JOURNAL OF VIROLOGY, Mar. 1976, p. 1038-1042

Vol. 17, No. 3
Printed in U.S.A.

NOTES

Nuclear Membrane Changes in Herpes Simplex Virus-Infected BHK-21 Cells as Seen by Freeze-Fracture

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Received for publication 15 September 1975

The freeze-fracture technique, which produces high-resolution replicas of large internal faces of membranes, was used for an ultrastructural study of the nuclei of herpes simplex virus-infected BHK-21 cells and mock-infected controls. Crystalline arrays of viral nucleocapsids were found in the nucleoplasm of infected cells, and numerous nuclear membrane "blebs" and protrusions were observed. The numerous areas of membrane distortions were not found to contain nuclear pores. In addition, specific areas of normal protein intramembranous particles are deleted from certain areas of the nuclear membrane as a result of herpes simplex virus, type 2, infection.

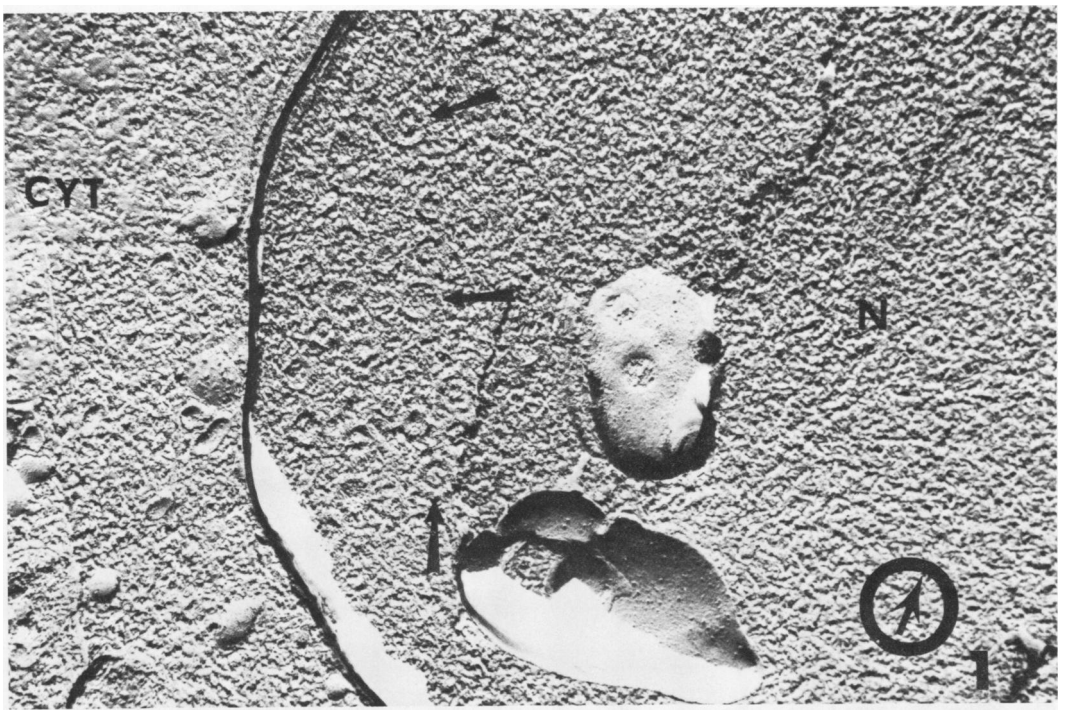
The membranes of cells productively infected with herpesviruses undergo distinct morphological, biochemical, and immunological changes as viral replication proceeds from the early stages of infection to the production of new virions and eventual host cell destruction. The nuclear membrane, in particular, becomes highly modified during the course of viral replication and is considered to be the primary site of envelopment for most herpesviruses (3, 11, 13, 14). The structural events that occur at the nuclear membrane before envelopment have been observed in detail by electron microscopy, using the conventional thin-sectioning technique. They include the deposition of an electron-dense, amorphous material on parts of the inner lamella of the nuclear membrane and the development of numerous protrusions and indentations over the membrane surface (4, 10).

