Feline Syncytium-Forming Virus: Identification of a Virion Associated Reverse Transcriptase and Electron Microscopical Observations of Infected Cells

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SUMMARY

The maturation of feline syncytium-forming virus (FSFV), a member of the foamy virus sub-family (Spumavirinae), has been studied by electron microscopy of thin sections of infected feline embryo (FEA) cells. The initial event observed was formation of crescent-shaped nucleoids at the plasma membrane. As budding progressed, the nucleoid became circular in outline with an electron-lucent centre in fully mature extracellular particles. These observations suggested that the maturation of FSFV in fully permissive FEA cells resembled that of C-type RNA tumour viruses, rather than the B-type mouse mammary tumour virus. In this respect FSFV may be distinct from other foamy viruses. However, like other foamy viruses FSFV possessed reverse transcriptase activity. Polymerase activity co-sedimented with infectivity in an equilibrium density gradient and exhibited a preference for poly(rA).oligo(dT)$_{10}$ over poly(dA).oligo(dT)$_{10}$ as exogenous template.

Feline syncytium-forming virus (FSFV) is a member of the Spumavirinae (foamy viruses), a sub-family of the Retroviridae (Fenner, 1976). On biological and morphological grounds FSFV appears to be a typical foamy virus (Hooks & Gibbs, 1975).

Simian and hamster foamy viruses have been shown to possess reverse transcriptase activity (Scolnick et al. 1970; Parks et al. 1971; Hruska & Takemoto, 1975) and we now describe some characteristics of a reverse transcriptase activity associated with partially purified FSFV, confirming an earlier undocumented report (Todaro et al. 1971). Analysis of fractions from a sucrose gradient showed that reverse transcriptase activity and plaque-forming ability were coincident.

It has been proposed that simian, bovine and human foamy viruses mature initially by the production of an intracytoplasmic particle which migrates to the cell membrane and becomes enveloped as the internal nucleoid of the mature virion, (Clarke et al. 1969a, b; Hooks et al. 1972, 1973; Malmquist et al. 1969; Boothe et al. 1970; Achong et al. 1971). This type of maturation was also characteristic of mouse mammary tumour virus (MMTV; Dalton, 1972), where it involved the envelopment of intracytoplasmic A-particles to produce the B-type virion. It has been reported that FSFV matured in the same manner. This interpretation, however, was based upon electron micrographs of cells in an advanced state of degeneration and conclusions about precise location of structures were uncertain (Riggs et al. 1969; Hackett & Manning, 1971). In this report we present evidence to show that the internal nucleoid of FSFV is formed at the cell surface in a manner analogous to the maturation of the C-type tumour viruses, at least in feline embryo cells.

Feline embryo (FEA) cells and FSFV were provided by Dr O. Jarrett of the Department of Veterinary Pathology, University of Glasgow and propagated in Eagle's minimum essential medium supplemented with 10% foetal calf serum (EFC$_{10}$). The virus used in these studies was purified by three sequential re-isolations from single plaques to obtain a genetically homogeneous stock.
Infectivity assays were performed by inoculating subconfluent monolayers of FEA cells in 50 mm Petri dishes with 0.2 ml of each virus dilution. After absorption at room temperature for 45 min the monolayers were washed twice with 5 ml of EFC_10 to remove the inoculum. Another 5 ml EFC_10 were added and the plates incubated at 37 °C for 4 to 5 days. Plaques were visualized after fixation using Giemsa stain.

It has been reported previously that FSFV possessed a reverse transcriptase activity (Todaro et al. 1971), However, since no data had been presented we decided to examine the reverse transcriptase activity of partially purified FSFV. FSFV was purified and tested for DNA polymerase activity in an exogenous reaction, involving two different templates; poly(rA).oligo(dT)_{10} or poly(dA).oligo(dT)_{10}. The culture fluid from approx. 4 x 10^7 FSFV-infected FEA cells was removed and clarified by centrifugation at 2000 rev/min in an M.S.E. Coolspin centrifuge. The virus was then pelleted from the culture fluid by centrifugation at 15000 rev/min for 30 min at 4 °C in a Beckman AL30 rotor. The pellet was suspended in STE (0.02 M-tris/HCl, pH 7.4, 0.1 M-NaCl, 0.001 M-EDTA), then layered on to a pre-formed 20 to 60 % sucrose gradient in STE. After centrifugation to equilibrium at 30000 rev/min in a Beckman SW 50.1 rotor for 16 h at 4 °C, the gradients were fractionated from the bottom. Each fraction was tested for both infectivity and DNA polymerase activity using both of the exogenous templates.

