

## Presence of a motile tubular vacuole system in different phyla of fungi

BETTYE REES, V. A. SHEPHERD AND ANNE E. ASHFORD

School of Biological Science, The University of New South Wales, Sydney, 2052, Australia

Actively growing cultures of organisms representative of the major mycelium-forming fungal and fungus-like protoctistan phyla were examined for the presence of a motile tubule and vacuole system similar to that found in *Pisolithus tinctorius*. The system was visualized by accumulation of 6-carboxyfluorescein and fluorescence microscopy. All members of the Eumycota examined had a similar but not always identical system. This included eight other members of the Basidiomycotina, four of the Ascomycotina, three of the Zygomycota and two of the Deuteromycotina, and indicates that the system is widespread. The Oomycota on the other hand, exhibited a different 6-carboxyfluorescein-accumulating system – a very fine tubular reticulum that was confluent with vacuoles which also accumulated fluorochrome. The tubular reticulum in members of the Oomycota was very fragile and could only be shown in growing hyphae with streaming cytoplasm and no apparent structural damage.

Actively growing hyphae of *Pisolithus tinctorius* (Pers.) Coker & Couch accumulated 6-carboxyfluorescein (CF) following transport of the non-fluorescent 6-carboxyfluorescein diacetate (CFDA) across the cell membrane and its hydrolysis to fluorescent CF inside the cells (Shepherd, Orlovich & Ashford, 1993a, b). In *P. tinctorius* CF accumulated in a motile tubular vacuole system. The tubules extended and retracted for many micrometres and rapidly transported globules of the fluorochrome along the hyphal tip-cells and into the penultimate cells by a movement that superficially resembled peristalsis. Vacuoles in these cells contain polyphosphate and it has been speculated that this tubule and vacuole system may be involved in transfer of polyphosphate and other phosphorus compounds in this region (Orlovich & Ashford, 1993; Ashford, Ryde & Barrow, 1994).

The presence of elongate vacuoles with recurrent dilations and the interconnection between tubules and vacuoles were confirmed by transmission electron microscopy of serial sections of freeze-substituted hyphae of *P. tinctorius* (Shepherd *et al.*, 1993a). Similar elongate vacuoles have been described from electron micrographs of freeze-substituted hyphae of other members of the Basidiomycotina. These include *Laetisaria arvalis* Burds. (Hoch & Howard, 1980), *Sclerotium rolfsii* (Roberson & Fuller, 1988), and the teliomycetes *Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth. (Hoch & Staples, 1983) and *Gymnosporangium juniperi-virginianae* Schwein. (Mims, Roberson & Richardson, 1988). In several species, elongate vacuoles are reported to be disrupted by conventional chemical fixation with glutaraldehyde and to round up into strings of vesicles (Hoch & Staples, 1983; Orlovich & Ashford, 1993), so that they would not be likely to be observed frequently in electron micrographs of chemically fixed material.

In the Ascomycotina, electron micrographs of freeze substituted hyphae of *Endothia parasitica* (Murrill) P. J. & H. W. Anderson and *Magnaporthe grisea* (T. T. Hebert) Barr show elongate tubules with alternate dilations and constrictions (Newhouse, Hoch & MacDonald, 1983; Bourett, Piccollelli & Howard, 1993). Narrowed bridge-like interconnections between vacuoles are found in *Fusarium acuminatum* Ellis & Everh. hyphal tip-cells (Howard & Aist, 1980), while stroma and haustoria in *Erysiphe graminis* DC. and *Venturia inaequalis* (Cooke) Wint. (Dahmen & Hobot, 1986) reveal vacuoles with granular inclusions but without any apparent interconnecting tubules. Elongate vacuoles have also been shown in freeze substituted hyphae of *Pythium ultimum* (Hoch, 1986) in the Oomycota. Taken as a whole these data suggest that a tubular vacuole system like that in *Pisolithus tinctorius* may be widespread in fungi, but may have been missed using conventional electron microscopy. Furthermore, single electron micrographs cannot give an indication of the full extent, interconnectedness or motility of such a system. It was therefore considered appropriate to investigate whether a tubular vacuole system similar to that in *P. tinctorius* is present in a range of fungi, including examples from most of the major taxa, using accumulation of CF to detect the system and to investigate its appearance and the nature of its movements in living hyphae.