In this study, we have followed, by the freeze-fracture technique, the changes that occur in the nuclear membrane of BHK-21 cells after infection with herpes simplex virus (HSV).

This technique has been shown to cleave unit membranes down the middle, exposing two novel membrane fracture faces (2, 8, 15) and permitting the observation of large expanses of internal fracture faces of both the inner and outer lamella of membranes. In addition, this technique allows the observation of changes in the intramembranous subunits (intramembranous particles) of cells under experimental conditions. Specifically, we have shown that smooth, particle-free areas occur on the internal fracture faces of the nuclear membrane of HSV-infected BHK-21 cells and that membrane deformation does not involve nuclear pores.

HSV-2 (strain 196) was originally obtained from William Rawls, Baylor College of Medicine, and stocks were passed at least 10 times in BHK-21 cells in our laboratory before freeze-fracture studies.

BHK-21 cells were grown in Eagle basal medium with Earle balanced salt solution (EBME) containing 10% fetal calf serum, 100 U of penicillin per ml, and 100 μg of streptomycin sulfate per ml. Under these conditions, cultures reached confluency in 2 to 3 days, after which they were maintained on EBME containing 5% fetal calf serum. Confluent monolayers were washed with phosphate-buffered saline and infected at a multiplicity of infection of 10 to 20 PFU. After adsorption for 2 h, the monolayers were washed twice and incubated at 37 C in maintenance medium. Control cultures were handled in the same manner, except that virus was not added. After 21 h of incubation, at which time cytopathic effect was complete, the medium was removed and the monolayers were fixed for 1 to 2 h in 2.5% glutaraldehyde in Millonig's buffer (5), pH 7.2, at room temperature. Fixed monolayers were removed from the glass surface by gentle scraping and pelleted by low-speed centrifugation. Pellets were allowed



