The Microsporidian Spore Invasion Tube. III. Tube Extrusion and Assembly

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ABSTRACT The polar filaments within microsporidian spores discharge as tubes with subsecond velocity. Populations of discharging tubes of *Glugea hertwigi* spores pulse-labeled with latex particles for 1–3 s were consistently devoid of label at the distal ends; discharging tubes were completely labeled after 30- to 60-s exposure to latex. This experiment indicates that discharge tubes grow at the tip. Completely assembled discharge tubes consisted of single, empty cylinders; however, incompletely discharged tubes had a cylinder-within-a-cylinder profile at the distal ends. This observation indicates that the discharge tube material emerges at the distal end by an eversion process. Finally, studies with cinematic Nomarski interference optics of spore tubes extruding across a water-air interphase indicate that all the material emerging from the growing tip of the tube is incorporated into the wall of the discharge tube. Evidence indicates that the polar filament of undischarged spores is a homogeneous coil of polar tube protein equivalent to the polar tube protein in discharged tubes.

Microsporidia have the capacity to inoculate a cell from a spore stage into a host cell by means of an invasion tube (1–3, 8). Microsporidian parasites are intracellular eukaryotes with a spore stage equipped with an extrusion apparatus (EXA). Upon stimulation, the EXA discharges a long (50–150 μm), thin (0.1 μm) tube with subsecond velocity (2). The tube is the vehicle by which the internal, infective cell is transferred from the spore to the susceptible host cell (5). Studies with light optics have indicated that the invasion tube is formed by an eversion process (3, 8). However, the assumption was made that discharge tubes are preassembled within the spore before extrusion. It was reported in an earlier paper (6) that it was unlikely that preassembled tubes were everted at the time of discharge, because the anticipated cylinder-within-a-cylinder arrangement was not apparent in incompletely discharged invasion tubes. However, this study will show that invasion tubes can indeed form a cylinder-within-a-cylinder profile during spore discharge. The principal assembly site for a stable tube is the distal end of the growing tube.

MATERIALS AND METHODS

Collection of Spores

Spores of *Glugea hertwigi* were recovered from xenoma cysts of infected rainbow smelt *Osmerus mordax* captured from Lake Erie and processed at the Lake Erie Fisheries Research Station, Wheatley, Ontario, Canada. Spores were centrifuged and washed until a pure white spore sediment was obtained (6). The purity of the pellet was checked by light microscopy.

Inducing Spore Discharge

Spore tube discharge was studied with Nomarski interference and phase microscopy. Spores were placed in a thin drop of alkaline distilled water on glass with 1 μM ionophore A23187 (Eli Lilly and Co., Inc., Indianapolis, Ind.). *G. hertwigi* spore extrusions were achieved at acid pH with the use of a French press (4). Spores were placed under 20,000 pounds of pressure and prepared for transmission electron microscopy according to the procedure reported earlier (6).

Pulse-labeling with Latex Particles

G. hertwigi spores were placed in hatching medium with 0.1 or 0.3 μm polystyrene spheres (Sigma Chemical Co., St. Louis, Mo.) for 2 s to 2 min and washed from the glass slide. Spores with discharged tubes were left on the slide and subsequently viewed with light microscopy. For electron microscopy, slides were coated with 0.5% Formvar and the spores and label were applied in the manner described above. The Formvar was then stripped off the slides and chosen areas were placed on copper grids, stained with uranyl acetate (procedure described below), and viewed with a Jeol 100 CX electron microscope.

Negatively Stained Preparations

Because discharged tubes are stable in sodium dodecyl sulfate (6), the tubes were incubated in this detergent (3%) for 15 min and washed with distilled water. The spores, with the attached discharged tubes, were applied to Formvar-coated grids, and the grids were passed through 2% (wt/vol) aqueous uranyl acetate and examined with an electron microscope.

RESULTS

Polar Tube Protein (PTP) in Undischarged and Discharged Spores

The term "polar filament" is used in this paper to designate

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the crystalline coil of PTP in unhatched spores before extrusion into discharge tubes. The discharged tubes were composed of PTP arranged as a cylinder (Figs. 4 and 5). The PTP in the cylinder and the polar filament appeared homogeneous. Polar filaments did not extrude from *G. hertwigi* spores in acid medium unless exposed to external pressure; spores exposed to 20,000 lbs/in^2 pressure displayed two kinds of filament extrusion: (*a*) through the polar aperture in the form of a typical discharged tube and (*b*) as a filament that had burst through the lateral walls of the spore. The filament did not evert into a tube when bursting through the lateral walls of spores.

