Herpes-Type Virus Particles Associated with a Fungus

Abstract. *A culture of the fungus* Thraustochytrium, *isolated from an estuary, was infected by an enveloped virus. The nucleocapsid measured 110 nanometers in diameter and contained a core of DNA. The virus replicated in the nucleus. These findings strongly suggest that the particles are a herpes-type virus.*

Fungi have been reported to act as vectors (*1*) or as hosts (*2*) for viruses. Heretofore no enveloped virus has been reported to infect fungi. We now report the presence of a probable herpes-type virus infecting cultures of an estuarine monocentric fungus, *Thraustochytrium* sp. (family, Thraustochytriaceae; order, Saprolegniales).

The fungus was isolated from the York River Estuary, Virginia (salinity about 20 parts per 1000). The culture was maintained and zoospores obtained according to methods described elsewhere (*3*). Cultures were grown for 36 hours at 20°C, flooded with autoclaved estuarine water of approximately 20 parts per 1000 salinity, and then kept at room temperature (about 22° to 23°C). At various intervals, the water overlay was decanted and the suspended cells prepared for electron microscopy. The cells were fixed for 20 minutes at room temperature in 1 percent glutaraldehyde followed by a 5- to 120-minute postfixation in 1 percent OsO_4 (*3*). The fixed suspension of cells was pelleted in a clinical centrifuge, enclosed in agar, and embedded in glycol methacrylate (*4*) for ultrastructural cytochemistry and in Durcupan ACM (Fluka) for other observations. Digestions with deoxyribonuclease (Sigma, type DN-EP) and ribonuclease (Nutritional Biochemical Co., crystallized five times) were accomplished on thin sections by the methods of Zambernard and Vatter (*5*) and Nass and Nass (*6*).

