Replication of Simian Foamy Virus in Monkey Kidney Cells

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The structure of foamy virus, its mode of maturation, and the origin of vacuoles in monkey kidney cells are described.

The morphology of a virus (strain MK5) obtained from rhesus monkey kidney cultures and propagated in HEp-2 cells has been described by use of ultrathin sections (2) and negative staining techniques (1). During the latter investigation, the agent was shown to be a type 1 foamy virus; since this virus is a frequent contaminant of rhesus monkey kidney cultures (4), it seemed worthwhile to examine its mode of replication in its natural host tissue in order to compare these results with those previously obtained in HEp-2 cells (2). This examination revealed host-dependent variations in the morphogenesis of the virus which are described in this communication.

Cultures of primary rhesus monkey kidney cells (assumed to be free from extraneous viruses since they showed no spontaneous cytopathic effect, failed to haemadsorb chick or guinea pig erythrocytes, and contained no agent which could be detected by ultrathin section) were infected with strain MK5 or a foamy virus freshly isolated from rhesus monkey kidney cultures. After 4 to 6 days, when about 50% of the monolayers showed "foamy" degeneration, the cells were scraped from the glass, fixed with 1% osmium tetroxide for 2 hr at 4 C, and embedded in Maraglas; the sections were then stained with uranyl acetate and lead citrate.

Cells were not synchronously infected so that various stages of virus formation were found in a single preparation. Groups of the internal components of the virus were commonly seen in the cytoplasm. The internal components were 50 to 60 nm in diameter, surrounded by an ill-defined halo, and associated with a matrix which stained slightly more densely than the surrounding cytoplasm (Fig. 1). At a presumably later stage in the process of virus maturation, the internal components were associated with extensive areas of the endoplasmic reticulum (Fig. 2), but a few were found at the plasma membrane. They matured by budding (Fig. 2, arrow) and thus acquired an envelope derived from a cell mem-

brane. Although the budding process could be initiated in association with morphologically normal endoplasmic reticulum, the endoplasmic reticulum tended to lose ribosomes and become dilated to form vacuoles (Fig. 3). In this figure, a few remaining ribosomes are indicated (upper arrows); the membrane is associated with budding virus (lower arrow), whereas the vacuoles contain mature particles. Maturation also occurred at the plasma membrane and at the outer nuclear membrane, thus producing complete virus outside of the cells (Fig. 8) and between the two nuclear membranes (Fig. 4).

In severely damaged cells, the cytoplasm contained little except large vacuoles or clear spaces traversed by membranes. These membranes were still associated with budding or mature particles (Fig. 5). A few vacuoles contained virus with damaged or incomplete envelopes (Fig. 6) which may represent lysosomal digestion.

Mature particles (Fig. 7, 8) had a diameter of 90 nm and consisted of internal components enveloped by a membrane bearing projections. The triple-layered structure of the membrane was resolved in many particles, especially in those which were extracellular, even after incorporation into the virus envelope. No significant change was seen to occur in the complete particles after their release, although in a few particles the nucleoid was slightly eccentric (Fig. 7).

Monkey kidney cells inoculated with foamy virus and examined by light microscopy are characterized by the formation of large vacuoles (3). These vacuoles are seldom seen in infected HEp-2 cells (Clarke, *unpublished data*) and this observation is given significance by the present findings. These findings show that in MK cells virus matures mainly at the endoplasmic reticulum which dilates in response to the infection thus forming vacuoles, whereas in HEp-2 cells maturation occurs mainly at the plasma membrane (2).

The two types of infected cells differed in that the cytoplasm of MK cells usually contained

FIG. 1. Group of cytoplasmic internal components is seen in a relatively densely stained matrix. Bar in the figure represents $1\ \mu\text{m}$. $\times 30,000$.

FIG. 2. Internal components are seen in the cytoplasm associated mainly with the endoplasmic reticulum but also with the plasma membrane. An occasional particle is budding through the endoplasmic reticulum (arrow). Bar in the figure represents $1\ \mu\text{m}$. $\times 28,000$.

FIG. 3. Mature particles (i.e., enveloped internal components) are seen in "vacuoles." The outer surfaces of these vacuoles still retain a few ribosomes (top arrows). A budding particle is also shown (lower arrow). Bar in the figure represents 500 nm. $\times 36,000$.

FIG. 4. Two nuclear membranes are seen at the left, where the internal component is budding from the cytoplasm into a space between them to form a mature particle. Free internal components are seen in the cytoplasm, and a group of mature particles is visible (center) between the nuclear membranes. Bar in the figure represents 1 μ m. $\times 72,000$.

FIG. 5. Cytoplasm of a degenerated cell is shown; the membranes are associated with budding and mature particles. Bar in the figure represents 500 nm. $\times 40,000$.

FIG. 6. Vacuole which is filled with mature particles is shown. The envelope of many of these particles is damaged or incomplete. Bar in the figure represents 500 nm. $\times 50,000$.

FIG. 7. Extracellular mature particles are shown. The two particles on the left lack projections, and the unit membrane structure of the envelope is seen. This is visible, though less distinct, in the particles which possess projections. Bar in the figure represents 100 nm. $\times 100,000$.

FIG. 8. Mature extracellular particles are seen. The unit membrane structure of the envelope is visible even in the presence of projections. Bar in the figure represents 100 nm. $\times 100,000$.

many internal components but no inclusions, whereas in HEp-2 cells relatively few internal components were seen and these tended to be associated with large accumulations of granular material (2). The structure of the membrane of the virus envelope also appeared to depend on the host tissue; thus, in contrast to the present findings it was seen as a single line in HEp-2-grown virus (2). It is possible that the triple-layered stage may represent only an intermediate stage of the formation of the envelope, but since the unit membrane was best resolved in extracellular particles, i.e., in what is presumably relatively "old" virus, this is unlikely.

It was suggested that strain MK5 resembled Bittner virus in some respects (2), but the persistent failure to demonstrate a double-shell

structure in the internal component of MK5 makes it likely that the two agents belong to distinct virus groups.

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