Vesicular Stomatitis Virus Maturation Sites in Six Different Host Cells

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(Accepted 16 December 1969)

SUMMARY

Six different cell types, L, Vero, HeLa, BHK21, PK(H13) and CF, were infected with vesicular stomatitis virus. A minimum of 100 individual virus-containing cells of each type was scored for the sites of viral maturation as observed by electron microscopy of thin sections. The principal site of viral maturation was the intracytoplasmic vacuolar membranes for PK(H13) and the plasma membrane for L and Vero cells. Both types of membranes served as sites for HeLa and BHK21 cells. It is concluded that the site of maturation of vesicular stomatitis virus is a host-dependent phenomenon.

INTRODUCTION

Vesicular stomatitis virus (VSV) has been shown to mature at two sites, the plasma membrane and the intracytoplasmic vacuolar membranes. Howatson & Whitmore (1962) described the plasma membrane as the principal site of virus maturation in L cells, but also noted a few virus particles in intracytoplasmic vacuoles. Mussgay & Weibel (1963) demonstrated maturation of the virus at the plasma as well as intracytoplasmic vacuolar membranes in KB cells. Similar observations were made by Schultze & Liebermann (1966) in infected bovine kidney cells and by David-West & Labzoffsky (1968) in chick fibroblasts (CF). In an earlier study, we found that the principal site of VSV maturation in pig kidney (PK) and CF cells was the intracytoplasmic vacuolar membranes (Hackett et al. 1968). Comparisons of various studies by other investigators on the morphogenesis of vesicular stomatitis virus are hampered by the many experimental variations in cell type, time of observation and multiplicity of infection.

METHODS

Virus. The Ogden isolate of the New Jersey serotype of vesicular stomatitis virus was used. The virus was grown in CF cells under dilute-passage conditions in order to minimize the amount of auto-interfering particles present, and assayed on CF monolayers as previously described (Hackett, 1964).

Cells. A pig kidney cell line, PK(H13), and primary chick embryo fibroblasts (CF) were grown in Earle's saline with 5% lactalbumin hydrolysate and 5% foetal bovine serum added. BHK21, HeLa, Vero, and L cell lines were grown in Eagle's medium with 5% foetal bovine serum and 10% tryptose phosphate broth. All these cell lines were obtained from the Cell Culture Division of the Naval Biological Laboratory.

Infection of cells. Cells from young, non-confluent monolayer cultures were suspended with trypsin-versene, washed twice and resuspended in Eagle's medium. The cells were counted in a Coulter Counter and infected with vesicular stomatitis virus at an input multi-
plicity of 10 p.f.u./cell. Adsorption was allowed to proceed at 4°C for 30 min. with gentle agitation. The unadsorbed virus in the inoculum was then inactivated by treating for 15 min. at room temperature with sufficient specific antiserum to neutralize 10^5 p.f.u. of virus. Antiserum was then removed by washing the cells three times with phosphate buffered saline (PBS). The cells were resuspended to 10^4 cells/ml. in Eagle's medium and incubated at 37°C. Samples were removed at intervals for assay of infectious virus and electron microscopy.

**Production of antisera.** Antisera against vesicular stomatitis virus were produced in rabbits. Each animal received three weekly subcutaneous injections of an emulsion of equal volumes of concentrated virus and Freund’s complete adjuvant. Serum was collected 14 days after the final inoculation and 1:100 dilution of the serum was found to neutralize 10^6 p.f.u. of virus.

**Electron microscopy.** Cells for electron microscopy were pelleted and prepared as previously described (Zee, Hackett & Talens, 1968). The cell samples to be surveyed for budding virus were taken just prior to the peak of virus release (7 hr), so that possible readsorption of newly produced virus to cell surfaces would be minimal. Surveys of sectioned cells were made at 8,000 x magnification with a Zeiss EM 9A electron microscope. A minimum of 100 individual cells of each type were observed and classified for the site(s) of viral maturation.

