

## Dictyosomes in Vegetative Hyphae of *Pythium ultimum*<sup>1</sup>

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The Golgi apparatus is a cell component consisting of inter-associated dictyosomes. Dictyosomes are stacks of plate-like cisternae characterized by the following features: 1) cisternal membranes free from ribosomes; 2) a peripheral system of 300 to 500 Å diam tubules; 3) shaggy vesicles attached to some of the tubules; 4) smooth-surfaced vesicles attached to peripheral tubules and functioning in secretion; 5) intercisternal elements appearing as rod-like structures 70 to 80 Å in diam; 6) morphological polarity (2, 6, 7, 15). The above characteristics hold for a wide range of plant and animal dictyosomes.

Among higher fungi, reports of Golgi apparatus are restricted to the Ascomycete *Neobulgaria pura* where cells of the inner ectal excipulum have a single perinuclear dictyosome (9). However, dictyosomes are widespread among lower fungi (3, 4, 5, 8, 12, 14) including the Oomycetes. This report concerns the occurrence and structure of dictyosomes in the vegetative hyphae of *Pythium ultimum* Trow, a phytopathogen in the order Peronosporales.

### Materials and Methods

Cultures of *P. ultimum* were grown at 27° C in Petri plates containing potato dextrose agar (Difco) overlaid with permeable cellophane. Mycelia were fixed at room temperature (25° C) by flooding cultures with 1) 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7), 2) 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7), or 3) 1% potassium permanganate. Immediately, small portions of mycelium were transferred to fixative in vials (1 to 8 hours for osmium tetroxide, 0.5 to 1 hour for glutaraldehyde, and 1 to 2 hours for potassium permanganate). All glutaraldehyde-fixed material was post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7) for 1 to 8 hours. The fixed materials were washed in distilled water, dehydrated in a graded ethanol and acetone series, embedded in an Araldite epoxy resin mixture (13), and polymerized in a nitrogen atmosphere at 70° C for about 24 hours.

Thin sections were cut using a Porter-Blum MT-2 ultramicrotome with a diamond knife. Materials fixed with osmium tetroxide were post stained with aqueous barium permanganate or uranyl acetate and lead citrate. Sections were examined with a Philips EM/200.

### Observations and Discussion

Dictyosomes of *P. ultimum* are composed of 3 to 5 plate-like cisternae (Figs. 1, 2, 4, 5, 6) 1 to 1.5 μ in diam. The cisternal peripheries appear fenestrated or tubular in face view (Figs. 2, 3). Connected to

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the tubules are structures (ca. 500 Å diam) having a fuzzy or shaggy appearance due to the presence of a nap-like electron-dense coating (Figs. 2, 4). These correspond to shaggy vesicles. Vesicles of similar structure in the area around the dictyosomes appear unattached (Fig. 4, arrow). In contrast to rough-surfaced endoplasmic reticulum (ER, Fig. 6), recognizable ribosomes are absent from the surface of the dictyosomes (Figs. 1, 2, 3, 4, 5, 6). In these features the dictyosomes of *P. ultimum* are similar to those of higher plants (2, 6, 7, 15).

*P. ultimum* dictyosomes occur throughout the cytoplasm, usually associated with the nuclear envelope (Figs. 1, 2, 4, 5) and/or ER (Fig. 6). This frequent perinuclear positioning may indicate a more general association of dictyosomes with ER (7) which includes the nuclear envelope. When perinuclear, the dictyosomes are often associated with that portion of the nuclear membrane adjacent to the nucleolus (Fig. 5). Perinuclear positioning, although uncommon in higher plants, is of general occurrence in lower forms (1, 3, 4, 7, 9, 12, 14).

The region between the dictyosomes and the nuclear envelope or ER is free from ribosomes (Figs. 1, 2, 4, 6). This region contains numerous small vesicles (Figs. 1, 2, 4, 5, 6) and extensions of the outer membrane of the nuclear envelope (Figs. 1, 4, 5). Projections from the nuclear envelope beside dictyosomes have been reported in other organisms. On the basis of a study with *Neobulgaria pura*, Moore and McAlear (9) suggest that the cisternae are formed by a series of vesiculations of the outer membrane of the nuclear envelope. Electron micrographs of *Aphanomyces euteiches* (14) show extensions of the outer nuclear membrane. Blebbing of the outer nuclear membrane toward the associated dictyosome also occurs in brown algae (1). Projections from the nuclear envelope in the region adjacent to the dictyosomes may represent a general phenomenon characteristic of lower plants.

