Morphogenesis of Venezuelan Equine Encephalomyelitis Virus

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Received for publication 19 June 1969

Morphogenesis of Venezuelan equine encephalomyelitis virus was studied by means of electron microscopy. Virus-specific structures (factories, viroplasts) were found at early stages of infection; these structures were composed of fibrillar and cylindrical formations, aggregates of ribosomes, and viral nucleoids. The latter emerged from fibrillar and cylindrical structures. Aggregates of viral nucleoids were found in the cytoplasm and occasionally in the nuclei of virus-infected cells. Viral envelopes and mature virions were formed on the cell membranes and on the membranes of intracellular vacuoles. Anomalous forms of virions (both polygenomic and oligogenomic) were observed.

Venezuelan equine encephalomyelitis (VEE) virus, a member of the group A arboviruses, propagates in several primary and continuous cell cultures (3). This virus is a convenient model for morphological, biological, and biochemical studies because of its short cycle of reproduction, accumulation in high titers, and plaque titration technique. It has been shown earlier that VEE virions have a diameter of 60 to 75 nm, and they consist of a lipoprotein envelope and a spherical core (the nucleoid) with a diameter of 30 to 40 nm (5).

This paper presents an electron-microscopy study of VEE virus morphogenesis.

MATERIALS AND METHODS

Virus and cells. The SPF strain of VEE virus, propagated in chick embryo fibroblasts (11), was used in this study. The plaque method described by Porterfield (8) was employed for virus titration. A method for determination of one-cycle growth of the virus was described previously (10).

Preparation of specimens for electron microscopy. Chick embryo fibroblasts grown in petri microdishes were infected with the virus and assayed at various intervals after infection. Cell monolayers were fixed in 1% glutaraldehyde in phosphate-buffered saline for 5 min, then in 1% osmium tetroxide in a Veronal-acetate buffer for 40 min. Thereafter, the monolayers were treated as described earlier (1). The monolayer embedded in a polymethacrylate disc was examined in a phase-contrast microscope. Ultrathin sections of cell groups and single cells were cut with an LKB-4800 ultratome. The sections were contrasted by the method of Karnovski (4) and examined with a JEM-7

electron microscope at instrumental magnifications of 5,000, 30,000, 50,000, and 70,000.

RESULTS

The single cycle of VEE virus reproduction in chick embryo fibroblasts infected at high multiplicity with the virus lasted from 7 to 8 hr. The latent period lasted 3 to 3.5 hr; maximal release of virus into culture fluid was observed by the end of the cycle. The yield of virus was 1,500 to 3,000 plaque-forming units (PFU) per cell (11).

A cytopathic effect as revealed by the phase contrast method was noted, usually after the end of the reproductive cycle of the virus, between 10 and 16 hr after infection. Staining of the cells with fluorescent antibody revealed virus-specific proteins at 4 to 5 hr after infection (10).

Early stage of virus reproduction. The cells infected with VEE virus at input multiplicities of 50 to 200 PFU per cell were studied at various intervals after infection—from 30 min to 16 hr. It was noted that morphological features found within the first 8 hr were also observed at later times of infection, thus showing that virus multiplication became asynchronous after the first cycle.

Virus particles entering the cells could be revealed within 30 min after infection and later. VEE virions may enter the cell by means of viropexis (Fig. 1a).

Fibrillar structures were detected in the cytoplasm by 4 to 6 hr after infection (Fig. 1b). They were never found in noninfected cells.

Structures composed of fibrillar components,

FIG. 1. Cells at 30 min (a) and 6 hr (b) after infection. (a) Part of a cell surface with entering virions. $\times 40,000$. (b) Area of a cell with tubular structures. $\times 200,000$.

aggregates of ribosomes, and viral nucleoids were also observed in the cytoplasm at later stages of infection (Fig. 2a). The fibrillar component consisted of osmiophilic fibrils and cylindrical structures with a diameter of 30 to 45 nm and a length of 1 to 2 μ m (Fig. 2c, d). In some parts of the cylindrical structures, the osmiophilic fibrillar component formed hemispheres 25 to 30 nm in diameter (Fig. 2e).

Aggregates of the cylindrical structures in the hyaloplasm were often arrayed in a parallel way and localized near the mitochondria (Fig. 2b, c, d). Clumps and linear arrays of polyribosomes were regularly revealed in this area. Linear polyribosomes contained up to 25 to 30 ribosomes and reached the maximal length of 30 to 45 μ m (Fig. 2f).

Formation of nucleoids. Nucleoids of VEE virus

were formed in close proximity to the fibrillar component emerging from the hemispheres of the cylindrical structures (Fig. 3a to c). As a rule the nucleoids had an oval shape with a diameter of 30 to 40 nm. Several nucleoids had the shape of hexagons or pentagons. When a nucleoid was oriented in the plane of the ultrathin section, an osmiophobic hollow with a diameter of 4 to 8 nm was revealed, the whole nucleoid being composed of osmiophilic fibrils packed in a certain way (Fig. 5h). Nucleoids newly formed in the cytoplasm were often localized in close proximity to the polyribosomes (Fig. 3d, e) and mitochondria (Fig. 2b) and formed aggregates surrounding cytoplasmic vacuoles (Fig. 3f, g).

In some preparations, similar structures were also revealed by the end of the period of observation (14 to 16 hr) in the nuclei of infected cells

FIG. 2. Cells at 12 (d, e, f) and 16 (a, b, c) hr after infection. (a) Virus-induced structures (arrows) in the cytoplasm. N, nucleus; m, mitochondria. $\times 4,000$. (b) Fragment of a. Virus-induced structures (Vi), endoplasmic reticulum (enr), mitochondria (m), viral nucleoids at the periphery of a mitochondrion (arrows). $\times 30,000$. (c) Area of tubular structures (ts), ribosomes (r), mitochondria (m). $\times 90,000$. (d) Fragments of cylindrical structures. $\times 200,000$. (e) Formation of a nucleoid (arrow) at the end of a cylindrical structure. $\times 220,000$. (f) Structures resembling linear polyribosomes. $\times 210,000$ ribosomes.