**RESULTS**

**Growth of vesicular stomatitis virus**

Typical single-step growth curves of VSV in six different cell types are shown in Fig. 1. Infectious virus increases rapidly after adsorption and in all cases reaches maximum titre in 8 to 10 hr. The slope of the growth curve is similar for all six cell types, although the total virus produced varies slightly. These experiments were carried out under the same conditions, so that the electron microscopic survey could be done on cell samples taken during the period where virus maturation was approaching its maximum. The 7 hr samples of infected cells were chosen for this purpose. It is expected that the amount of cell-associated virus should be maximal and the opportunity for release and readsorption of virus minimal. In order to verify that the 7 hr sample was representative of morphogenesis for that cell type, early (4 to 5 hr) and late (10 hr) samples were also examined.

**Site of maturation of vesicular stomatitis virus in six different host cells**

Virus particles were observed attached to the plasma membrane in infected L cells (Fig. 2a). A continuation of the viral envelope with the host cell membrane is clearly visible (Fig. 2b).

Virus particles were found within intracytoplasmic vacuoles of several types of infected cells studied. Membrane bound vacuoles in the cytoplasm of infected PK(H13) and BHK21 cells containing randomly oriented virus particles are illustrated in Fig. 3. Virus particles in the cytoplasmic vacuoles sometimes appear to be tightly packed side-by-side at the membrane boundary. This phenomenon is shown in Fig. 4, in which virus particles were observed in the process of budding at the vacuolar membrane of an infected CF cell. In the various host cells observed, with the exception of PK(H13) cells, virus particles were found to mature at the plasma membrane as well as at the intracytoplasmic membranes. Fig. 5 shows an infected Vero cell with virus particles maturing at both the plasma and vacuolar membranes.

Morphologically identifiable virus particles were present in over 90% of the cells
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observed. Surveys were made at low magnification, so that only those sections which contained a whole cell were included. One hundred separate cells of each type were photographed, and enlargements were studied in order to distinguish between virus rods and pseudopodia at the cell surface. Each cell was scored for the position of maturing virus particles: plasma membrane, intracytoplasmic membrane, or both. The findings are summarized in Table I for samples taken at 7 hr, the peak of viral production. Similar results were obtained when early and late samples were examined.

![Growth curves of vesicular stomatitis virus in six types of host cells.](image)

Virus was found budding from the plasma membrane in 84% of the L cells observed (54% at the plasma membrane only and 30% at the intracytoplasmic vacuolar membrane and plasma membrane). Vero cells and HeLa cells had a similar range at the plasma membrane. Although L and Vero cells show some virus (16%) at intracytoplasmic membranes, the major part of their virus yield matures by budding from the plasma membrane. In HeLa cells, virus was observed at both sites in 60% of the cells and virus particles were maturing at either one or the other site in 40% of the cells.
BHK₂¹, CF and PK(H₁₃) cells display different maturation patterns for vesicular stomatitis virus from those described above. Virus was observed to mature at intracytoplasmic vacuolar membranes only in 56% of BHK₂¹ cells; at the plasma membrane only in 14%.

Fig. 2. (a) Vesicular stomatitis virus budding from plasma membrane of an infected L cell; (b) Fusion of cell membrane with that of the budding particle.
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and at both sites in 30% of those cells. CF and PK(H13) cells are the most extreme among the cell types tested. Eighty-nine per cent of the CF cells and 99% of PK(H13) cells showed the vacuolar membrane as the sole site of virus maturation.