Stability of Discharged PTP

Discharged, assembly PTP resisted dissociation in 3.0% sodium dodecyl sulfate (SDS), 1.0% Triton X-100, 1–10% H_2O_2,

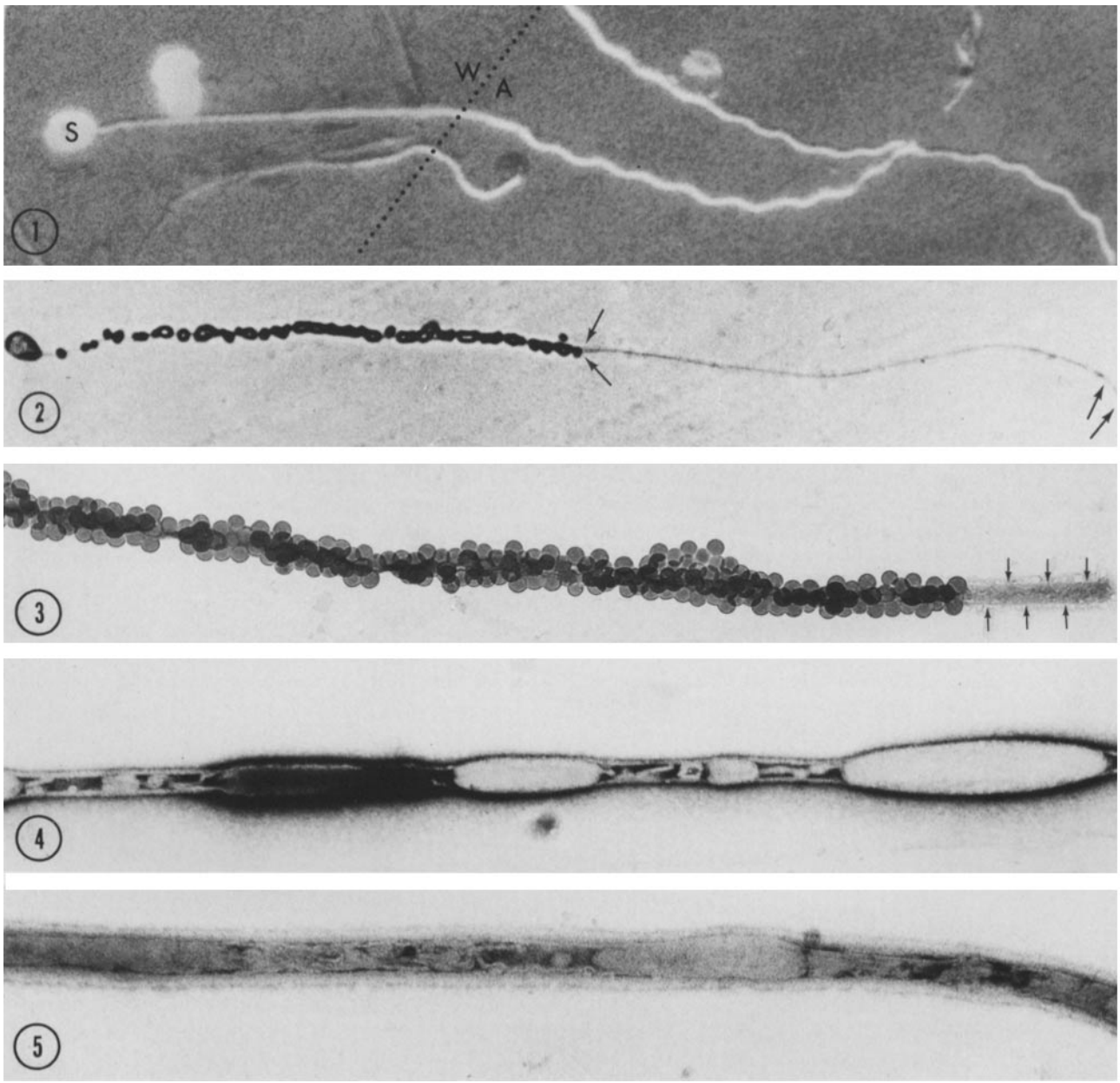

FIGURE 1 Nomarski interference optics of *G. hertwigi* spores (s) discharging across water-air (*W*=*A*) interphase (indicated by dotted line). Twisting motion of extruding tube apparent on air side. × 3,000.