Assays for reverse transcriptase were carried out in a 100 μl final volume containing 50 mM-manganese acetate, 50 mM-potassium chloride, 35 mM-tris/HCl, pH 8.3, 10 mM-dithiothreitol (DTT), 50 mg BSA, 0.1 % NP-40, 1 mM-ATP, 5 μCi methyl 3H-thymidine triphosphate (TTP, Radiochemical Centre, Amersham. sp. act. 30 Ci/mmol) and 2 μg of either poly(rA).oligo(dT)_{10} or poly(dA).oligo(dT)_{10} (Miles, Ltd). After incubation at 37 °C for 120 min, an equal volume of ice-cold 20 % trichloracetic acid (TCA) containing 8 % sodium pyrophosphate was added. After 10 min on ice the whole reaction mixture was transferred to a 2.5 cm Whatman No. 1 filter paper disc. This was then washed six times in ice-cold 5 % TCA containing 4 % sodium pyrophosphate, twice in ethanol, twice in diethyl ether, then air-dried and the radioactivity was estimated by liquid scintillation counting.

The results of the DNA polymerase reactions were plotted as the ratio of the amount of radioactivity (ct/min) incorporated into acid insoluble material in 120 min (Fig. 1). The results show that maximum reverse transcriptase activity was located at the same density in an equilibrium gradient as maximum infectivity of FSFV, indicating that reverse transcriptase activity was associated with the virion of FSFV.

No virus-like particles were visible under the electron microscope after negative staining of gradient fractions, because of the low number of infectious units present (i.e. a maximum infectivity of approx. 10^6 p.f.u./ml). Occasionally virus-like particles could be identified after negative staining of the 30000 rev/min pellets (our unpublished data). However, they were only observed rarely and positive identification of these structures as FSFV was not possible. For this reason the morphology of FSFV could be studied only by thin sectioning of infected cells.

For transmission electron microscopy FEA cells infected 4 to 5 days previously with a low m.o.i. were scraped from the dish into fresh medium, pelleted at 2000 rev/min for 5 min in an M.S.E. Coolspin centrifuge and fixed in 2.5 % glutaraldehyde for 30 min and osmium tetroxide for 30 min. Dehydration was performed through 30, 50, 70, 90 and 100 % alcohol and the fixed cells were then placed in 1:1 alcohol:propylene oxide, followed by two changes of 100 % propylene oxide (30 min each). The cells were then infiltrated for 24 to 72 h with resin (Epon or Araldite) in a 1:1 propylene oxide:resin mixture, followed by 100 % resin. They were finally embedded in fresh resin and polymerized for at least 72 h at 68 to 70 °C. Thin sections of this embedded material were cut on an LKB ultramicrotome and stained with lead citrate and uranyl acetate.
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Fig. 1. Co-sedimentation of reverse transcriptase activity and infectivity. FSFV was partially purified from the medium of infected FEA cells. Each fraction of a 20 to 60% sucrose gradient was tested for infectious virus (●—●) and DNA polymerase activity. The ratio of TCA-precipitable counts incorporated using poly(rA).oligo(dT)$_{10}$ or poly(dA).oligo(dT)$_{10}$ as template (i.e. the ratio rA:dA) was calculated and plotted (○-○). The background counts were approx. 200 ct/min, and the peak of incorporation using the (rA) template was 2232 ct/min at fraction 13.

