Growth of Rubella Virus in BHK21 Cells: Electron Microscopy of Morphogenesis

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SUMMARY

The growth of rubella virus in suspended BHK21 cells was studied by electron microscopy under conditions of one-step growth. After a latent period of 15 hr the first new virus particles were found with their number increasing successively till about 25 hr. This corresponded well with the infectivity assays obtained from these samples.

The virus is formed through two pathways; first, by budding into vesicles of the Golgi-apparatus which then are emptied outside the cells; second, to a lesser extent by budding directly from the marginal cell membrane. No accumulation of nucleoids in the cytoplasm is detected, but they are formed at the site of budding.

INTRODUCTION

The structure of the rubella virus has for a long time remained unclear primarily because of the difficulty in obtaining preparations with concentrations of particles high enough to allow successful electron microscopy. Since the results by Best et al. (1967), Holmes & Warburton (1967) and Holmes et al. (1968) describing enveloped, round particles, with a distinct nucleoid, about 60 nm. in diameter, several other workers have come to the same conclusions about the structure. For references see Seminar on Rubella Virus; co-chairmen: W. E. Rawls and A. Vaheri, First Int. Congr. Virol. Progr. Med. Virol. (in the Press).

This study was undertaken with the intention of clarifying morphogenesis of the rubella virus under conditions of one-step growth of the virus.

METHODS

Conditions for growth of rubella virus in BHK21/13S cell suspension cultures, plaque assay, virus identification and sterility tests have been described in the previous paper (Vaheri et al. 1969). The cell-associated virus was assayed after the infected cells had been washed twice in phosphate buffered saline (Dulbecco & Vogt, 1954) and subjected to three cycles of rapid freezing and thawing. For identification of virus the peak-titre samples were subjected to specific neutralization tests.

For electron microscopy specimens were taken simultaneously with the samples for infectivity assay (Fig. 1). Each specimen contained about 10 to 15 x 10⁶ cells, which were pelleted by low speed centrifugation. Similar samples from uninfected cultures were taken at 0, 3, 24, 48 and 72 hr. The pellets were fixed (unwashed) with 3 % glutaraldehyde for 90 min. at 4° (Sabatini, Bensch & Barnett, 1963). The prefixation
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was followed by a 60 min. fixation with 2 % OsO₄ at 4°, dehydration through progressive ethanol series and embedding in Epon 812 (Luft, 1961). Thin sections were cut with a Porter-Blum MT I ultramicrotome. The sections were either stained with lead citrate (Reynolds, 1963) or double stained with uranyl acetate and lead citrate (Frasca & Parks, 1965). Electron micrographs were taken with a Siemens Elmiskop 1A microscope at original magnification of 10,000 to 30,000 and enlarged as desired. The diameter values of rubella virus were based on at least 100 particles.

RESULTS

The preservation of the cellular morphology was fair in these experiments. No changes due to the growth conditions or preparative process could be detected in the control samples. There was, however, both in the control preparations and in the infected ones a small number of cells at different stages of degeneration; this appeared most frequently as vacuolation of the cytoplasm and condensation of nuclear material.

Morphology of rubella virus in thin sectioned material

All infected preparations contained spherical viruses measuring from 50 to 74 nm. (average 59 nm.) in diameter 15 hr or more after infection. The particles were enveloped, having a distinct core. The envelope was of unit membrane structure showing, however, a somewhat fuzzy surface. The core (30 to 35 nm. in diameter) was round and had an electron-lucid centre. (Pl. 1 B).

At a later stage there appeared more variation in the morphology of rubella virus. Distorted, elongated, almost filamentous forms were frequently seen. The variation in size was also greater than during the early stages of virus formation.

Latent hamster virus

A BHK 21 cell-associated virus of the type earlier described by Bernhard & Tournier (1964) was occasionally found in the control and infected preparations. This virus was mostly seen in the cisternae of rough-surfaced endoplasmic reticulum. The diameter of this virus was 70 to 90 nm. The distinct core was connected to the envelope by clearly detectable spokes (Pl. 1 C).

Early morphological changes in RV infected cells

No definitive changes in the cellular morphology as a sign of infection could be detected during the first 10 to 15 hr. The smooth-surfaced membrane structures, appeared to increase, especially in the Golgi apparatuses. This change became quite clear in those samples having the highest virus titres (24 to 30 hr).