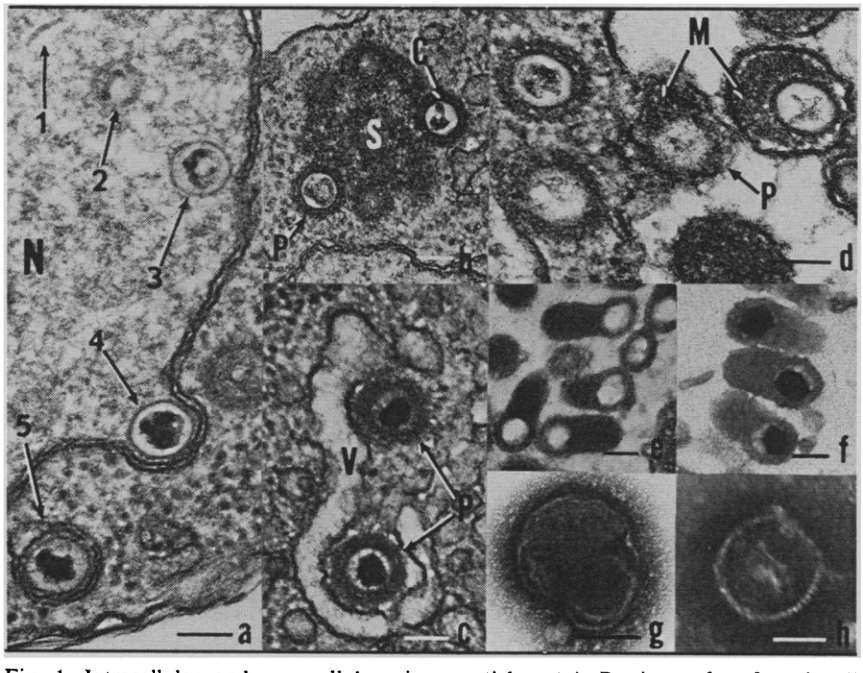

Fig. 1. Intracellular and extracellular virus particles. (a) Portions of a fungal cell show several developmental stages of the virus. Condensed material (1), partially formed particles (2), low-density cores surrounded by a capsid (3), and a nucleocapsid in the process of budding into the cytoplasm (4) can be found within the nucleus (N). Newly emerged cytoplasmic particles (5) are surrounded by the two unit membranes of the nucleus (bar = 0.1 μm). (b) Cytoplasmic particles (P) acquire a surface electron-opaque coat (C) at ribosome-free, electron-opaque sites (S) in the cytoplasm (bar = 0.1 μm). (c) Surface-coated cytoplasmic particles (P) appear to be budding into a vacuole (V) (bar = 0.1 μm). (d) A surface-coated cytoplasmic particle (P) appears to be in the process of budding from the plasmalemma. Additional electron-opaque material (M) seems to be acquired during budding (bar = 0.1 μm). (e and f) Thin sections of extracellular particles were embedded in glycol methacrylate and treated with deoxyribonuclease for 30 minutes (e) or ribonuclease for 2 hours (f). Deoxyribonuclease treatment removes the cores, making them electron-lucent, but the cores are still electron-opaque after ribonuclease treatment (bars = 0.1 μm). (g) The enveloped particle was stained with aqueous 1 percent uranyl acetate (bar = 0.1 μm). (h) The unenveloped particle was stained with 0.8 percent potassium phosphotungstate (*p*H 7.0). The capsid appears to have a core and a hexagonal profile (bar = 0.05 μm).

Evidence of virus production was not observed until approximately 5 hours after the cultures were flooded with estuarine water. Shortly after 5 hours and up to approximately 20 hours after flooding, partially formed particles as well as virus particles with electron-opaque cores surrounded by capsids were observed in the nuclei. These latter particles measured about 110 nm in diameter and possessed a capsid about 10 nm thick (Fig. 1a). During this period virus particles with electron-opaque cores were also observed budding from the nucleus into the cytoplasm (Fig. 1a). The cytoplasmic particles were at first surrounded by the two unit membranes of the nuclear envelope (Fig. 1a), but appeared to be coated subsequently with an electron-opaque material (approximately 25 nm thick) at ribosome-free, electron-opaque sites in the cytoplasm (Fig. 1b). These surface-coated nucleocapsids without a surrounding envelope were frequently encountered free in the cytoplasm (Fig. 1d). Observations indicated that the virus envelope was acquired by budding into cytoplasmic vacuoles (Fig. 1c). It is also possible that the envelope can be acquired during egress from the cell (Fig. 1d). The extracellular particles measured about 130 nm in diameter at the region of the nucleocapsid and were about 280 nm long. The envelope was about 6.8 nm thick. These particles had additional electron-opaque material that was apparently acquired during envelopment (Fig. 1d).

Deoxyribonuclease treatment of thin sections of the particles removed the cores, rendering the area within the capsids electron-lucent (Fig. 1e). Ribonuclease treatment of thin sections for up to 8 hours had no apparent effect on the cores (Fig. 1f).

Numerous attempts were made to increase the titer and to concentrate the particles for negative-staining studies, but these were generally unsuccessful. Negative stains penetrated a few enveloped (Fig. 1g) and unenveloped (Fig. 1h) capsids, which were found

in pellets obtained after the supernatant from low-speed centrifugation of the water overlay was centrifuged at 100,000*g* for 60 minutes.

Although the number of capsomeres could not be enumerated, many properties of these particles—the presence of the envelope, the core of DNA, the nature of the replicative cycle, the likely cubic symmetry, and the diameter of the capsid—strongly suggest that the particles are a herpes-type virus belonging to the family Herpesviridae (*7*).