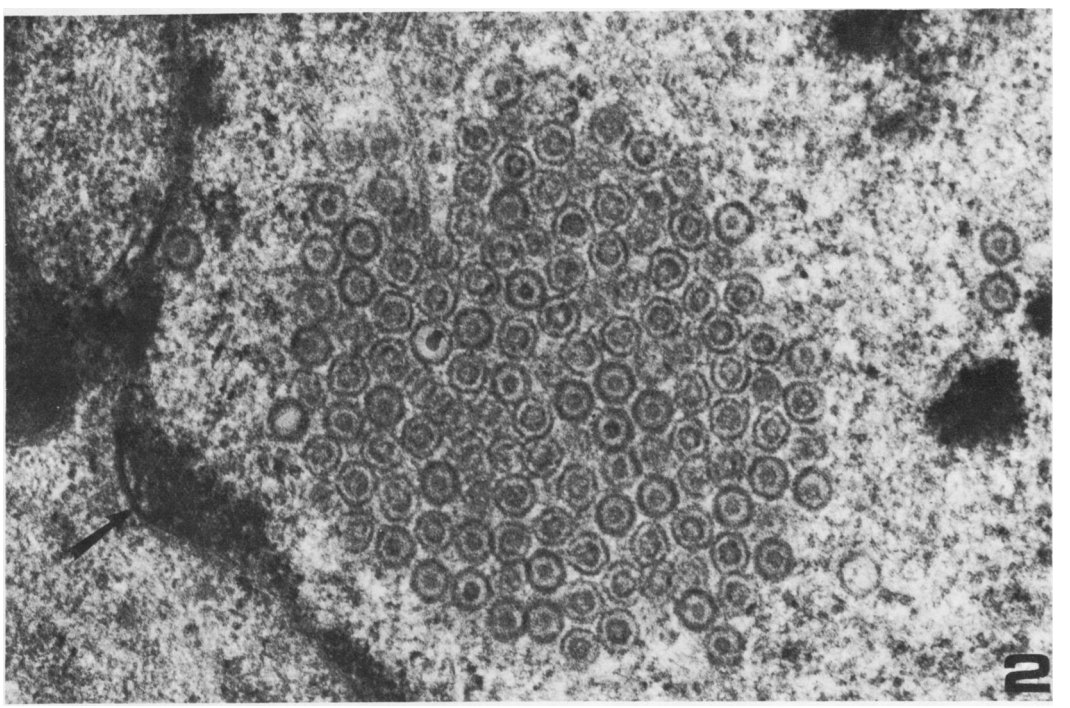


FIG. 1. *Electron micrograph of freeze-fractured BHK-21 cells after infection with HSV-2 and incubation for 21 h at 37 C. A crystalline array (arrow) of viral nucleocapsids is seen in close approximation to a segment of nuclear membrane. N, Nucleoplasm; Cyt, cytoplasm. Encircled arrow in this micrograph and in those that follow indicates the direction of shadow casting.* ×52,000.

FIG. 2. *Electron micrograph of HSV-infected BHK-21 cells after conventional thin-sectioning, showing nucleocapsid crystalline array and deposit of amorphous material along nuclear membrane (arrow). Note similarity to Fig. 1.* ×56,500.

to stand overnight in 30% glycerol at 4 C, after which they were quick-frozen in Freon-12 and freeze-fractured by the method of Moor and Mühlethaler (6) in a Balzar 360M freeze-etch device (Balzar's AG, Liechtenstein). Replicas were examined in a Philips 300 electron microscope at an accelerating voltage of 80 kV.

In describing results and in labeling micrographs, outer membrane refers to fracture faces of the outer lamella of the nuclear membrane; inner membrane refers to fracture faces of the inner lamella.

Figure 1 is a replica of a crystalline array of HSV nucleocapsids as it appears in the nucleoplasm of a freeze-fractured BHK-21 cell, in close proximity to the nuclear membrane. The hexagonal shape of the nucleocapsids corresponds very closely to that seen in thin sections of infected nuclei (Fig. 2). The size of freeze-fractured nucleocapsids (100 to 120 nm) is approximately the same as sectioned nucleocapsids. A membrane protrusion and deposition of amorphous material on the inner surface of the nuclear membrane are also seen in Fig. 2.

