JOURNAL OF VIROLOGY, Feb. 1977, p. 802-805

Vol. 21, No. 2
Printed in U.S.A.

Macromolecular Synthesis in Cells Infected by Frog Virus 3

VI. Frog Virus 3 Replication Is Dependent on the Cell Nucleus

RAKESH GOORHA, DAWN B. WILLIS, AND ALLAN GRANOFF*

Laboratories of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

Received for publication 16 August 1976

Previous evidence indicated that frog virus 3 (FV3), an icosahedral DNA virus, replicates exclusively in the cytoplasm. However, data presented here demonstrate that FV3 does not replicate in UV-irradiated or enucleated chicken embryo or BSC-1 cells and that virus-specific DNA synthesis is not initiated in such cells. Primary transcription was not detected in infected enucleated cells. These results demonstrate that a functional nucleus is essential for FV3 replication.

Previous studies suggested that frog virus 3 (FV3), an icosahedral DNA virus, replicates exclusively in the cytoplasm (2, 3, 6). By light microscopy, feulgan-positive cytoplasmic inclusion bodies are seen in infected cells. Electron microscopic studies have shown foci of infection in the cytoplasm that first appear as electron-translucent areas containing fine grains and surrounded by degenerate mitochondria. Later in infection, virus particles are seen in crystalline arrays in the cytoplasm, and extensive budding of FV3 takes place at the plasma membrane. Autoradiography also indicated that FV3 DNA replicates in the cytoplasm and that virions mature there (9, 10). On the other hand, virus particles have been detected by electron microscopic examination in cell nuclei in the later stages of infection (3, 7). However, since FV3 causes rapid and severe inhibition of host macromolecular synthesis (5, 9, 10), virus particles were seen only after the completion of one or more cycles of replication. Replicating viral DNA is associated with the nuclear fraction of disrupted cells, but this DNA is removed by centrifugation of the nuclear fraction through a sucrose solution (9). Therefore, it is not clear whether the nucleus plays an active role in FV3 replication or whether the nuclear association of viral DNA occurs during cell disruption and the presence of virus particles in the nucleus is the result of cell damage. Several investigators have used UV-irradiated or enucleated cells to study nuclear involvement in influenza virus, vesicular stomatitis virus (VSV), and vaccinia virus replication (1, 4, 12). Employing the same approaches, we now show that a viable nucleus is required for FV3 replication.

BSC-1 and BHK-21/13 cells were grown as monolayers at 37°C in Eagle minimum essential medium containing non-essential amino acids and 10% fetal calf serum. Chicken embryo (CE) cells were prepared from 11-day-old embryos and were grown at 37°C in minimal essential medium containing 5% fetal calf serum, 3% sodium bicarbonate, and 1% lactalbumin hydrolysate. CE cells were passed serially and, for enucleation, cells between passages 5 and 20 were used. A clonal isolate of FV3 was grown in fathead minnow cells at 30°C as described previously (11), vaccinia virus was grown on the chorioallantoic membrane of 11-day-old CEs, and VSV was grown in BHK cell monolayers. Virus titers were determined by plaque assay: vaccinia virus in primary CE cells (37°C), VSV in BHK cells (37°C), and FV3 in fathead minnow cells (25°C).

For enucleation experiments, CE or BSC-1 cells were grown to semiconfluency on 2.5-cm round plastic cover slips and enucleated with cytochalasin B by the procedure of Pennington and Follett (12). After enucleation, cells were incubated for 1 h in standard medium at 37°C. One cover slip from each group was then either stained with Giemsa or examined by phase-contrast microscopy to estimate efficiency of enucleation and cell loss after centrifugation. The proportion of cells enucleated was more than 97%; cell loss varied from 22 to 30% with CE cells and was less than 10% with BSC-1 cells. Enucleated or nucleated cells were infected with FV3 at a multiplicity of 10 PFU/cell, with VSV at 5 PFU/cell, and with vaccinia virus at 10 PFU/cell.

No increase in virus titer was detected after infection of enucleated CE cell monolayers with FV3 (Fig. 1). Cells treated with 10 μg of cytochalasin B per ml for 20 min, but not centrifuged, supported normal replication, showing that cytochalasin B per se had no effect on the

replication of FV3 in CE cells. In contrast to the results with FV3, enucleated cells supported VSV replication, in agreement with previous work (4), although the yield of virus was lower than in control cells. The loss of up to 30% of CE cells from the cover slips during centrifugation could account, in part, for the reduced yields of VSV.

