-REx cells (10⁶) were treated with tetracycline 12 h before infection (●), at the same time as infection (■) or were mock treated (▲). Cells were infected with SFV4 at an m.o.i. of 0.2, and the samples were collected at the indicated time points and titrated by a standard plaque assay on BHK-21 cells. The experiment was repeated twice with no significant difference between the datasets (Student’s t-test). (b) HEK293 T-REx, T-REx-nsP1 and T-REx-nsP16D cells (10⁶) induced with tetracycline for 12 h or mock induced were infected with SFV1-d1EGFP1 VLPs at an m.o.i. of 5. The percentage of d1EGFP-positive cells (determined at 12 h p.i. using a BD LSR II cell sorter) in mock-induced samples was taken as 1 for the corresponding induced cell line. (c) HEK293 T-REx, T-REx-nsP1 and T-REx-nsP16D cells (10⁶) induced with tetracycline for 12 h or mock induced were infected with SFV4 at an m.o.i. of 0.6. The samples were collected at 8 and 12 h p.i. and titrated. The titre of the virus stock obtained from the mock-induced cells (open columns) was taken as 1 for the corresponding stock from induced cells. Dark-shaded columns represent the relative titre of the virus stock from induced cells at 8 h p.i. and light-shaded columns represent the relative titre of the virus stock from induced cells at 12 h p.i. All experiments were repeated at least twice with closely similar results. (b, c) Each column represents the data of three experiments (means ± SD). An asterisk indicates a statistically significant difference (P<0.05; Student’s t-test).
inhibited the synthesis of genomic RNA. In contrast, induction of nsP1\textsuperscript{6D} expression resulted in a strong reduction of both genomic and sgRNAs (Fig. 6). The results obtained by Northern blot analysis and those obtained by analysis of marker protein expression from recombinant viruses correlated and indicate that expression of nsP1 and nsP1\textsuperscript{6D} exerts different effects on SFV replication.

**DISCUSSION**

Inducible expression of recombinant protein from stable cell lines has several advantages over systems based on transient expression. Firstly, induction with tetracycline causes no damage to the cells, reaches efficiencies close to 100\% and, on its own, does not affect SFV infection. Secondly, the level of recombinant gene expression is
Expression of nsP1 in HEK293 T-REx cells interfered with virus multiplication, resulting in reduced levels of virus genomic RNA, reduced levels of replicase proteins, delayed synthesis of structural proteins and reduced levels of infectious virus. SFV infection of induced T-REx-nsP1 cells closely resembled the Arg183 mutant of SV, which, due to a defect in negative-strand RNA synthesis, generates fewer replicate complexes but has enhanced sgRNA synthesis (Fata et al., 2002). It can be speculated that nsP1 expression results in lower numbers of virus replicate complexes due to competition with P123 for binding of nsP4 (Shirako et al., 2000; Salonen et al., 2003), incorrect incorporation into replicate complexes, inhibition of P123 or P1234 binding to membranes or interference with the translocation of replicate complexes. A reduction in replicate complex formation would result in reduced RNA replication, reduced levels of genomic RNA and reduced levels of replicase proteins, as was observed. The delayed accumulation of structural proteins could also be explained by a reduction in the number of virus replication complexes. Fewer replication complexes would result in a delay in the early synthesis of subgenomic transcripts and structural polyproteins. However, with time, perhaps these could, as was observed, accumulate to the levels seen in control cells (no nsP1 expression) due to exhaustion of metabolites or saturation of synthesis systems. An alternative explanation for the accumulation to ‘normal’ levels by 12 h of the subgenomic transcripts and structural polyproteins would be that nsP1 expression actively promotes increased production of sgRNA. Whatever the explanation, in nsP1-expressing cells, sufficient structural proteins were expressed and genomic RNA packaged to result in relatively high yields of virions.

In Hela cells, stable expression of mutated forms of nsP1 of SV(LM21) interferes with SV infection (Li et al., 1997). Similarly, the inhibition of SFV multiplication by nsP16D was also significant, but was different to nsP1. The extent of inhibition was far less than observed with the terminal deletion mutants of SV(LM21). The more prominent inhibitory effects of SV nsP1 expression observed by Li et al.
may be the consequence of their much higher recombinant protein expression levels. The inhibitory effect of nsP1\textsuperscript{6D} was relatively mild at early stages of infection but increased over time. That the effects of expression of nsP1 and nsP1\textsuperscript{6D} on SFV infection were different suggests that the mechanism(s) through which these proteins affect SFV infection may be different. Indeed, several differences in biological properties of nsP1 and nsP1\textsuperscript{6D} have been revealed. nsP1\textsuperscript{6D} cannot be palmitoylated, is less tightly bound to cellular membranes and a significant fraction of it is localized in the cytoplasm of transfected cells (Ahola et al., 2000; Žusinaite et al., 2007). SFV nsP1\textsuperscript{6D} is also unable to interact with nsP4 (Žusinaite et al., 2007). It cannot therefore compete with P123 for binding of nsP4 and may therefore be less efficient than wt nsP1 at blocking early replicase complex formation, resulting in the observed lower suppression of genomic RNA synthesis and non-structural protein expression. However, location of nsP1\textsuperscript{6D} on membranes could still result in incorporation into replicase complexes or affect replicase membrane interactions and thereby reduce replicative efficiency. nsP1\textsuperscript{6D} could still compete with replicase complexes for substrates for cap synthesis, as has also been suggested for one of the truncated forms of nsP1 of SV(LM21) (Li et al., 1997).

ACKNOWLEDGEMENTS

The authors thank Valeria Lulla, Amanda Boyd and Liane Ulper for their help and advice. This work was supported by ESF Grants no. 5055 and 6609, by the European Union SFvectors Program and grant 067575 from the Wellcome Trust.

REFERENCES


http://vir.sgmjournals.org 1465


