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Is Cytoskeleton Involved in Vesicular Stomatitis Virus Reproduction?

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CER cells infected with vesicular stomatitis virus showed a morphology similar to that observed after cytochalasin B treatment. Temperature-sensitive mutants affected in envelope protein maturation did not induce those morphological changes at a nonpermissive temperature. In addition, the cytoskeleton was not implicated in vesicular stomatitis virus reproduction.

Infection by vesicular stomatitis virus (VSV, an enveloped virus RNA$^-$ strand) leads to a more or less pronounced rounding of host cells, depending on the cell type infected (1, 13). As this morphological change may reflect underlying modifications of the host cytoskeleton (24), it was of interest to determine which cytoskeleton elements would be involved in this modification and which viral components would induce this process. Although experiments were performed with both BHK and CER cells (21), only data for CER cells are presented in this note. These cells spread to a great extent on the substrate (Fig. 1a), whereas the others elongated and formed bundles.

A progressive retraction of cells began within 2 h postinfection with a VSV wild-type strain; cells appeared thicker in the center, and borders were more clearly defined (Fig. 1b). After 6 h, the cells became spherical, adhering to the substrate with long, thin cytoplasmic processes extending from the original cell borders (Fig. 1c). For determining whether a modification of cell morphology after VSV infection was due to parental virions or to newly synthesized viral material, infected cells were treated after adsorption with 25 μg of cycloheximide per ml to block protein synthesis. After 6 h, cells were still well spread (Fig. 1g). Translation thus appears to be required for the morphological changes observed after viral infection.

Experiments with effectors which directly or indirectly affect cytoskeleton organization were performed to attempt to mimic the effect of VSV (wild-type) on cell morphology and to determine whether an intact cytoskeleton is necessary for viral reproduction.

Cytochalasin B is known to alter the distribution and contractile functions of microfilaments (23). Treated cells, whether infected or not, became rounded but still adhered to the substrate with retracting processes (Fig. 1d). This spidery appearance had already been observed on noninfected cells of different types (8, 22, 24). The similarity between VSV and the cytochalasin B effect led us to propose that VSV affects cell morphology by disrupting the microfilament organization; a reduction in the number of bundles containing actin filaments had already been shown in infected BHK cells (19). At concentrations ranging from 10 to 104 μM, cytochalasin B had no effect on viral production (Table 1). As at higher concentrations aqueous solutions of the drug led to the formation of crystals, higher concentrations were not used. The absence of inhibition had also been observed in BHK cells. The same results have recently been reported with MDBK cells (8). Thus, organized microfilaments are not required for transcription, replication, translation, or assembly of viral components.

Colchicine and colcemid are known to disrupt microtubules (2). After 1 h of exposure to colchicine, the cells still layering on the substrate exhibited a large indentation of their borders. This morphology had already been observed with different cell types (5, 24). Infected cells treated with colchicine and observed at 6 h postinfection retained this characteristic floral appearance (Fig. 1e). Colcemid at concentrations ranging from 17 to 135 μM and colchicine at concentrations ranging from 15 μM to 2 mM had no effect on viral production (Table 1). The same results were obtained with BHK cells. These results suggest that microtubules do not play a major role in the reproduction of VSV and that the spidery appearance of infected cells is not a prerequisite for viral production.

Local anesthetics are known to act on the assembly of microtubules and microfilaments, both of which are involved in the transmembrane control of cell surface receptor distribution (16, 17). Cells, whether infected or not and treated with dibucaine or tetracaine, became slightly vacuolated. Even at 6 h postinfection, infected treated cells retained the same mor-