The CF-accumulating system in *P. tinctorius* is thought to be similar to tubular endosomal and lysosomal systems in cultured animal cells (Shepherd *et al.*, 1993a). It is generally characterized as a series of clusters of vacuoles interconnected by tubules which can form a three dimensional reticulum. Characteristic zonation occurs in the tip-cell, there being an apical region with small vacuoles and tubules, a nuclear region in which only tubules are generally found, and a basal region

**Table 1.** Species investigated for the presence of a motile tubular vacuolar system

Organism	Nutritional status	Source	Growth medium	Incubation temperature °C	Age at analysis	Slide or Plate (S/P)
<b>Eumycota</b>						
<b>Basidiomycotina</b>						
<i>Agaricus carneus</i> (Heinem. & Gooss.-Font.)	Sapr	UNSW 026	PDA	26	3 wk	P
<i>Armillaria mellea</i> (Vahl.: Fr.) P. Kumm.	Path	UNSW 460	PDA	26	4 wk	P
<i>Fistulinella mollis</i> (Cleland) Watling	Not known	UNSW 043	MMN	21	3 wk	P
<i>Nidularia</i> sp.	Sapr	UNSW 045	PDA	26	8 d	P
<i>Paxillus involutus</i> (Batsch: Fr.) Fr.	EctoM	UNSW 056	MMN	21	3 wk	P
<i>Sclerotium rolfsii</i> Sacc.	Path	RBG 728	PDA	26	2 d	S
<i>Sphaerobolus</i> sp.	Sapr	UNSW 044	PDA	26	8 d	P
<i>Suillus granulatus</i> (L.: Fr.) O. Kuntze	EctoM	UNSW 027	MMN	21	4 wk	P
<b>Ascomycotina</b>						
<i>Claviceps</i> sp.	Path	UNSW 693	PDA	26	7 d	P
<i>Hymenoscyphus ericae</i> (Read) Korf & Kern.	EricM	UNSW 013	MMN	26	3 wk	P
<i>Monilinia fructicola</i> (G. Winter) Honey	Path	UNSW 691	PDA	26	7 d	S
<i>Peziza whitei</i> (Gilkie) Trappe	EctoM	UNSW 050	PDA	26	2 d	P
<b>Deuteromycotina</b>						
<i>Aspergillus niger</i> Tiegh.	Sapr	UNSW 208	PDA	26	2 d	S
<i>Penicillium expansum</i> (Fr.: Link) Gray	Sapr	UNSW 214	PDA	26	2 d	S
<i>Candida albicans</i> (C. P. Robin) Berkhout	OpPath	AMMRL 36.83	RIAT	26	3–7 d	S
<b>Zygomycota</b>						
<i>Rhizopus stolonifer</i> (Ehrenb.: Fr.) Lind	Sapr	UNSW 716	PDA	26	2 d	S
<i>Thamnidium elegans</i> Link	Sapr	UNSW 203	PDA	26	3 d	S
<i>Zygorhynchus moelleri</i> Vuill.	Sapr	UNSW 205	PDA	26	3 d	P
<b>Oomycota</b>						
<i>Phytophthora cinnamomi</i> Rands	Path	DAR 58978	PDA	26	3 d	S
<i>Pythium irregulare</i> Buisman	Path	DAR 63895	PDA	26	18 h	S
<i>Saprolegnia parasitica</i> Coker	Path	*H 303	GYPS	26	17 h	S

Abbreviations: Nutritional status – Sapr, Saprotroph; Path, Pathogen; EctoM, Ectomycorrhizal; EricM, Ericoid-mycorrhizal; OpPath, Opportunistic Pathogen. Media – PDA, Potato Dextrose Agar; MMN, Modified Melin-Norkrans Agar; RIAT, Rice Infusion Agar with Tween 80; GYPS, Glucose Yeast Extract-Peptide-Salts Medium.