Dictyosomes of *P. ultimum* exhibit structural polarity. The cisterna immediately adjacent to the nuclear envelope or ER is discontinuous (Figs. 1, 2, 5). The cisternae in median position are continuous and tend to be compressed (Figs. 1, 6). Cisternae at the distal pole are discontinuous (Figs. 4, 5, 6) but appear more swollen than those at the proximal pole (Figs. 2, 6).

### Summary

Dictyosomes of the Golgi apparatus of *Pythium ultimum* are shown to have characteristics in common with dictyosomes of higher plants. The existence of tubular cisternae and attached shaggy vesicles are reported for the first time in a fungus. The dictyosomes are distributed throughout the cytoplasm but usually are in association with the nuclear envelope or ER. Dictyosome polarity is also demonstrated.

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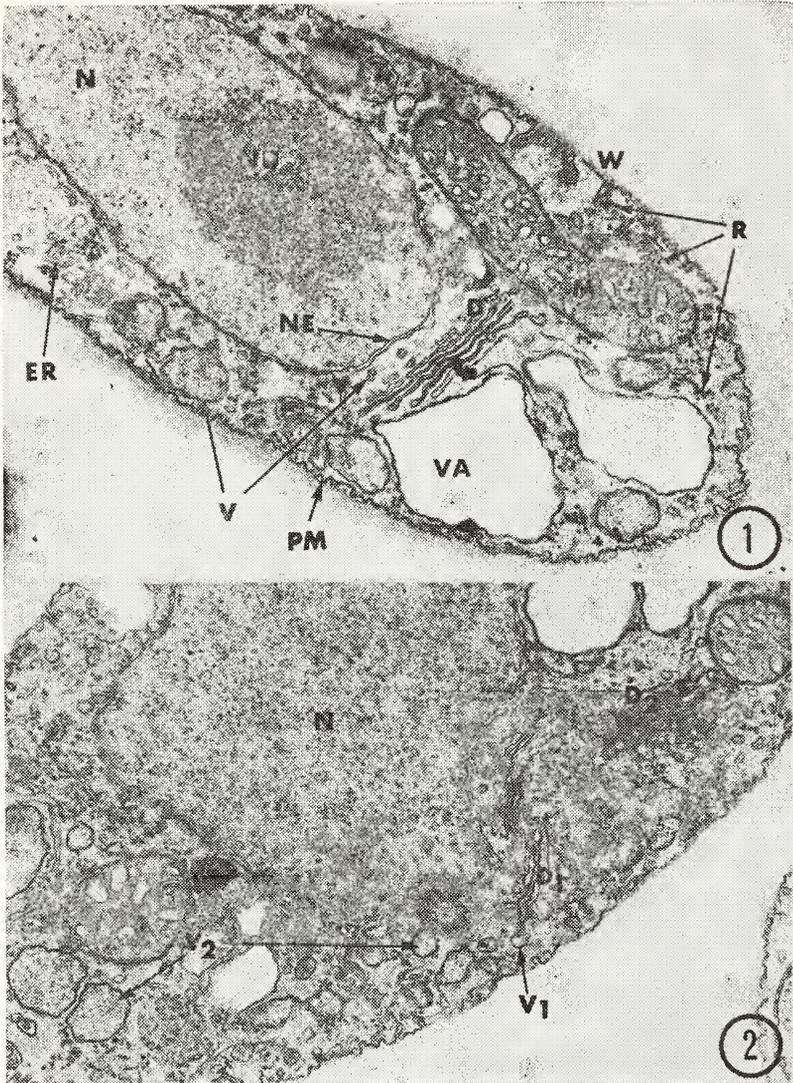
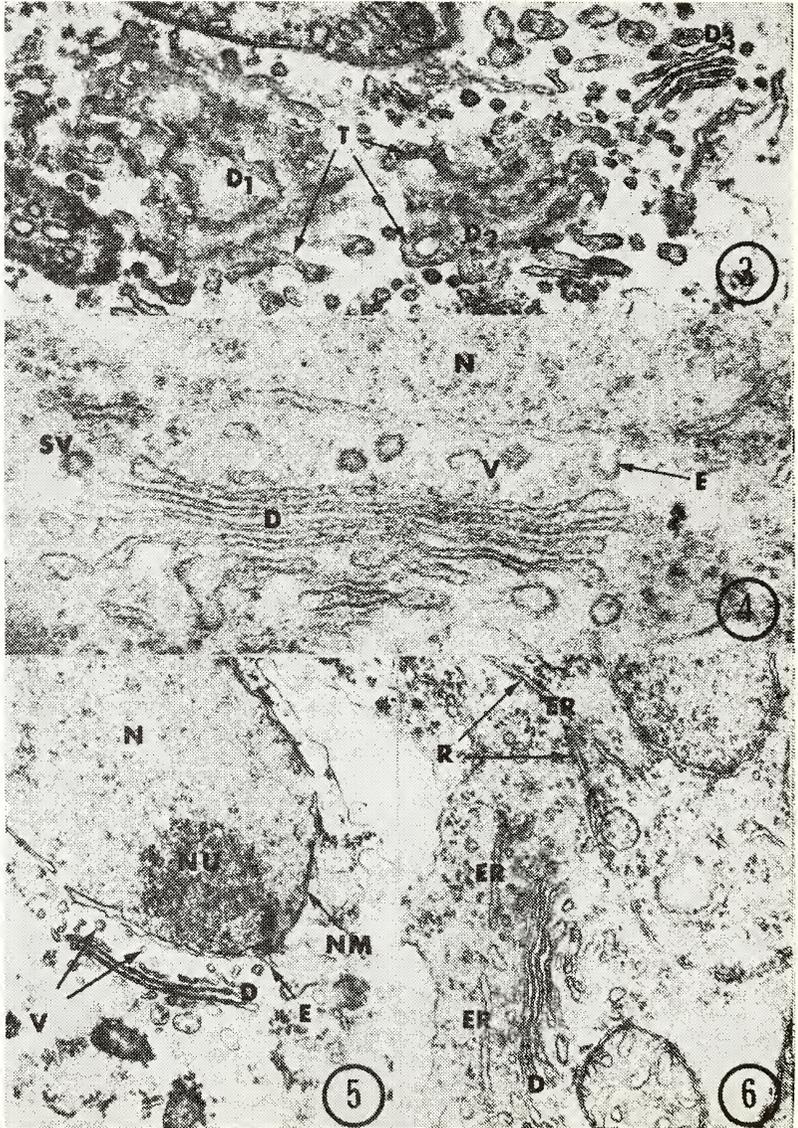


