Infectious DNA from Cells Infected with Feline Syncytium-forming Virus (Spumavirinae)

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SUMMARY

DNA isolated from cells infected with FSFV (a foamy virus) is infectious when tested on susceptible cells. The virus produced by this infectious DNA is identical to the original infecting virus in terms of plaque and virion morphology and serology.

Feline syncytium-forming virus (FSFV) has been isolated from both normal and diseased cats (Riggs et al. 1969; Jarrett, Hay & Laird, 1974). In morphological, biological and biochemical characteristics, FSFV appears to be a typical member of the Spumavirinae (formerly foamy viruses) a sub-family of the Retroviridae (Fenner, 1976). FSFV, in common with other members of the Retroviridae, has been shown to possess an RNA-dependent DNA polymerase activity (Parks & Todaro, 1972; our unpublished observations). This fact, together with the isolation of infectious proviral DNA from cells infected with representative members of the other sub-families of the Retroviridae (Hill & Hillova, 1972; Haase et al. 1976) led us to investigate whether cells infected with FSFV contained infectious proviral DNA molecules. In this communication we report the isolation of DNA from cells infected with FSFV which, upon transfection to susceptible cells, produces c.p.e. and progeny virus identical to the original infecting virus.

Feline embryo (FEA) cells were grown in the Glasgow modification of Eagle’s minimum essential medium (MEM; Macpherson & Stoker, 1962) supplemented with 10% foetal calf serum (EFC10). FSFV was propagated by infecting sub-confluent FEA monolayers in slowly rotating 80 oz bottles.

DNA was extracted from FEA cells grown at 37 °C, either mock-infected, or infected 4 to 5 days previously with FSFV at a multiplicity of infection of 0.1, by a modification of the method of Marmur (1961). Essentially, when infected monolayers showed approx. 70 to 80% of cells involved in syncytial c.p.e., the medium was removed and the cells scraped into Dulbecco’s phosphate buffered saline (PBS), washed twice with PBS, then resuspended in 0.2 M-NaCl, 0.02 M-tris-HCl, pH 7.4, 0.001 M-EDTA (NTE). Boiled protease (Sigma Chemicals, type VI) and SDS were added to final concentrations of 500 μg/ml and 0.5% respectively. The mixture was heated to 80 °C for 10 min, then incubated at 37 °C for 2 h. The digest was then extracted twice by gentle mixing with equal volumes of phenol saturated with NTE and twice with chloroform:iso-amyl alcohol (24:1). The extract was then dialysed overnight against NTE buffer before the addition of RNase A (Sigma Chemicals, boiled for 3 min) to a concentration of 500 μg/ml and 0.5% respectively. The mixture was heated to 80 °C for 10 min, then incubated at 37 °C for 2 h. The digest was then extracted twice by gentle mixing with equal volumes of phenol saturated with NTE and twice with chloroform:iso-amyl alcohol (24:1). The extract was then dialysed against NTE buffer before the addition of RNase A (Sigma Chemicals, boiled for 3 min) to a concentration of 500 μg/ml; incubation was carried out at 37 °C for 3 h. The digest was then extracted twice with equal volumes of phenol saturated with NTE, and then chloroform:iso-amyl alcohol extractions performed until protein was no longer observable at the interface (approx. 2 to 4 times). The extract was then dialysed against 0.1 x SSC (SSC = 1.5 M-sodium chloride, 0.15 M-sodium citrate, 0.15 M-citric acid, pH 7.5) before being stored at -20 °C. DNA concentrations were estimated using an assay based on the specific binding of mithromycin to double-stranded DNA (Hill & Whateley, 1975). Calf thymus DNA was...
Short communications

Table 1. Effects of various treatments on the infectivity of DNA from infected cells and FSFV