\* Held by A. Hardham, Plant Cell Biology Group, Research School of Biological Sciences, Australian National University.

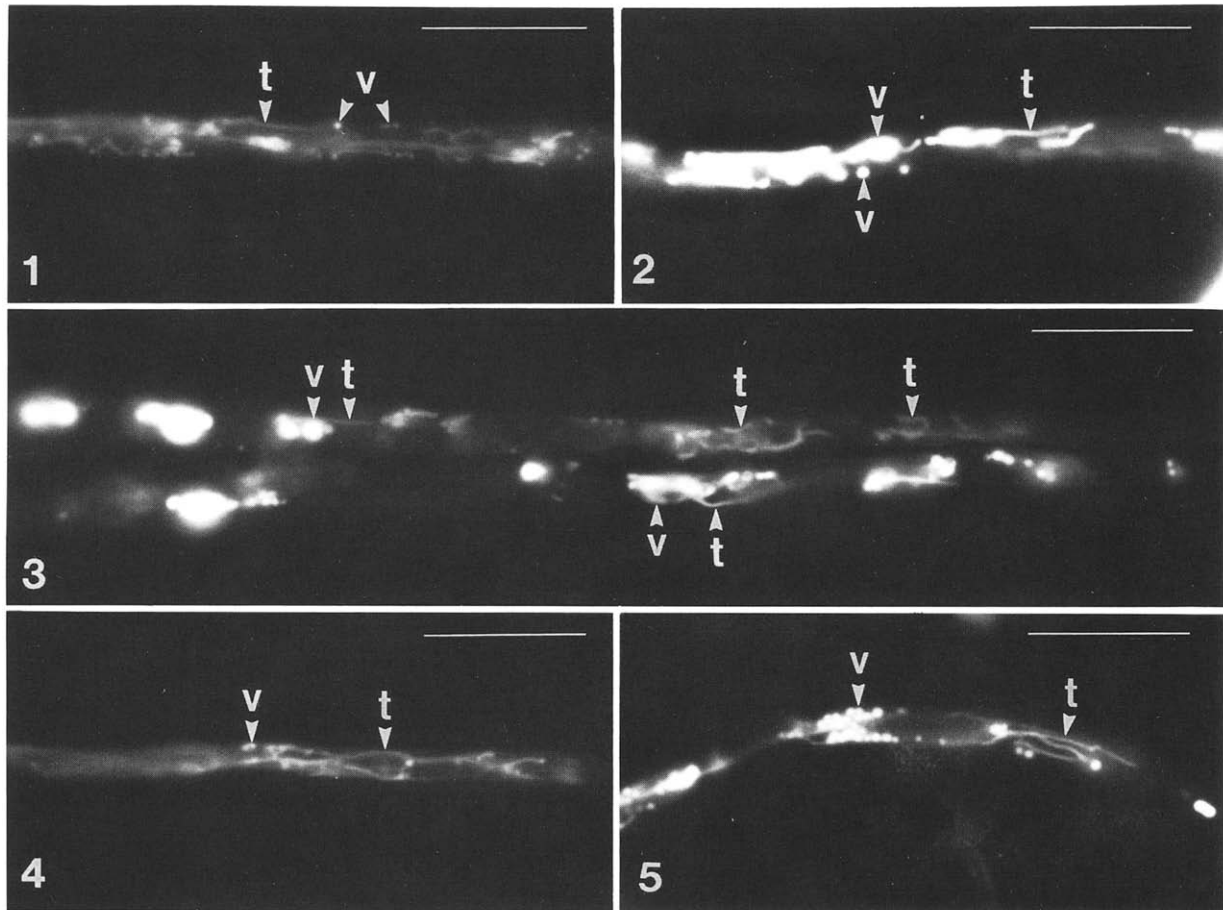
closest to the septum, where there are either almost exclusively tubules, or several large vacuoles interconnected by tubular bridges. Movement of the tubules occurs intermittently. It is independent of cytoplasmic streaming and frequently runs counter to it (Shepherd *et al.*, 1993a). With these criteria in mind, actively growing cultures of organisms which exhibit a mycelial style of growth were investigated for the presence of a similar system using CF accumulation. The taxonomy used here follows the scheme of Kendrick (1985) for the Eumycota and that of Dick (1990) for the Oomycota.