FIG. 3. Cells at 6 (a, d, e), 12 (b, c), and 16 (f, g) hr after infection. (a, b, c) Formation of nucleoids (arrows) of the virus from elements of the cylindrical structure. $\times 650,000$; $440,000$, and $180,000$, respectively. (d, e) Formation of nucleoids near the structures resembling ribosomes. $\times 400,000$. (f, g) Aggregates of nucleoids in the hyaloplasm and around the cytoplasmic vacuoles. $\times 200,000$ and $115,000$, respectively.

FIG. 4. Cells at 16 hr after infection. (a) Fragment of a. $\times 340,000$. (b) Area of a nucleus of a cell infected with VEE virus. Aggregates of viral nucleoids localized in the nucleus. $\times 60,000$. (c) Part of the nucleus of an infected cell. Aggregations of the fibrillar component and viral nucleoids at various stages of formation. $\times 180,000$. (d, e) Formation of viral nucleoids. $\times 400,000$.

FIG. 5. Cells at 10 to 16 hr after infection. (a, b, c, d, e, f, g) Subsequent stages of release of viral nucleoid from the cell and formation of the virion. C, cytoplasm; cm, cytoplasmic membrane. $\times 400,000$. (h) The intracellular virion. An osmiophobic cavity in the center of the virion. $\times 1,100,000$. (i, j, k) Variants of the release of the virus from the cell. $\times 400,000$.

FIG. 6. Cells at 10 to 16 hr after infection. (a) A chain of virions which was apparently formed as a result of the subsequent release of linearly oriented virions. $\times 230,000$. (b, c, d, e, f, g) Various forms of polygenomic virions. $\times 400,000$. (h) Formation of anomalous forms of virions. $\times 340,000$. (i) Extracellular aggregation of anomalous forms of virions. $\times 400,000$.

(Fig. 4a to e). In this case, areas of the osmophilic fibrillar component morphologically similar to that of the cytoplasmic one were revealed. Intranuclear aggregates of viral nucleoids often occupied vast areas of the nucleus having a diameter of 3 to 5 μ m.

Formation of virions. Formation of virions took place on the cell surface with the participation of the cell membrane or in the cytoplasmic vacuoles with the participation of their membranes. The envelope of the virions is formed of the cytoplasmic or vacuole membranes (Fig. 5a to g, and i to k; 6a).

An average diameter of the virion was 4.5 to 4.8 μ m. Fig 6b to g present variations of virions, including virions which consist of two or three nucleoids. The diameter of such polygenomic forms exceeded 10 μ m.

Incomplete forms of the virus with a diameter of 6 to 7 μ m were also revealed. The nucleoid of incomplete virions was presented, not as a spherical body, but as a thread with a diameter of 4 to 5 nm. Such incomplete forms were found both in the process of formation on the cell membrane (Fig. 6h) and outside the cell (Fig. 6i).

DISCUSSION

The data presented correlate with some results obtained by other investigators in the study of morphogenesis of arboviruses. Virus-specific intracellular structures were found in cells infected with Western equine encephalomyelitis virus (6); formation of virions on the vacuole membranes has also been found (2). The structure of the virion envelope of VEE virus (5) is similar to that of Sindbis virus (7). The incomplete forms were also described for dengue fever virus (9). The findings described here seem to be in some contradiction with our previous statement (5) about the helical symmetry of the nucleocapsids of VEE virus (Fig. 5h). However, the picture can be interpreted in different ways until evidence is found as to whether free ribonucleic acid or ribonucleoprotein is packed inside the viral core.

A plausible model of VEE virus morphogenesis based on the data presented here is shown in Fig. 7.

An extracellular virion [Fig. 7 (1)] attaches to the cell [Fig. 7 (2)] and enters the cell by means of viropexis [Fig. 7 (3, 4)]. Viral ribonucleoprotein or ribonucleic acid is released from the virion [Fig. 7 (5)] and induces virus-specific synthesis. As a result, virus-specific structures appear in the cytoplasm [Fig. 7 (6, 7)] and probably in the nucleus [Fig. 7 (7a)]. The virus-specific structures are designated as "factories" or "viroplasts." It is assumed that these structures are

FIG. 7. Morphogenesis of Venezuelan equine encephalomyelitis virus.

the sites of the synthesis of viral ribonucleic acid and protein and formation of nucleoids of virions [Fig. 7 (8, 8a)]. Viral nucleoids are localized around the cytoplasmic vacuoles [Fig. 7 (9)], near polyribosomal accumulations or near the mitochondria [Fig. 7 (10)]. Formation of virions takes place with the participation of the vacuole membranes [Fig. 7 (11)] or the cell membrane [Fig. 7 (12)]. The complete virions [Fig. 7 (13)], as well as anomalous forms of virions [Fig. 7 (14, 15)], are released from the cell.

There are many points in this model which need to be elucidated. It is not clear how virus-specific tubular structures are formed and how the cell contributes to their formation. One may hypothesize that both the synthesis of virus ribonucleic acid and core protein and the formation of the viral nucleoid occurs in these structures; however, this needs further study. Some doubts arise about the possibility of the formation of the virus nucleoids in the cell nucleus. We consider, therefore, the suggested model as an

initial step in the study of morphogenesis of VEE virus.

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