(a)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Origin of DNA</th>
<th>+ DMSO (average of 4 plates)</th>
<th>- DMSO (average of 4 plates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>Mock-infected cells</td>
<td>o</td>
<td>0</td>
</tr>
<tr>
<td>No treatment</td>
<td>Infected cells</td>
<td>42.5 (± 11.5)§</td>
<td>4 (± 2.2)</td>
</tr>
<tr>
<td>DNase† (30 min at 37 °C)</td>
<td>Infected cells</td>
<td>33.75 (± 15.8)</td>
<td>3 (± 2.7)</td>
</tr>
<tr>
<td>RNase‡ (30 min at 37 °C)</td>
<td>Infected cells</td>
<td>20.75 (± 12.2)</td>
<td>4 (± 1.5)</td>
</tr>
<tr>
<td>Protease‡ (30 min at 37 °C)</td>
<td>Infected cells</td>
<td>o</td>
<td>N.D.§</td>
</tr>
<tr>
<td>Hind III</td>
<td>Infected cells</td>
<td>o</td>
<td>N.D.</td>
</tr>
<tr>
<td>Bgl II</td>
<td>Infected cells</td>
<td>o</td>
<td>N.D.</td>
</tr>
<tr>
<td>Xba</td>
<td>Infected cells</td>
<td>o</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

(b)

FSFV incubated at 37 °C for 30 min 120.25 (± 6.7)
FSFV incubated at 37 °C for 30 min + protease‡ 0
FSFV incubated at 37 °C for 30 min + sonication (60 s) 112.5 (± 8.4)

* ± standard deviation. † 50 µg/ml. ‡ 500 µg/ml. § N.D. = not done.

used as a standard. The mol. wt. of DNA prepared by this method was routinely > 30 × 10⁶ when estimated by agarose gel electrophoresis.

Transfection assays were performed using the calcium phosphate precipitation technique developed by Graham & van der Eb (1973) both with and without the modification of Stow & Wilkie (1976). Twenty µg of DNA, treated as in Table 1, was diluted into HEPES buffered saline (HeBS, 20 mM-HEPES; BDH Chemicals), 140 mM-NaCl, 5 mM-KCl, 0.5 mM-Na₂HPO₄, 6 mM-glucose, pH 7.0) and 2 mM-calcium chloride added to a final concentration of 130 mM, and a final volume of 800 µl. Two hundred µl of this solution (equivalent to 5 µg of DNA) was placed on to each of 4 sub-confluent monolayers in 50 mm Petri dishes seeded 48 h previously with 0.33 × 10⁶ FEA cells. After 40 min at 37 °C Eagle's MEM supplemented with 2% foetal calf serum was added, and the incubation continued for 3 h 20 min at 37 °C. These timings are those optimal for the infectivity of HSV-1 DNA (Stow & Wilkie, 1976). In the experiments employing calcium phosphate precipitation techniques alone, the monolayers were washed twice with Eagle’s MEM before the addition of fresh EFC10 and incubation at 37 °C. When the modification of Stow & Wilkie (1976) was employed 1 ml of 15% dimethyl sulphoxide (DMSO) in HeBS was added to each dish after the first wash. After 4 min at room temperature the DMSO was removed and the monolayers washed once with Eagle’s MEM before the addition of EFC10 and incubated at 37 °C. After one day the medium was replaced with fresh EFC10. After 5 days at 37 °C the plates were stained with Giemsa and the number of plaques scored. In the experiments using virus (see Table 1 b) the treatment of the cells was identical except the DNA was replaced with 2.4 × 10⁶ p.f.u. of FSFV.

DNA was treated with restriction endonucleases Hind III, Bgl II and Xba (Table 1) using the methods described by Wilkie & Cortini (1976).

Monolayers to be used for electron microscopy were fixed in 2.5% glutaraldehyde when
Fig. 1. (a) C.p.e. produced after infection of FEA cells with FSFV infectious DNA. (b) Virus particle formed after infection of FEA cells with FSFV infectious DNA.
c.p.e. involved 60% of cells. Post-fixation was in 1% osmium tetroxide for 30 min at room temperature. Dehydration was performed in a graded series of alcohols and cells were embedded in araldite resin. Ultrathin sections were stained with uranyl acetate and lead citrate.

