Effect of Non-permissive Temperature on the Assembly of Frog Virus 3 Particles

By M. Mercedes Bragaglia, Gabriella Campadelli-Fiume and M. La Placa

Istituto di Microscopia Elettronica Clinica, Università di Bologna, Via Zamboni 16, I-40126 Bologna, Italy and
Istituto di Microbiologia, Università di Bologna, via San Giacomo 12, I-40126 Bologna, Italy

(Accepted 31 January 1974)

SUMMARY

Cells infected by FV-3 and incubated at the non-permissive temperature of 33 °C were examined by electron microscopy. In the cytoplasmic virus matrices, only irregularly shaped vesicle-like, polyhedral and tubular capsids were present. Normal particles were produced quickly by infected cells when shifted to the permissive temperature.

INTRODUCTION

Frog Virus 3 (FV-3) belongs to a group of polyhedral deoxyriboviruses which replicate in the cytoplasm of infected cells but differ from poxviruses (Granoff, Came & Breeze, 1966; Kelly & Robertson, 1973; Kucera, 1973). FV-3 replicates also in phylogenetically unrelated mammalian host cells at temperatures between 12 and 32 °C; replication is optimal at 30 to 31 °C (Gravell & Granoff, 1970). At temperatures above 32 °C, no infective virus is formed (Granoff et al. 1966), although several virus functions are still expressed.

Granoff (1969) demonstrated the accumulation of virus antigens and DNA, by immunofluorescence and Feulgen staining respectively, in the cytoplasmic inclusion bodies of FV-3 infected cells incubated at 33 °C. The biochemical and biophysical studies by Kucera (1970) showed that at incubation temperature progressively above the permissive threshold, some virus-specific functions were differentiated by their thermal sensitivity and that, in particular, cultures incubated at 33 °C produced virus DNA which acquired no capsids.

To clarify these early thermosensitive events which prevent the formation of mature virus particles, we have performed an electron microscopic study of the morphogenesis of FV-3 particles in infected cells incubated at 33 °C, which is 1 °C above the permissive limit.

We include studies on virus particle assembly in cell cultures incubated at 33 °C and then shifted to a permissive temperature in the presence or absence of an inhibitor of protein synthesis.

METHODS

BHK 21/13 cells were grown at 37 °C on mica coverslips in Leighton tubes in Eagle’s basal medium containing 10 % bovine serum. Fully grown monolayers were infected with FV-3 at a multiplicity of 10 p.f.u./cell and incubated for 45 min at 26 or 33 °C for virus
adsorption. The virus inoculum was then removed, the monolayers were washed and Eagle's medium without serum was added. Infected cells were then incubated in a water bath at 26 or 33 °C for 15 or 18 h.

Duplicate specimens were fixed with 1.7 % glutaraldehyde in 0.1 M-phosphate buffer solution, pH 7.2, postfixed in 1 % OsO₄ and embedded in Durcupal ACM. All these operations were carried out on the monolayers adhering to the mica coverslips, from which embedded cells were easily removed at the end of the procedure (Laschi & Rizzoli, 1968).

Ultrathin sections, double stained with uranyl acetate and lead citrate, were examined in a Siemens Elmiskop 1 electron microscope.

RESULTS

The morphological aspects of the assembly of mature virus particles of FV-3 at permissive temperatures have been described (Darlington, Granoff & Breeze, 1966; Bingen-Brendel, Triper & Kirn, 1971) and are summarized here to facilitate comparison with the results obtained at the non-permissive temperature of 33 °C.

In the cytoplasm of cells infected by FV-3 and incubated at 26 °C, virus inclusions are demonstrable about 6 h after infection. They appear as juxtanuclear roundish areas, with a filamentous matrix lighter than the surrounding cytoplasm, bordered by mitochondria and ribosomes. Capsids first appear within the matrix 9 h after infection and always show a regularly hexagonal profile even if incomplete. At about 12 h after infection, an electron-dense core containing virus DNA appears inside the hexagonal capsids. The nucleocapsids measure 120 to 130 nm in diam. (Fig. 1). Enveloped virus particles are produced later by the budding of nucleocapsids through the cytoplasmic membranes (Fig. 1, arrow), and are 165 to 200 nm in diam.

In our experiments, cells infected by FV-3 and incubated at 33 °C showed cytoplasmic sites of virus synthesis similar in number, size and matrix to those present at permissive temperatures. The most striking differences were shown by the morphology of the capsids appearing in infected cells kept at 26 or 33 °C. At 33 °C only irregular and pleomorphic structures developed in the sites of virus synthesis; these were from 75 to 220 µm in diam. (Fig. 2). Fewer than 1 % showed the normal hexagonal profile of the empty capsids formed at permissive temperatures.

Tubular forms were also present with the vesicle-like structures inside the virus matrices. In sections these were generally not well defined at the apices (Fig. 3 and 4), but some showed the regular polyhedral pattern at one end (Fig. 5). Most of the rod-shaped structures were 50 to 60 nm in diam. but about 20 % reached 100 nm; lengths ranged from 200 to 550 nm.

The internal material of these vesicle-like and rod structures had electron densities similar to that of normal empty capsids and most probably contained no DNA.

Polyhedral and hexagonal structures containing a central electron-dense core were detected rarely, on the average one per ten cells observed (Fig. 3, arrow).