Pennington and Follett (12) reported that infection of enucleated BSC-1 cells by vaccinia virus leads to virus-specific DNA and protein synthesis, but that infectious virus is not produced. Therefore, it was possible that FV3-specific macromolecular synthesis occurred in enucleated CE cells, even though no infectious virus was produced. To examine this, enucleated CE cells were infected with FV3 and labeled for 15 min with [^{3}H]thymidine (10 μCi/ml) at intervals after infection. Vaccinia virus was used as a positive control. FV3 DNA synthesis was not detected in enucleated CE cells; in contrast, vaccinia virus DNA was synthesized under similar experimental conditions (Fig. 2).

Enucleated CE cells were also examined for their ability to support FV3 RNA synthesis. At intervals after infection, enucleated CE cells were labeled for 15 min with [^{3}H]uridine (10 μCi/ml). No virus-specific RNA synthesis could be detected in enucleated, infected cells, whereas control cell monolayers treated with cytochalasin B, but not centrifuged, supported viral RNA synthesis (Fig. 3). Similarly, enucleated BSC-1 cells supported neither FV3 replication nor virus-specific DNA or RNA synthesis (data not shown).

Another way to look at nuclear requirements for viral replication is to test UV-irradiated cells for their ability to replicate virus (1). The data (Fig. 4) show that FV3 did not replicate in

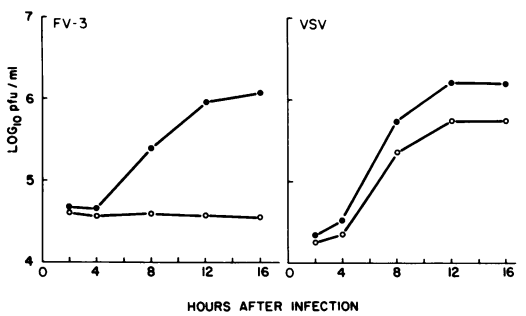

FIG. 1. *FV3 and VSV replication in enucleated CE or control cells. CE cells were enucleated by cytochalasin B as described in the text. Enucleated cells were infected either with FV3 or VSV, and at the indicated times cells from one cover slip of each group of infected cultures were suspended in 1 ml of Eagle minimal essential medium and disrupted by sonic treatment for 2 min. FV3 was plaque assayed on fathead minnow cells at 25°C, and VSV was plaque assayed on BHK cells at 37°C. Control cells received cytochalasin B (10 μg/ml) for 20 min but were not centrifuged. Symbols: ●, control cells; ○, enucleated cells.*

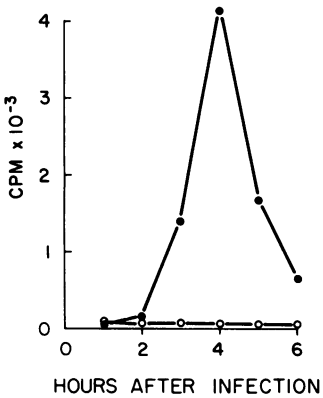

FIG. 2. *FV3 and vaccinia viral DNA synthesis in enucleated CE cells. Enucleated CE cells were infected with vaccinia or FV3, and at the indicated times enucleated cells were labeled with [^{3}H]thymidine (10 μCi/ml). After a 15-min labeling period, cells were washed three times with phosphate-buffered saline, suspended in 1.0 ml of reticulocyte standard buffer, and disrupted by sonic treatment. DNA was precipitated by the addition of 2 ml of 10% trichloroacetic acid to each sample. The precipitate was filtered on membrane filters (Millipore Corp.) and washed three times with 5% trichloroacetic acid. The filters were dried, and radioactivity was determined in a Packard Tri-Carb scintillation spectrometer. Symbols: ○, FV3; ●, vaccinia virus.*