Figure 3 shows a large expanse of the inner

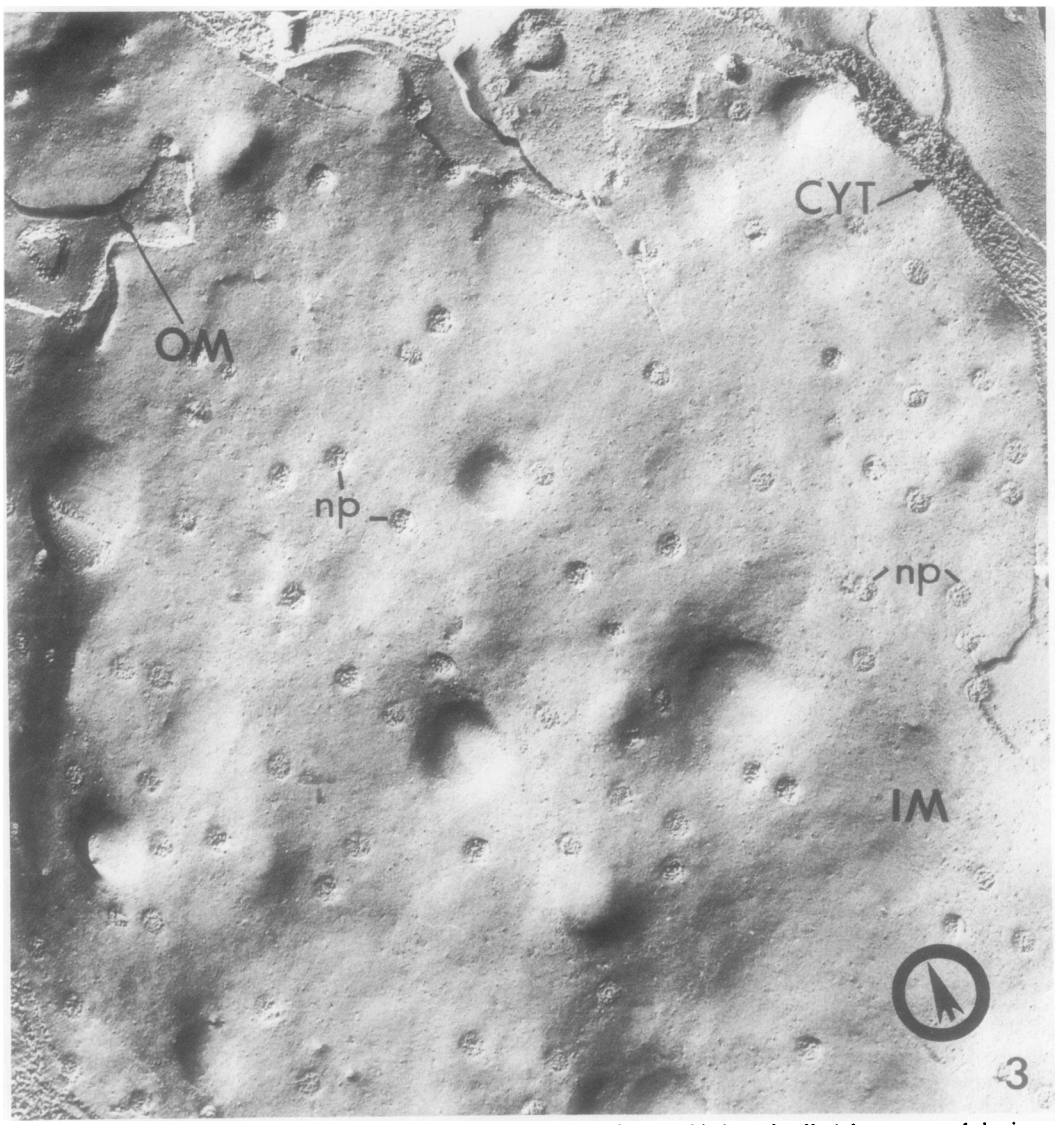


FIG. 3. *Electron micrograph of freeze-fractured nuclear membrane of infected cell. A large area of the inner nuclear membrane (IM) shows numerous protrusions and blebs, which arise as a result of virus infection. OM, Outer nuclear membrane; np, nuclear pores.* ×*41,000.*