Examination of thin sections of FSFV-infected FEA cells revealed virus particles during various stages of maturation. Fig. 2(a) shows a virion at a late stage of maturation which reveals details of virion structure. A central nucleoid (n) is surrounded by a membrane (m) and spike projections (s). Fig. 2(b) shows a representative virion (l) at a late stage of maturation with the internal nucleoid fully formed. Also shown in Fig. 2(b) is a virion (e) at an earlier stage of maturation, revealing an incomplete nucleoid (n). This type of maturation is also evident in Fig. 2(c) which shows a crescent-shaped nucleoid (n) forming under an area of membrane into which virus projections have been inserted. Although these electron micrographs are interpreted as sequential stages in maturation, it is possible that the differences observed may merely reflect different planes of sectioning. These data indicate that FSFV buds from FEA cells in a manner similar to the C-type RNA tumour viruses.

In contrast to these findings simian, human and bovine foamy viruses have been reported to mature in a manner similar to that described for B-type RNA tumour viruses (Clarke et al. 1969a, b; Malmquist et al. 1969; Boothe et al. 1970; Achong et al. 1971; Hooks et al. 1972, 1973), i.e. the nucleoid appeared to be completely formed within the cytoplasm and no incomplete nucleoids were observed associated with the cell membrane. Some reports have identified nucleoid-like bodies within the cytoplasm of FSFV-infected cells (Riggs et al. 1969; Hackett & Manning, 1971). However, in these reports the micrographs were of cells in an advanced state of degeneration, which makes uncertain any conclusions concerning
Fig. 2. FSFV infected FEA cells were prepared and sectioned for electron microscopy as described in the text. (a) A virion at a late stage of maturation revealing a central electron-lucent nucleoid (n) surrounded by a membrane (m) and spike projections (s). (b) FSFV particles at both early (e) and late (l) stages of maturation. (c) An early stage of maturation with an incomplete crescent-shaped nucleoid (n).

the precise locations of these nucleoid-like bodies. The possibility of membranes adjacent to these bodies in another unsectioned plane could not be excluded. Data presented by McKissick & Lamont (1970) indicated the presence of ‘C-type’ budding particles in canine fibrosarcoma cells infected with FSFV. Our observations suggest that FSFV may normally mature in this fashion, although it is possible that different strains of FSFV may behave differently in this respect.
FSFV multiplied in BHK-21, HEP-2 and HeLa cells as efficiently as it did in FEA cells and with reduced efficiency in another eight mammalian cell lines (D. J. Chiswell & C. R. Pringle, unpublished data). It will be necessary therefore, to compare the maturation of FSFV in permissive and semi-permissive cells. If FSFV indeed does mature normally in all cells by formation of crescent-shaped nucleoids at the plasma membrane it would represent a major difference between FSFV and other foamy viruses.

We have presented results which confirm that FSFV possesses a virion RNA-dependent DNA polymerase. This polymerase showed a preference for poly(rA). oligo(dT)$_{10}$ as opposed to poly(dA). oligo(dT)$_{10}$ in an exogenous DNA polymerase assay. This is a characteristic of virus reverse transcriptase (Baltimore & Smoler, 1971; Borrow et al. 1972; Goodman & Spiegelman, 1971; Robert et al. 1972; Smith & Gallo, 1972; Wells et al. 1972). Reverse transcriptase activity co-migrated with infectivity in equilibrium sucrose density gradients, indicating that the enzyme was present in or on the FSFV virion. The ratio of radioactivity (ct/min) incorporated using each template was similar to that obtained with hamster foamy virus (Hruska & Takemoto, 1975) and simian foamy virus, type 6 (Liu et al. 1977).

The presence of a virion-associated reverse transcriptase, together with the finding of infectious DNA in FSFV-infected FEA cells (Chiswell & Pringle, 1977, 1978) confirmed that FSFV was a genuine member of the Retroviridae. Although the maturation of FSFV may be different from that of other foamy viruses, in other respects it is a typical foamy virus. Like FSFV all members of the Spumavirinae may replicate via an intermediate proviral DNA stage.

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MRC Virology Unit
Institute of Virology
Church Street
Glasgow G11 5JR, Scotland

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