Sindbis Virus Maturation

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Sindbis virus, a group A arbovirus, is composed of a nucleocapsid and a glycolipoprotein membrane (Pfefferkorn & Hunter, 1963a; Strauss et al. 1968; Schelle & Pfefferkorn, 1969). The membrane protein is specified by the virus genome (Pfefferkorn & Clifford, 1964; Strauss et al. 1968; Strauss, Burge & Darnell, 1969) whereas the phospholipids and carbohydrate are derived from the host of origin (Pfefferkorn & Hunter, 1963b; Burge & Strauss, 1969; Strauss, Burge & Darnell, 1970) during maturation by budding through the membranes of cytopathic vacuoles (CPV) or the plasma membranes of the infected cell (Grimley, Berezsksky & Friedman, 1968; Hackett et al. 1968; Holmes, Wark & Warburton, 1969; Nakai, Shand & Howatson, 1969). We have studied the maturation of Sindbis virus with the use of ferritin-conjugated antibody.

After exhaustive adsorption with normal chick embryo cells, the globulin fraction precipitated from rabbit anti-Sindbis serum (Keckwick, 1940; Strauss et al. 1960) was conju-
Chick embryo cells infected with Sindbis virus showed CPV of types I and II as described by Grimley \textit{et al.} (1968). Virus nucleoids surrounding CPV II were not labelled with virus conjugated with ferritin (Singer, 1959; Hsu, Rifkind & Zabriski, 1963; Rifkind, Hsu & Morgan, 1964). Chick embryo cells infected with 1 p.f.u./cell were harvested 12 h after infection when the cells were collected, washed and fixed in 1\% (v/v) glutaraldehyde. After washing, the cells were treated with 10\% (v/v) dimethyl sulfoxide, frozen in an ethanol-dry ice bath, and thawed in 0.5 ml of the ferritin conjugated antibody. Two-tenths ml of 1/25 guinea-pig complement (Hyland, Los Angeles, California) was added and after 15 min at 37°C the cells were washed three times, fixed again in 1\% glutaraldehyde, then postfixed in 1\% (w/v) osmium tetroxide, dehydrated and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate before examination in a Hitachi HS 7s electron microscope.

Fig. 2. Cell membranes binding ferritin-conjugated antibodies with Sindbis virus particle immediately below.
antibodies. The virus particles which had matured by passage through cell membranes were labelled heavily (Fig. 1). In other studies (Nii et al. 1968), herpesvirus-infected cells showed extensive ferritin labelling of nuclear and cytoplasmic membranes. Infection by mumps virus (Duc-Nguyen & Rosenblum, 1967) resulted in grossly labelled, differentiated plasma membranes, whereas infection by influenza virus (Duc-Nguyen, Rose & Morgan, 1966) induced progressive labelling of the entire cell membrane. In our studies the membranes of cells infected with Sindbis virus did not bind virus antibodies except where virus nucleocapsids were immediately below (Fig. 2). Intact virus particles were labelled by the ferritin conjugated antibody only after acquisition of the virus envelope or during active budding (Fig. 3). Burge & Pfefferkorn (1967) showed by haemadsorption that determinants specific of Sindbis virus were in the infected cell surface within 2.5 h of infection. However, the size of
the red cell precluded any determination of the area of cell surface which ultimately acquired virus specificity.

Our studies using the electron microscope showed that Sindbis virus could be labelled with ferritin-conjugated antibody only after maturation of the virus envelope. Host cell membranes did not bind anti-virus globulins until virus nucleocapsids were immediately beneath them. Our results suggest that either (a) the incorporation into the cell membrane of the glycoprotein of the virus envelope is under the control of the progeny nucleocapsid or (b) the progeny nucleocapsids are assembled adjacent to those portions of the membrane which have incorporated virus determined components.

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REFERENCES


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Short communications


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