Figure Legends

## PLATE I

Figure 1. A vegetative hypha of *P. ultimum*. Nucleus (N), nucleolus (NU), nuclear envelope (NE), mitochondrion (M), dictyosome (D), vesicle (V), vacuole (VA), plasma membrane (PM), hyphal wall (W), ribosome (R), and endoplasmic reticulum (ER). Glutaraldehyde-OsO<sub>4</sub> fixation. X 29,000.

Figure 2. Dictyosome (D<sub>1</sub>) with what may be an attached secretion vesicle (V<sub>1</sub>) and similar vesicles (V<sub>2</sub>) free in the cytoplasm. A second dictyosome (D<sub>2</sub>) is seen in face view. Glutaraldehyde-OsO<sub>4</sub> fixation. X 30,000.



## PLATE II

Figure 3. Face ( $D_1$ ), oblique ( $D_2$ ) and cross sectional ( $D_3$ ) views of dictyosomes showing tubules (T) at the peripheries. Glutaraldehyde- $\text{OsO}_4$  fixation. X 35,000.

Figure 4. Shaggy vesicles (SV) attached to cisterna. Extension (E) on outer nuclear membrane (NM) and small vesicles (V) occur between dictyosome (D) and nucleus (N). Glutaraldehyde- $\text{OsO}_4$  fixation. X 85,000.

Figure 5. A perinuclear dictyosome (D) adjacent to a nucleolus (NU). Extension (E) of the outer nuclear membrane (NM) and small vesicles (V) shown between the nucleus (N) and the dictyosome.  $\text{OsO}_4$  fixation. X 22,000.

Figure 6. Dictyosome (D) contrasted to rough surfaced endoplasmic reticulum (ER). Arrow indicates ribosomes (R). Glutaraldehyde- $\text{OsO}_4$  fixation. X 32,000.