Early virus formation

The first virus particles were found in samples taken 15 hr after infection. Single particles were seen outside the cells but more often in vesicles of the Golgi apparatuses (Pl. 1 A). From 15 to 25 hr after infection there were increasing numbers of virus particles in accord with the increase of p.f.u. in these samples (Fig. 1). These particles were found in the Golgi apparatuses as well as outside the cells. The nuclei were free of virus. Particles were found in approximately 30 to 50 % of the Golgi apparatuses.
A. Rubella virus infected BHK 21 cells sampled 15 hr after infection. The Golgi apparatus contains a virus particle (arrow). B. A group of rubella virus particles at the cell membrane. One particle is seen budding typically at the tip of a cytoplasmic projection. The nucleoid is assembled at the site of budding. C. A portion of an infected cell showing that rubella virus is also formed in cells containing latent BHK 21 virus (arrow).

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(Facing p. 48)
Cytoplasm of a BHK-21 cell sampled 22 hr after infection showing numerous enlarged, virus-containing Golgi apparatuses in close vicinity to the centriole.

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Rubella virus-induced degeneration seen as increasing vacuolation of the cytoplasm.

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During this stage of infection the general cell morphology was not much affected except for the increase in the smooth membrane structures (Pl. 2).

Virus particles at different stages of budding through the membranes were often observed in the Golgi apparatuses (Pl. 2), and also at the cell membrane (Pl. 1B). Ring-shaped nucleoids appeared at the site of budding but no accumulation of nucleoids could be detected in the cytoplasm. Simultaneously with the accelerating virus production the Golgi apparatuses increased in size and membrane content. Rubella virus particles could occasionally be seen developing in cells already containing latent hamster virus (Pl. 1C).

![Growth curve of rubella virus in suspension cultures of BHK 21/13S cells.](image)

**Fig. 1.** Growth curve of rubella virus in suspension cultures of BHK 21/13S cells.

Late virus formation

From about 25 hr after infection the infected cells started to vacuolize and the cells appeared to have degenerated (Pl. 3). The Golgi apparatuses became swollen and resulted in large vacuoles which often contained a great number of virus particles as well as amorphous material. The particles in these vacuoles often showed greater distortion than those seen during the early virus production. Elongated, almost filamentous forms were occasionally seen (Pl. 3). Outside the cells, often attached to the cell membrane, virus aggregates could be found together with debris. In the 60 to 70 hr samples many cells were totally broken down, and in the remnants virus particles were found.

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DISCUSSION

The morphogenesis of the rubella virus seemed to occur only in the cytoplasm of the infected cells. The mature virion was formed by a budding process, and the nucleoid was detected only at the site of budding. This budding occurred during the early virus formation into Golgi vesicles and apparently also, though observed only during the later stage of infection, on the cell membrane. Contrary to the results of Holmes et al. (1968) and Murphy, Halonen & Harrison (1968), our observations indicate that budding into cytoplasmic vacuoles was more prominent during early virus formation (till 25 hr) although a quantitative evaluation is hazardous. The increase in the number of Golgi apparatuses as well as their gradual enlargement during the infection suggests that they are some kind of virus assembly centres in the cell. For some enveloped viruses, especially of the arbo-group, a similar characteristic of budding both into cytoplasmic vacuoles and at the cell membrane has been proposed. On the other hand, Acheson & Tamm (1967) have suggested that with Semliki Forest virus only virus budded from the cell membrane was complete, which appears not to be the case with rubella virus.

The nucleoid of the rubella virus seen in sectioned material is very distinct and clearly separated from the envelope. It is, however, of interest to note that when purified viruses were treated with deoxycholate no distinct nucleoids were seen in negatively stained preparations (Vaheri et al. 1969). In contrast, Semliki Forest virus under the same conditions clearly showed the nucleoid (von Bonsdorff, C.-H. & Kääriäinen, L. Seminar on Arboviruses; co-chairmen; B. Blascovic and J. Casals, First Int. Congr. Virol., Progr. Med. Virol. (in the Press)). This seems to indicate that these nucleoids, although morphologically alike in sectioned material, must have some difference in structure.

The occurrence of the BHK21 associated virus (Bernhard & Tournier 1964, Thomas et al. 1968) in both infected and uninfected control preparations did not, in our opinion, disturb the interpretation of the results to any great extent. Morphologically this virus was easily distinguishable from the rubella virus by the larger size and the distinct spokes connecting the nucleoid with the envelope. The only disadvantage of the presence of this virus was that it made the interpretation of the rubella virus specific cellular changes, particularly during early infection, difficult. It is interesting that rubella virus seemed to multiply without interference in cells already containing the BHK21-virus.

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REFERENCES


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