FIGURE 2 Phase optics of discharging tube of *G. hertwigi* spore pulse-labeled for 2–3 s with 0.3-μm latex particles. Arrows indicate end of latex labeling and end of discharged tube. × 1,500.

FIGURE 3 Negatively stained discharging *G. hertwigi* tube pulse-labeled for 2–3 s with 0.1-μm latex particles. Arrows indicate unlabeled end of tube. × 30,000.

FIGURE 4 Negatively stained, SDS-washed, incompleted discharged tube of *G. hertwigi* spore. Note tube-within-a-tube profile. × 30,000.

FIGURE 5 Negatively stained, SDS-washed, incompletely discharged *G. hertwigi* tube. Cylinder-within-a-cylinder profile apparent. Some indications of subunits apparent. × 45,000.

5–8 N H_2SO_4, 1–2 N HCl, chloroform, 1.0% guanidine HCl, 0.1 M proteinase K, 8–10 M urea, 50 mM $NaCO_3$, and 50 mM $MgCl_2$; however, assembled PTP was reduced with various concentrations of 2-mercaptoethanol, 1% dithiothreitol, 6% urea with 0.1 M proteinase K, and 0.1 M proteinase K in 1.0% guanidine hydrochloride. Tubes of assembled PTP immersed in 0.05–0.1 M $CaCl_2$ exhibited a semifluid state by sprouting branches that fused with other assembled PTP tubes. This phenomenon was not observed with discharged tubes of PTP exposed to equivalent concentrations of $MgCl_2$ or KCl.

Nomarski Interference Microscopy of Discharge Tubes Extruded across a Liquid-Air Interface

G. hertwigi spores, near the air-water interface on glass slides, discharged polar tubes across the interface onto the air side (Fig. 1). These discharging tubes were slowed significantly, from 0.5 to 15 s, during the passage over glass. A small proportion of these discharging tubes formed irregular profiles. The specific positions along the lengths of these tortuous tubes remained fixed as new material continued to emerge and assemble at the tip (Fig. 9). According to Nomarski optical images of tube growth at the air side of a water-air interphase, all the material emerging at the tip was assembled into newly formed tube.

Pulse-labeling of Discharging Polar Tubes with Latex Particles

G. hertwigi spores were induced to discharge invasion tubes in small aqueous pools with latex particles (0.1 or 0.3 μm) on glass slides. Because the tubes attached to glass, it was possible to rinse away unbound latex in addition to the unhatched spores. 10–15% of discharging *G. hertwigi* spores immersed in latex for 1–3 s consistently were devoid of bound latex at the distal ends (Figs. 2 and 3). The latex was observed only on the basal portions of the discharged tubes. However, extruding spores exposed for 1–5 min to the latex pool had discharged tubes that were completely labeled.

Negatively Stained, Extruded Discharged Tubes Prewashed with SDS

SDS-washed, negatively stained polar tubes were examined for protein order in completely assembled tubes (CAT) and in incompletely assembled tubes (IAT). SDS was effective in washing the tubes before staining, since the assembled PTP was not reduced by the detergent. CAT consisted of a single cylinder of completely assembled PTP; an interior component was not observed in lumens of CAT. However, SDS-washed IAT had various aggregations of partially assembled PTP subunits within the lumen of the assembled outer cylinder. The internal aggregations of subunits were indistinguishable from the units comprising the outer cylinder (Figs. 4–7).

DISCUSSION

This study provides information on the extrusion of microsporidian polar filaments into invasion tubes. Evidence indicates (*a*) that the discharging polar filament assembles into a tube at the growing tip and (*b*) that the filament and tube are composed of a principal component, PTP.