In broad outline, the events that occur during the replicative cycle are not unlike those found in other herpes viruses (*8, 9*). Several unusual features of the replicative cycle were noted, but most of these have been reported to occur in various other herpes-type viruses. Cytoplasmic particles surrounded by two unit membranes have been reported by Shipkey *et al.* (*8*). The nuclear membrane is considered the site for permanent envelopment of most herpes-type viruses; but temporary envelopment by nuclear membranes, as well as the cytoplasmic acquisition of a coat, have been reported for the viruses associated with frog renal adenocarcinoma (*10*). Many herpes-type virions are described as essentially spherical, varying from 145 to 205 nm in diameter (*11*). The extracellular particles found in *Thraustochytrium* sp. cultures are elongate (280 nm long), with the capsids eccentrically located in the envelopes (Fig. 1, d to f).

Herpes-type viruses have not been described in organisms other than the vertebrates. The presence of these particles in a fungus considerably extends the host range for this viral group [similar particles may be present in another monocentric phycomycetous fungus, *Schizochytrium aggregatum* (*12*)]. The infection of a unicellular organism by a herpes-type virus may prove useful as a tool for studying host-virus interactions; this is especially significant because some herpes viruses have oncogenic activity (*13*). As far as we are aware, all viruses infecting fungi thus far studied have cores of double-stranded RNA, and not of DNA as is found in the virus infecting *Thraustochytrium* sp. Viruses infecting marine fungi have also not been previously described.

No method to assess the viral titer has been devised, but observations of thin sections, as well as our inability to obtain concentrated virus pellets despite numerous attempts, suggest that it is low. In spite of the apparent low titer and the low number of infected cells found in the electron microscopic preparations (approximately 16 percent of 500 cell profiles examined were found to contain particles), repeated attempts have failed to yield single zoospore cultures free of the viruses. No virus particles have yet been observed in mature fungal thalli or in suspensions of zoospores obtained less than 5 hours after the cultures were flooded. These results suggest that the fungal cultures are "virogenic," with some cells becoming productive under as yet unidentified permissive conditions during certain phases of the fungal life cycle.

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Inhibition of Bacterial Adherence by Secretory Immunoglobulin A: A Mechanism of Antigen Disposal

Abstract. *Preparations of secretory immunoglobulin A (S-IgA) isolated from human parotid fluid specifically inhibited the adherence of* Streptococcus *strains to epithelial cells. Since bacterial adherence is a prerequisite for colonization of mucous surfaces, S-IgA–mediated inhibition of adherence would limit bacterial colonization. This mechanism can explain how secretory immunoglobulins function in the disposal of bacterial antigens.*

Secretory immunoglobulin A (S-IgA) is the predominant class of antibody in secretions bathing mucous membranes (*1, 2*). S-IgA has been demonstrated to serve a protective function against viral infections, but the mechanism by which S-IgA antibodies function in the disposal of bacterial antigens is not understood (*1, 2*). Although S-IgA has been reported to bind to and agglutinate bacteria to which it is specifically directed, S-IgA is not generally considered to be bactericidal, to mediate complement-dependent bacterial lysis, to bind to macrophages, or to enhance phagocytosis (*1, 2*).

It is possible that by merely binding to, and aggregating bacteria, S-IgA may exert an immune protective action. Bacteria colonizing an exposed mucous surface must attach to the surface to prevent being washed away by bathing secretions. Bacterial species exhibit surprising specificity in the degrees to which they can attach to surfaces, and this property directly influences their colonization (*3–5*). The recognized ability of S-IgA to specifically bind to bacterial cells and effect their aggregation could influence their adherence and, hence, their colonization of a mucosal surface. We now report that human parotid S-IgA does, in fact, interfere with bacterial adherence to epithelial surfaces in vitro and our evidence suggests that this effect also occurs in vivo. This function per se would limit the colonization of indigenous organisms as well as exogenous pathogens on mucosal surfaces and could be the major mechanism of immunity mediated by secretory immunoglobulins.

Stimulated parotid saliva from a single donor was clarified, dialyzed, and lyophilized. Parotid S-IgA was separated from other salivary immunoglobulins and isolated by sequential chromatography on a column of Bio-Gel A-15