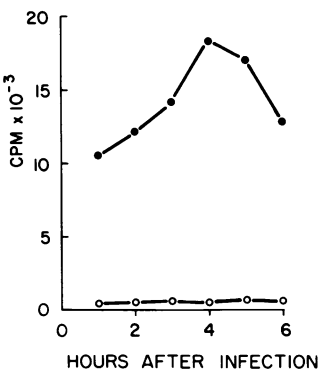

FIG. 3. *FV3 RNA synthesis in enucleated or control CE cells. Cells were infected with FV3 at a multiplicity of 10 PFU/cell. At the indicated times, enucleated or control cells were labeled for 15 min with [^{3}H]uridine (10 μCi/ml). Other details are as described in the legend to Fig. 2. Symbols: ○, enucleated cells; ●, control cells (received cytochalasin B but were not centrifuged).*

UV-irradiated cells, even though these cells were capable of supporting a significant level of VSV replication. To determine whether viral DNA was synthesized in the absence of the production of infectious virus, UV-irradiated cells were labeled for 60 min with [^{3}H]thymidine at 2 or 4 h after infection, and DNA was extracted with phenol-chloroform. The viral nature of the newly synthesized DNA in the infected cells was established by DNA-DNA hybridization. Nearly 35% of the [^{3}H]DNA extracted from purified virions hybridized to unlabeled FV3 DNA bound to the filters, and different concentrations of CE DNA had no effect on the hybridization of FV3 DNA (Table 1). The cross-hybridization of FV3 DNA to CE DNA was less than 0.6%. In addition, no significant amount of virus-specific DNA synthesis occurred in infected, UV-irradiated cells. In contrast, unirradiated CE cells infected with FV3 synthesized virus-specific DNA both at 2 and 4 h after infection.

Our results provide evidence for a functional nucleus as an essential cellular requirement for initiation of FV3 infection. In enucleated cells, FV3 failed to initiate even primary transcription. The possibility that disruption of cellular organization, which might provide a scaffolding for DNA and/or RNA replication or some other nonspecific effect of enucleation, was responsible for the failure of FV3 to initiate infection in enucleated cells is unlikely since UV-irradiated cells also failed to support FV3 replication or

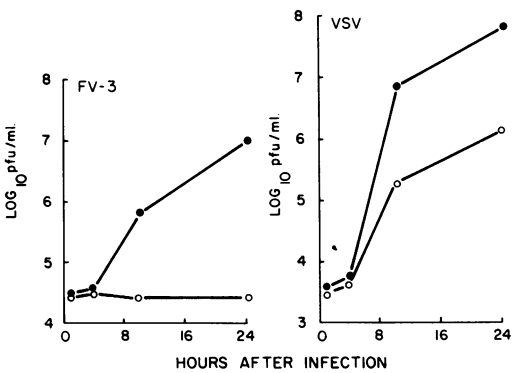

FIG. 4. *FV3 and VSV replication in UV-irradiated CE cells. CE cells were grown in monolayers in 100-mm dishes. For UV irradiation, the medium was removed and cells were exposed, in open petri dishes, to UV light (total dose: 1,000 ergs/mm^2). Control and UV-irradiated cells were infected either with FV3 or with VSV. Other details are as described in the legend to Fig. 1. Symbols: ●, unirradiated cells; ○, UV-irradiated cells.*

TABLE 1. *DNA synthesis in FV3-infected, UV-irradiated CE cells*[a]

Source of DNA	Amt (μg)	Radioactivity (cpm)	FV3 DNA bound to filter		CE cell DNA bound to filter	
			cpm hybridized	% hybridized	cpm hybridized	% hybridized
[^{3}H]DNA from purified FV3	0.01	1,412	517	36.5	9	0.6
	0.05	7,060	2,438	34.5	6	0.04
	0.1	14,120	5,287	37.5	8	0.005
[^{3}H]DNA from purified FV3 + CE DNA						
1 μg	0.1	14,120	5,307	37.6	6	0.04
2 μg	0.1	14,120	5,289	37.5	0	—[b]
5 μg	0.1	14,120	5,286	37.5	11	0.1
[^{3}H]DNA from uninfected CE cells	0.1	3,060	—	—	318	10.4
	0.2	6,120	—	—	673	11.0
	2.0	61,200	—	—	6,278	10.4
[^{3}H]DNA from FV3-infected cells						
2.0–3.0 h p.i.[c]	2.0	6,238	1,084	17.4	492	9.9
4.0–5.0 h p.i.	2.0	7,132	2,018	28.3	258	3.5
[^{3}H]DNA from UV-irradiated, FV3-infected cells						
2.0–3.0 h p.i.	2.0	516	6	1	46	9.0
4.0–5.0 h p.i.	2.0	489	0	0	41	8.4

[a] DNA from purified FV3 virions or infected cells was extracted with phenol-choloroform by the procedure of LaColla and Weissbach (8). The procedure of Raskas and Green (13) was followed for DNA-DNA hybridization.