Discharging Polar Filaments Assemble into Tubes at the Growing Tip

The following observations indicate that microsporidian tubes assemble by a flow of interior material which emerges at the tip. First, negatively stained, CAT were single cylinders, but IAT characteristically had an additional, less-defined internal cylinder of presumptive PTP. Second, discharging tubes pulse-labeled for 1–2 s with latex displayed distal ends free of label; however, discharging tubes exposed to latex for longer periods time (30–60 s) were always completely labeled. The

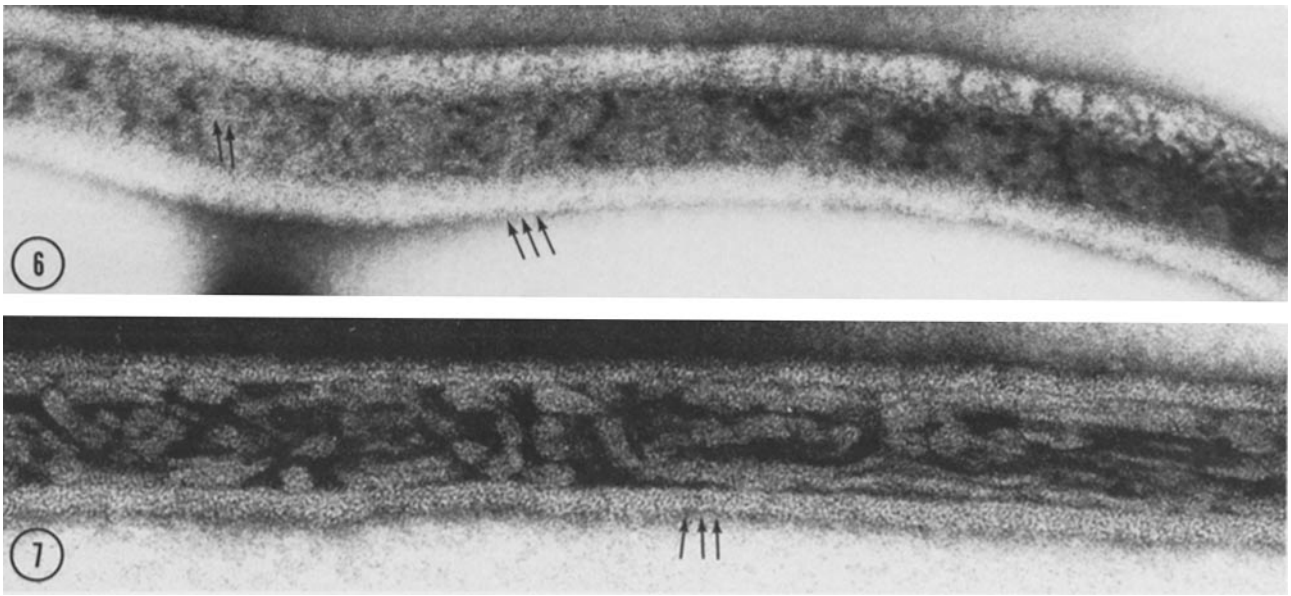

FIGURE 6 Negative staining of incompletely assembled discharged tube from *G. hertwigi* spore. Arrows indicate clusters of presumptive PTP within tube. × 200,000.

FIGURE 7 Negative staining of incompletely assembled tube of *G. hertwigi*. Arrows indicate outer cylinder. Aggregates of presumptive PTP are within tube. × 200,000.

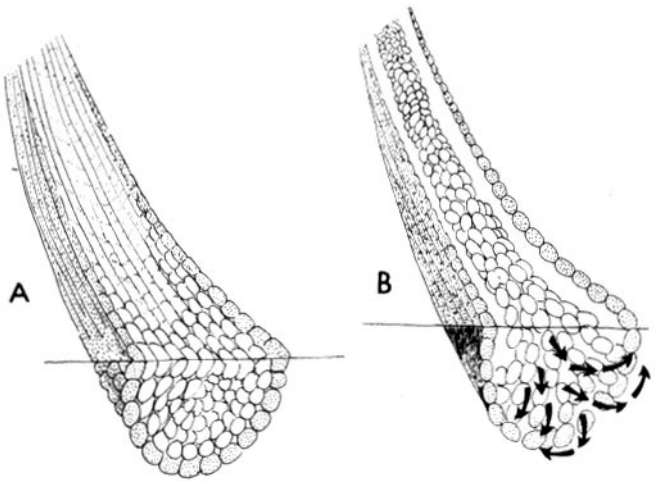

FIGURE 8 (*A* and *B*) Model for PTP assembly into discharge tubes. *A* indicates order of PTP within undischarged polar filament. *B* displays partially assembled internal component of PTP organizing into outer cylinder at the tip. PTP units depicted in model are greatly exaggerated in size to show more simply how extrusion works.