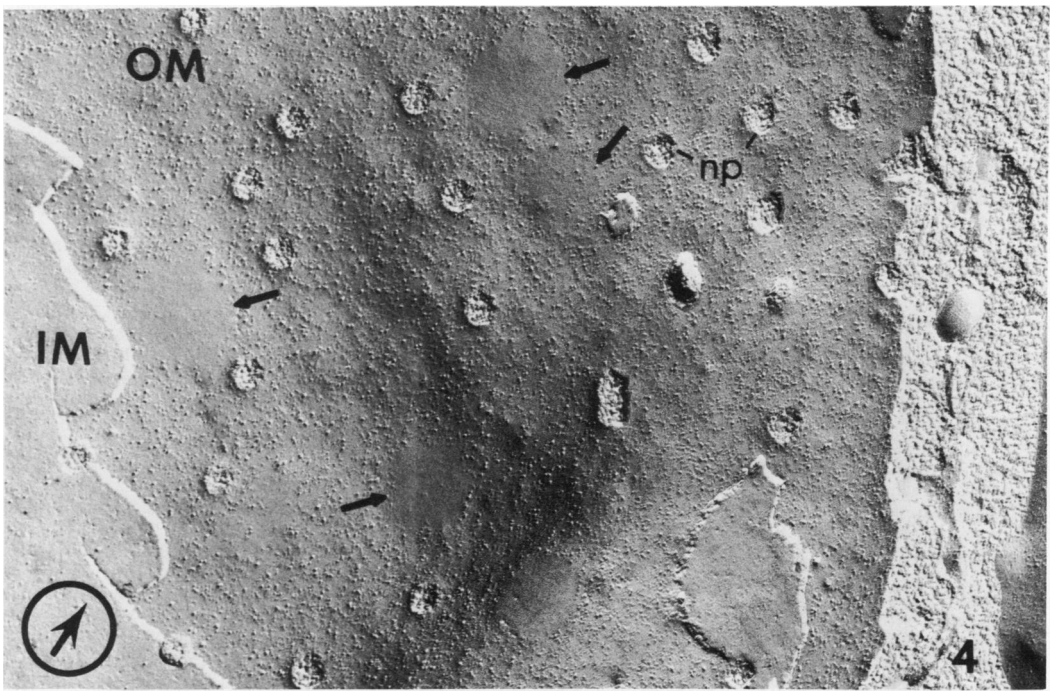


FIG. 4. *Electron micrograph of freeze-fractured nuclear membrane of infected cell, nucleoplasmic view. Distinct areas (arrows) of the outer fracture face of the outer nuclear membrane (OM) show an absence of the intramembranous particles that are a normal component of cellular membranes.* ×*44,700.*

nuclear membrane (outer fracture face) of an infected nucleus, as viewed from the nucleoplasmic side. Numerous indentations and protrusions, shown as concavities or convexities, are seen from this perspective and are apparently surface views of the blebs seen in thin sections (Fig. 2). Very lightly embossed concave areas were also seen, which may represent early stages of the larger concavities. Similar fracture faces in mock-infected cells do not show these concavities, or blebs. The size of these blebs ranges from 120 to 390 nm in diameter.

In this study, the nuclear pores of infected cells did not differ in size, morphology, or arrangement when compared with nuclear pores of mock-infected cells. Figure 3 shows that nuclear pores are found adjacent to the numerous blebs and indentations but are never a part of the indentations. This was a consistent observation throughout our study and seems to exclude involvement of nuclear pores during HSV envelopment.

Figure 4 is a view of the outer nuclear membrane (outer fracture face) as viewed from the nucleoplasmic side. Several areas of the outer membrane are completely free of the intramembranous particles, which are part of the normal, internal makeup of membranes. The size of these particle-free areas corresponds roughly to the size of the protrusions and concavities seen in Fig. 3. Examination of the internal fracture faces of mock-infected nuclei revealed no particle-free areas.

These small, intramembranous particles seen embedded in the inner fracture faces of membranes have been described in several systems. They appear to be protein or glycoprotein in nature (16) and are randomly and evenly arranged in most cells.

There are two possible explanations for our observation that particle-free areas occur in infected cells but not in mock-infected cells. The first explanation comes from the suggestion that the viral envelope is derived from areas of the nuclear membrane from which cellular proteins have been lost or displaced (1, 9). This is logical when it is considered that the intramembranous protein particles are capable of movement within the fluid domain of the membrane (12). Thus, the deposition of viral-induced antigens on the surface of the nuclear membrane (7) may result in the "displacement" or "movement" of the cellular protein particles away from the area of antigen binding.

A second explanation, which also deserves consideration, is that the particle-free areas represent areas of the nuclear membrane synthesized de novo from virus-specific proteins

inserted into existing cellular phospholipids. This appears to be unlikely since the particle-free areas occur in the fracture faces farthest from the interior of the nucleus and the maturing nucleocapsids.

This investigation was supported by Public Health Service grant CA 04036 from the National Cancer Institute.

We thank David Smith for his advice and use of equipment. Excellent technical assistance was provided by Noel D. Carter and Annalana Charla.

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