[b] Dashes indicate no hybridization.

[c] p.i., Postinfection.

DNA synthesis. In contrast, another cytoplasmic DNA virus, vaccinia, synthesizes viral DNA and proteins in enucleated cells (12). Thus, it appears that FV3 is completely dependent upon a nuclear function(s) for its replication in contrast to vaccinia virus, which can express most of its genetic information in enucleated cells. However, morphogenesis and assembly of both viruses occur in the cytoplasm. An understanding of compartmentalization of nuclear and cytoplasmic cell functions required for FV3 DNA replication and morphogenesis should provide insight into viral and cellular control mechanisms. Information on viral DNA replication will be the subject of another report (manuscript in preparation).

Ramona Tirey provided excellent technical assistance.

This work was supported by Public Health Service research grant CA 07055 and Childhood Cancer Research Center grant CA 08480 from the National Cancer Institute and by ALSAC.

LITERATURE CITED

1. **Barry, R. D., D. R. Ives, and J. G. Cruickshank.** 1962. Participation of DNA in the multiplication of influenza virus. Nature (London) 194:1139–1140.
2. **Bingen-Brendel, A., F. Tripier, and A. Kirn.** 1971. E'tude morphologigue sequentielle du development du FV 3 sur cellules BHK 21. J. Microsc. (Paris) 11:249–258.
3. **Darlington, R. W., A. Granoff, and D. C. Breeze.** 1966. Viruses and renal carcinoma of *Rana pipiens*. II. Ultrastructural studies and sequential development of virus isolated from normal and tumor tissue. Virology 29:149–156.
4. **Follett, E. A. C., C. R. Pringle, W. H. Wunner, and J. J. Skehel.** 1974. Virus replication in enucleate cells: vesicular stomatitis virus and influenza virus. J. Virol. 13:394–399.
5. **Goorha, R., and A. Granoff.** 1974. Macromolecular synthesis in cells infected by FV 3. I. Virus-specific protein synthesis and its regulation. Virology 60:237–250.
6. **Granoff, A., P. E. Came, and D. C. Breeze.** 1966. Viruses and renal carcinoma of *Rana pipiens*. I. The isolation and properties of virus from normal and tumor tissue. Virology 29:133–148.
7. **Kelly, D. C.** 1975. Frog virus 3 replication: electron microscope observations on the sequence of infection in chick embryo fibroblasts. J. Gen. Virol. 26:71–86.
8. **LaColla, P., and A. Weissbach.** 1975. Vaccinia virus infection of HeLa cells. I. Synthesis of vaccinia DNA in host cell nuclei. J. Virol. 15:305–315.
9. **McAuslan, B. R., and W. R. Smith.** 1968. Deoxyribonucleic acid synthesis in FV-3-infected mammalian cells. J. Virol. 2:1006–1015.
10. **Maes, R., and A. Granoff.** 1967. Viruses and renal carcinoma of *Rana pipiens*. IV. Nculeic acid synthesis in FV 3 infected BHK 21/13 cells. Virology 33:491–502.
11. **Naegele, R. F., and A. Granoff.** 1971. Viruses and renal carcinoma of *Rana pipiens*. XI. Isolation of frog virus 3 temperature-sensitive mutants; complementation and genetic recombination. Virology 44:286–295.
12. **Pennington, T. H., and E. A. C. Follett.** 1974. Vaccinia virus replication in enucleate BSC-1 cells: particle production and synthesis of viral DNA and proteins. J. Virol. 13:488–493.
13. **Raskas, H. J., and M. Green.** 1971. DNA-RNA and DNA-DNA hybridization in virus research, p. 248–270. *In* K. Maramorosch and H. Koprowski (ed.), Methods in virology, vol. 5. Academic Press Inc., New York.