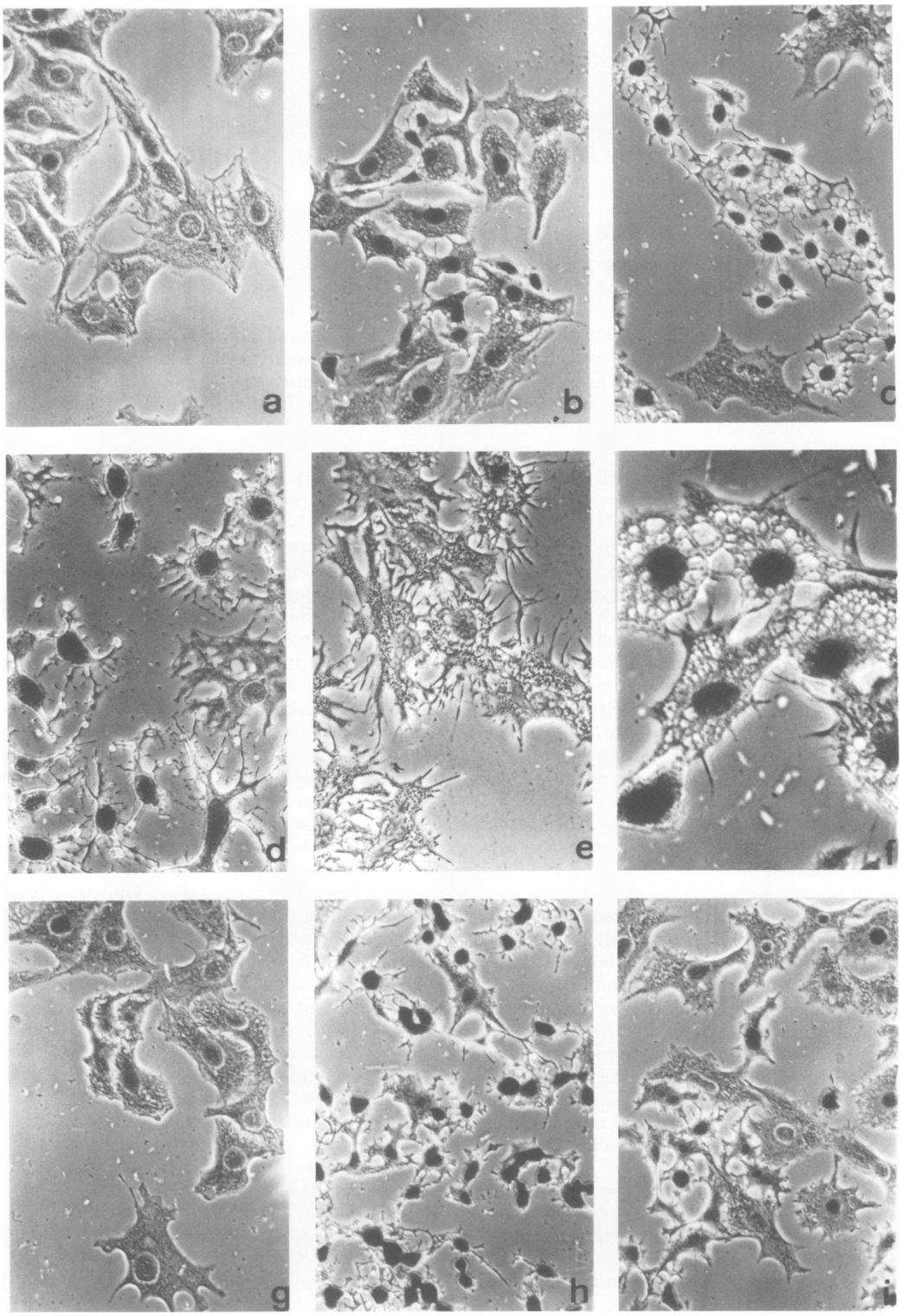

FIG. 1. *Morphology of infected CER cells untreated or treated with various compounds. Cells were incubated at 33.5°C in Eagle medium supplemented with 8% calf serum in tissue culture chamber (a). Cells infected with VSV (Indiana serotype from standard strain) (multiplicity of infection = 10 PFU/cell) were incubated either for 2 (b) and 6 (c) h or in media containing 26 μM cytochalasin B (Aldrich) (d), 62 μM colchicine (Prolabo) (e), 2.5 mM procaine (Merck) (f), and 25 μg of cycloheximide (Sigma) per ml (g) for 6 h. Cells infected with VSV mutants [tsO5(I) (h), tsO23(III) (i)] (multiplicity of infection = 10 PFU/cell) were incubated at 39.5°C in normal medium for 6 h. Samples were washed, fixed in absolute ethanol, and stained with the May-Grunwald-Giemsa method. Phase-contrast microscopy, ×320.*

TABLE 1. *Viral yields of VSV (wild-type)-infected CER cells at 8 h postinfection*[a]

Effector tested	Viral yield
Compound	
Control	7.0×10^8
Control + cytochalasin B (μM)	
26	6.5×10^8
52	7.0×10^8
104	7.3×10^8
Control	5.0×10^8
Control + colchicine (μM)	
16	5.2×10^8
32	5.4×10^8
62	5.2×10^8
Control	4.5×10^8
Control + colcemid (μM)	
17	4.5×10^8
34	4.3×10^8
68	4.5×10^8
Control	3.2×10^8
Control + cytochalasin B (μM) + colchicine (μM)	
52 + 62	3.0×10^8
Local anesthetic	
Control	7.1×10^8
Control + dibucaine (μM)	
4	7.0×10^8
16	6.9×10^8
62	7.6×10^8
Control + tetracaine (μM)	
4	7.0×10^8
16	6.9×10^8
62	7.6×10^8
Control	3.0×10^8
Control + procaine (mM)	
0.9	1.5×10^8
1.3	9.0×10^7
2.0	6.0×10^7
3.0	2.0×10^7
4.4	2.0×10^6
6.6	3.0×10^4
10.0	1.5×10^4