Table 1 shows the effects of various treatments on the infectivity of DNA isolated from infected cells, and compares these effects with the effect some of the treatments have on the infectivity of FSFV. The results show that although DNA extracted from uninfected FEA cells does not produce any plaques, DNA extracted from FEA cells infected with FSFV produces an average of 42.5 syncytial plaques per 5 µg of DNA. It also shows that the post-infection treatment of the monolayer with 15% DMSO increased the sensitivity of the assay five- to tenfold. The infectivity of this DNA is sensitive to DNase and sonication (sonication under these conditions reduced the size of DNA to < 0.5 x 10^6 daltons; our unpublished observations) but insensitive to RNase and protease (Table 1a—the reduction in the number of plaques after protease treatment is probably due to the effect of the protease on the cell monolayer). This is in contrast to the infectivity of FSFV which is sensitive to protease but insensitive to sonication (Table 1b). We conclude from these results, along with the sensitivity of the infectious DNA to restriction endonucleases Hind III, Bgl II and Xba (Table 1a), that the infectious material is DNA.

The morphology of the plaques produced by the infectious DNA is identical with those produced after infection of FEA monolayers with FSFV (Fig. 1a). To confirm that the virus produced by the transfection process is identical to the original FSFV used in the lytic infection, we used two additional criteria: (a) morphology of the virion in infected cells; (b) sensitivity of the infectious agent obtained from monolayers infected with DNA to FSFV specific antiserum. The FSFV antiserum, generously provided by Dr O. Jarrett, consisted of pooled serum from infected cats.

Fig. 1b shows an electron micrograph of virions released from a monolayer infected with infectious DNA. These are identical in morphology to virions produced from monolayers infected with FSFV (McKissick & Lamont, 1970; Jarrett et al. 1974; our unpublished observations).

Incubation of the infectious agent produced from monolayers infected with FSFV-infected cell DNA with a 1/25 dilution of FSFV specific antiserum reduced the infectivity from 7.75 × 10^8 p.f.u./ml to < 5 × 10^6 p.f.u./ml. Similarly, the yield from monolayers infected with FSFV was reduced in infectivity from 5.2 × 10^6 p.f.u./ml to < 5 × 10^6 p.f.u./ml.

Thus, DNA was extracted from cells infected with FSFV, which upon transfection produced morphologically and serologically identical virus to the original infecting FSF virus. Preliminary results using the Hirt extraction procedure (Hirt, 1967) indicate that at least some of this proviral DNA is integrated into the host cell genome (our unpublished data). Members of all three sub-families of the Retroviridae have now been shown to replicate through a DNA proviral stage. These findings substantiate the inclusion of the Spuma- virinae in the Retroviridae and show that the presence of reverse transcriptase is associated with transcription of the RNA virus genome into a DNA form even in lytic infections.

These results also confirm that the enhancement of DNA infectivity obtained by treating the cells with DMSO 4 h post-infection (Stow & Wilkie, 1976) applies to several different virus DNA systems. The phenomenon has now been observed with infectious DNA isolated from cells infected with FSFV, as well as with the infectious virion nucleic acids of HSV-1 (Stow & Wilkie, 1976) and adenovirus-5 (E. Frost and J. Williams, unpublished data).

From dose-response experiments, specific infectivities of up to 50 plaques per µg of DNA have been obtained with this assay (D. J. Chiswell & C. R. Pringle, in preparation). The fact
that this is higher than that reported for visna virus (Haase et al. 1976) and avian tumour viruses (Cooper & Temin, 1974) is probably a result of the increased efficiency of the transfection assay due to the DMSO treatment. We are now using this assay to investigate the genome structure of FSFV and the relationship between the proviral DNA and the host cell genome. Preliminary estimates of genome size indicate one of not more than $10 \times 10^6$ daltons, and experiments are in progress to obtain a more accurate estimate of size, along with information on the kinetics of the dose-response relationship and the infectivity of denatured DNA (D. J. Chiswell & C. R. Pringle, in preparation).

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REFERENCES


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