Virus maturation at the plasma membrane in PK(H13) cells must be very rare. In this host

Table 1. Maturation sites observed at 7 hr post infection in different host cells infected with vesicular stomatitis virus

<table>
<thead>
<tr>
<th>Cell types*</th>
<th>L (%)</th>
<th>Vero (%)</th>
<th>HeLa (%)</th>
<th>BHK21 (%)</th>
<th>CF (%)</th>
<th>PK(H13) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maturation sites</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Plasma membrane only</td>
<td>54</td>
<td>41</td>
<td>21</td>
<td>14</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Intracytoplasmic membrane only</td>
<td>16</td>
<td>17</td>
<td>19</td>
<td>56</td>
<td>89</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Both membranes†</td>
<td>30</td>
<td>42</td>
<td>60</td>
<td>30</td>
<td>10</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* Results are expressed as percentage of the total number of cells observed in each category.
† Per cent of cells showing virus particles at both sites simultaneously.

Fig. 3. A portion of the cytoplasm of an infected PK(H13) cell shows mature virus particles randomly oriented in one of the vacuoles. m, mitochondria, n, nucleus.
cell, well over 100 cells were examined, and one virus particle was found at the plasma membrane of one cell. In CF cells, virus at the plasma membrane was found in only 11% of the cells observed.

**DISCUSSION**

This study confirms the findings of other investigators that both plasma and intracytoplasmic vacuolar membranes serve as maturation sites for VSV (Howatson & Whitmore, 1962; Mussgay & Weibel, 1963; Reczko, 1961; Schulze & Liebermann, 1966), and demonstrates that the principal site of maturation is not the same for every host cell type. The discrepancies between previous reports have been attributed to several possibilities such as differences in cell types used (Davis-West & Labgoffsky, 1968) and rapid release of newly formed virus particles (Hackett *et al.* 1968).

In this study six different cell types were propagated and infected under similar conditions with one stock virus pool, and infected cells were surveyed for the site of virus maturation when the first cycle of viral replication was at its peak. Emphasis during the survey was placed on the number of different cells examined, rather than on the number of virus particles.
particles present in a given cell section. Observations made under these standardized conditions have shown that the maturation site for VSV is host-dependent.

It is clear that in PK(H13) cells, the site of maturation is the membrane boundary of intracytoplasmic vacuoles, and that the plasma membrane does not play a significant role in maturation of VSV under these conditions. These cells are, however, capable of supporting the growth of VSV to high titre in the same time interval as the other host cells tested.

Fig. 5. Portions of two Vero cells infected with vesicular stomatitis virus. One cell has virus particles inside cytoplasmic vacuoles and the other has virus particles budding from the plasma membrane.

The maturation sites of VSV are similar to other members of the Rhabdovirus group. Hummeler, Koprowski & Wiktor (1967) have observed that rabies virus matured on cell membranes by the process of budding. This occurs either in cytoplasmic vacuoles or, to a lesser extent, on the plasma membrane. Hart Park virus has been shown by Jenson et al.
(1967) to bud off from plasma membranes of infected neurones and glial cells in new-born mice suffering from a fatal encephalitis following intracerebral inoculation, and similar results have been reported for the related Flanders virus (Murphy, Coleman & Whitfield, 1966). Furthermore, there is some indication that different sites of virus maturation are present in different cells of red foxes infected with rabies virus. Virus particles matured in cytoplasmic vacuoles in cells of the central nervous system, but at the plasma membrane in the acinar cells of the salivary gland (Murphy et al. 1968). The basis for these differences is not known at the present time. Klenk & Choppin (1969) have shown chemical differences between the plasma membranes of primary monkey kidney cells, BHK21 and hamster kidney cells. This difference is reflected in the composition of the envelope of SV5 virus grown in these cells. Such differences in membrane composition and structure may be responsible for determining the maturation site of vesicular stomatitis virus.

This research was supported by General Research Support Grant 5457 from the National Institutes of Health, U.S. Public Health Service, Bethesda, Maryland, and by the Office of Naval Research.

We thank Mr Grant Holloway for his technical assistance. We also wish to thank Dr T. H. Dunnebacke and Dr F. L. Schaffer for reviewing the manuscript.

REFERENCES


(Received 13 November 1969)