Budding particles were never observed.

We investigated the possibility that the irregular structures which developed at 33 °C could become normal capsids by shifting the infected cells to a permissive temperature. When the FV-3-infected cells which had been incubated at 33 °C for 15 h were shifted to 26 °C, hexagonal empty capsids and regular nucleocapsids were already demonstrable with the irregular structures 1 h after the shift to the permissive temperature (Fig. 6). Two hours after the shift, more than 50 % of the particles present in the virus matrices were regular nucleocapsids (Fig. 7).
Morphogenesis of FV-3 at 33 °C

Fig. 1. Part of the cytoplasm of an FV-3-infected BHK cell incubated at 26 °C for 18 h. Nucleocapsids, arranged in a paraerystalline array, migrated from the virus matrix toward the periphery of the cell. Arrow shows a budding virus particle.

Fig. 2. Virus matrix of an FV-3-infected BHK cell incubated at 33 °C for 15 h. Only irregular vesicle-like capsids developed.
Fig. 3, 4, 5. Irregular vesicle-like and tubular structures can be seen inside the virus matrices of FV-3-infected BHK cells incubated at 33 °C for 15 h. In Fig. 3 the arrow shows a regularly hexagonal nucleocapsid.
Morphogenesis of FV-3 at 33 °C

Fig. 6. Part of a virus matrix of an FV-3-infected BHK cell incubated at 33 °C for 15 h and then shifted to 26 °C for 1 h. Regularly hexagonal nucleocapsids can be seen together with the vesicle-like structures.

Fig. 7. Virus matrix of an FV-3-infected BHK cell incubated at 33 °C for 15 h and then shifted to 26 °C for 2 h. Hexagonal capsids and nucleocapsids are more numerous than those irregularly shaped.
Concurrently with the appearance of regular nucleocapsids, budding virus particles were observed; these were more numerous 2 h after the shift to 26 °C.

Virus particles produced under such conditions were also tested for their infectivity. Duplicate FV-3-infected cell monolayers were incubated at 33 °C for 15 h, shifted to 26 °C for a further 2 h and frozen and thawed three times. The duplicate samples were pooled, centrifuged at low speed to remove cell debris, and the clear supernatant fluid titrated for virus infectivity. It was found that $5 \times 10^6$ p.f.u./ml were present in the supernatant fluid, as compared with $4.7 \times 10^6$ p.f.u./ml for cells kept at 26 °C for 17 h, and $9 \times 10^6$ p.f.u./ml for cells kept at 33 °C for 17 h.

In order to ascertain whether normal virus particles could assemble by using only the proteins synthesized at 33 °C, the following experiment was performed. FV-3-infected cells incubated at 33 °C for 15 h were further incubated at this temperature for 3 h in a pre-warmed medium containing 30 μg/ml of cycloheximide, which ensured a 95 % or greater reduction of protein synthesis. The infected cells were then rapidly cooled to 26 °C and incubated at this temperature for 2 h.

In the infected cells shifted to the permissive temperature after the block of protein synthesis, the formation of normal capsids and nucleocapsids was completely absent. Furthermore, and rather surprisingly, the virus matrices appeared empty and devoid of the irregular capsids formed at 33 °C (Fig. 8). Control FV-3-infected cells incubated at 33 °C for 18 h in the absence of cycloheximide showed the same irregular structures as described above.
**DISCUSSION**

Our results show that in FV-3-infected cells incubated at 33 °C: (a) cytoplasmic virus matrices develop with irregular capsids; (b) normal capsids and nucleocapsids are produced very rarely; (c) normal nucleocapsids and budding virus particles are observed soon after the shift of the infected cells to a permissive temperature, provided protein synthesis has not been blocked.

These observations are in agreement both with the report of Granoff (1969), who found intracytoplasmic inclusion bodies containing virus antigens in FV-3-infected cells incubated at 33 °C, and with the finding that the DNA of both wild type FV-3 and FV-3 temperature-sensitive mutants acquired no capsids if infected cells were incubated at non-permissive temperatures (Kucera, 1970; Purifoy, Naegle & Granoff, 1973).

Altogether these findings indicate that the non-permissive temperature of 33 °C hinders the formation of infective virus by acting at the stage of virus assembly, which therefore seems to be the step sensitive to the minimum increase of temperature above the upper permissive limit.

Moreover, they show that inhibition of protein synthesis prevents the production of normal virus particles upon shifting the infected cells to a permissive temperature.

The formation of irregular capsids has been already described for other viruses in various experimental conditions. Vesicles similar to the ones we have observed occur in cells infected by vaccinia virus in the presence of rifampicin (Moss et al. 1969). Polyheads having a rod-shaped form have been found during the growth cycle of T-even phages when the assembly process was in some way disturbed (Cummings, Couse & Forrest, 1970). Tubular aggregates formed also during the process of *in vitro* self-assembly of a spherical plant virus if it took place in the absence of RNA (Bancroft, 1970).

At the moment we have no explanation for the disappearance of the virus capsids in infected cells treated with cycloheximide and then shifted to a permissive temperature in the presence of the drug. A comparative study of the behaviour of FV-3 capsids and nucleocapsids in the presence of the drug is in progress.

This investigation was supported in part by a grant from the Consiglio Nazionale delle Ricerche, Rome.

**REFERENCES**


(Received 1 November 1973)