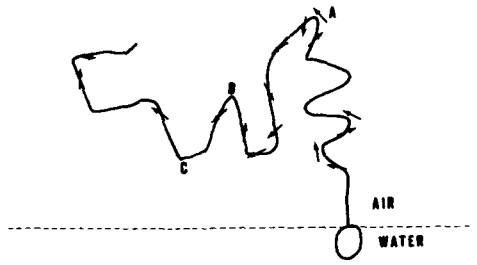

FIGURE 9 Diagram of *G. hertwigi* spore discharging a tortuous tube across air-water interphase. Direction of tube growth is indicated by arrows. Positions *A*, *B*, and *C* remained stationary as tube assembly continued at tube tip.

unlabeled distal ends indicate that the tube continued to assemble at the tip after the latex pool was removed from the extruding spores. Additional evidence is the behavior of tubes extruding onto glass across the water-air interphase. The specific points along the length of highly tortuous discharge tubes remained fixed in position as assembly continued at the distal end. The length of the tube would likely shift in position if assembly were localized at the base of the tube.

Core of the Polar Filament is PTP

The following observations indicate that PTP is the principal component in the polar filament. First, the polar filament consists of a homogeneous pattern of subunits ultrastructurally indistinguishable from that of the PTP in IAT and CAT. Second, CAT, IAT, and polar filaments respond in the same way to protein-reducing agents; for example, polar filaments and discharging tubes are stable in proteinase K and SDS. However, both are reduced with mercaptoethanol and dithiothreitol. Third, CAT and IAT are antigenic and electrophoretic equivalents (6); therefore, it seems likely that the subunits of CAT and polar filaments are the same, since polar filaments are transformed directly into IAT at the time of discharge. Finally, cinematic images of spore discharges clearly indicate that all the material emerging at tube tips is incorporated into the growing discharge tube.

How Does PTP Work?

A model for PTP assembly into discharge tubes is displayed in Fig. 8. PTP appears to flow through the lumen of the assembling tube as a monolayer. Assembled PTP polymer seems to achieve a higher order of stability upon emerging from the tip of the growing discharge tube. It is not clear what specific forces affect PTP assembly or stability; however, two factors are known to influence the behavior of PTP. Specifically, a pH shift can alter the stability of dissociated PTP; for example, PTP reduced to subunits will self-assemble into monolayers when acidified in vitro (6). Another factor, calcium, changes the behavior of discharge tubes. Calcium induces a coalescence of *G. hertwigi* discharge tubes into networks; other cations, Mg^{++}, Ba^{++}, Na^{+}, and K^{+}, do not produce tube coalescence (7).

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REFERENCES

1. Kramer, J. P. 1960. Observations on the emergence of the microsporidian sporoplasm. *J. Insect Pathol.* 2:433–439.
2. Lom, J., and J. Vavra. 1963. The mode of sporoplasm extrusion in microsporidian spores. *Prog. Protozool. Proc. Int. Congr. Protozool.* 487–489.
3. Ohshima, K. 1964. Stimulative or inhibitive substance to evaginate the filament of *Nosema bombycis* Nägeli. I. The case of artificial buffer solution. *Jpn. J. Zool.* 14:209–229.
4. Simpson, K. L., A. W. Wilson, E. Burton, F. Nakayama, and J. Chichester. 1963. Modified French press for the disruption of micro-organisms. *J. Bacteriol.* 86:1126–1127.
5. Weidner, E. 1972. Ultrastructural study of microsporidian invasion into cells. *Z. Parasitenkd.* 40:227–242.
6. Weidner, E. 1976. The microsporidian spore invasion tube. I. The ultrastructure, isolation, and characterization of the protein comprising the tube. *J. Cell Biol.* 71:23–34.
7. Weidner, E. and W. Byrd. 1982. The microsporidian spore invasion tube. II. Role of calcium in the activation of invasion tube discharge. *J. Cell Biol.* 93:972–977.
8. West, A. F. 1960. The biology of a species of *Nosema* (sporozoa:microsporidia) parasitic in the flour beetle *Tribolium confusum*. *J. Parasitol.* 46:747–754.