[a] Cell monolayers in petri dishes (diameter, 5 cm) were infected with 0.1 ml of a viral suspension containing 4.5×10^7 PFU (multiplicity of infection = 30 PFU/cell). The virus was allowed to adsorb for 40 min at room temperature, after which the cells were washed with 3 ml of cold buffer and were drained. A 3-ml amount of Eagle minimal medium containing 0.1% bovine serum albumin was added, and the medium was removed several minutes later to eliminate desorbed virions. Then, 3 ml of medium, with or without the compound to be tested, was added. Time zero corresponded to the onset of incubation. At different times postinfection, 0.1 ml of supernatant was collected and then titrated. When production was unaffected, the one step was similar to the control. Only results from 8 h postinfection were presented. It has been confirmed that each compound added to a viral suspension at the highest concentration used did not modify virus infectivity.

phology as uninfected treated cells. At concentrations ranging from 4 to 200 μM, viral production was normal (Table 1). At 250 μM, cells detached from the substrate, and viral production was inhibited by 50%. As viral production was also inhibited in artificially detached infected cells, no definite conclusion can thus be drawn for high concentrations. Cells treated with another local anesthetic, procaine, at 0.6 mM became slightly vacuolated, primarily at the cell periphery. Vacuolization was greater at 2.5 mM and led to an increase in cell volume. At a concentration of 10 mM, the cells became completely rounded and adhered only slightly to the substrate. Vacuolization had already been observed on different cell types (5). Infected cells treated with procaine, even at 6 h postinfection (Fig. 1f), retained the same morphology as uninfected cells. The following effects of procaine on viral production were observed in a dose-dependent manner: (i) there was a delay in the emergence of the first virions (1 h with 0.9 mM, 2 h with 2 mM, 4 h with 4.4 mM, and 6 h with 10 mM); (ii) at 8 h postinfection, the viral production decreased with increasing concentrations (Table 1); (iii) maximal viral production reached 10^9 PFU/ml with 2 mM procaine and no more than 10^5 PFU/ml with 10 mM procaine. This inhibitory effect of procaine on viral production either in CER cells or BHK cells is not a general property of local anesthetics as dibucaine and tetracaine had no effect. It has been reported that the combination of cytochalasin B and colchicine mimics the cellular effects of procaine (16). When these two drugs were added together (to 125 μM each), cells rounded up, but viral production was still normal. This result suggests that the action of procaine on VSV production is not a consequence of the effect of that drug on cytoskeleton depolymerization. In fact, further studies have shown that one of the effects of procaine is at the protein synthesis level, G protein synthesis being the most affected (F. Bussereau and N. Genty, manuscript in preparation). Procaine does not have a direct toxic effect leading to cell death as this effect is reversible on cell morphology as well as on viral production.

Temperature-sensitive (*ts*) mutants from the Orsay group belonging to complementation groups I, III, and V were used to explore the

involvement of the viral component in the modification of host cell morphology. At a permissive temperature, cell morphological changes and viral production were similar to those obtained with the wild-type strain. At a nonpermissive temperature, residual growth was practically zero; nevertheless, morphological changes occurred. They were dependent on the complementation group used. Mutants from complementation group I [*ts*O5(I), *ts*O53(I)], altered in their transcriptase activity (6, 9, 18), provoked the same changes as the wild-type strain (Fig. 1h) but with some delay; complete cell rounding was observed at 8 to 10 h postinfection. It has been shown previously that viral protein syntheses were required to induce cell morphology modification (compare Fig. 1g to 1c). As translation is severely depressed in these mutants, these results suggest that small quantities of newly formed viral proteins are sufficient to induce modifications of cell morphology. Mutants from complementation groups III and V (10, 11), altered in the matrix protein M [*ts*O23(III), *ts*O76(III)] and in the glycoprotein G [*ts*O45(V)], respectively, exhibited no morphological changes at 2 h postinfection. The changes were relatively minor at 6 h postinfection (Fig. 1i). At 20 h postinfection, 15 to 20% of the cells still adhered to the substrate; generally, at that time cells infected with either VSV wild type or group I *ts* mutants no longer adhered to the substrate. It is assumed that the integrity of cell shape requires the cooperation among protein components of the cell surface of the skeleton and integral constituents of the plasma membrane (4, 15, 20). The molecular organization of the host cell membrane changes with the appearance of viral antigens, inducing structural and functional changes in the infected plasma membrane (3, 7, 10, 12, 14). It has been shown that in cells infected with mutants of groups III and V, M and G proteins, respectively, do not reach the cell plasma membrane under nonpermissive conditions (10, 11). The present results suggest that the effect of the virus on cell morphology is at the level of the host cell plasma membrane, the insertion of newly synthesized viral antigens inducing a depolymerization of actin filaments.

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