## MATERIALS AND METHODS

Cultures of species selected from the major fungal subphyla and some of the mycelium-forming Oomycota were grown in the dark on media and at temperatures and for times calculated to produce optimal rates of growth (Table 1). Mycorrhizal fungi were grown on modified Melin-Norkrans (MMN) agar (Marx, 1969), saprotrophs and parasitic/facultative saprotrophs on commercial Difco Potato Dextrose agar (PDA), and the mycelium-forming yeast *Candida albicans* on Rice Infusion agar with Tween 80 (RIAT), chosen to encourage the formation of mycelium and chlamydospores (Taschdjian, 1957). A thin sector of agar containing the actively growing hyphae at the margin of each colony was immersed face down

in a solution of CFDA (Molecular Probes) at 20 µg ml<sup>-1</sup> (pH 4.8), and then rinsed briefly in reverse osmosis water before mounting on a slide. The time of exposure to the fluorochrome was varied from 1 to 15 min depending on the rate of accumulation of CF and fragility of the hyphae. The period of time for rinsing and mounting was also varied to allow cytoplasmic streaming to resume and the motile tubular vacuole system to become fully visible. Cultures varied in growth habit and wettability. Surprisingly, those cultures which were slow growing and hydrophobic and floated on the CFDA solution displayed a tubule system that was more easily observed and stable than those which sank. In contrast, many of the faster growing hydrophilic cultures had to be mounted and viewed immediately before the motile system disintegrated. For those cultures where the hyphae appeared very fragile and the tubule system disintegrated rapidly, the CFDA was made up in 0.025 M KH<sub>2</sub>PO<sub>4</sub> buffer (pH 5.0), and the preparations rinsed in the same buffer.

In order to avoid disrupting the cells with an accompanying loss of contents, many of the fungi (especially the aseptate ones) were grown and examined as slide cultures. A Petri dish containing a microscope slide supported on a glass rod was sterilised and the surface of the slide covered with a thin film of the appropriate growth medium. This was inoculated centrally with a small agar cube cut from the actively growing



**Figs 1–5.** Fluorescence micrographs showing pleiomorphic motile tubular vacuole systems in the Basidiomycotina. Bars, 10  $\mu\text{m}$ . **Fig. 1.** Typical system in the terminal cell of *Pisolithus tinctorius* showing small vacuoles (v) of various shapes and sizes with interconnecting tubules (t). **Fig. 2.** *Fistulinella mollis* demonstrating a similar system with wider tubules (t) and some vacuoles (v). **Fig. 3.** *Paxillus involutus* showing tubules (t) forming a reticulum and connecting with vacuoles (v). **Fig. 4.** A hypha of *Agaricus carneus* showing a well developed tubular network (t) with small vacuoles (v). **Fig. 5.** A hypha of *Armillaria mellea* showing a tubular reticulum (t) connected with clusters of vacuoles (v).

margin of a plate culture and sterile water was added to keep the preparation moist. The Petri dish was sealed and incubated as usual. The colony was flooded with CFDA on one side of the central inoculum, then rinsed, and the growing margin on both sides covered with a cover slip. This enabled observation of CF accumulation from high and low concentrations of CFDA. *Saprolegnia parasitica* cultures were grown on GYPS agar (Beakes & Gay, 1980). A small agar cube plus mycelium was cut from the colony and placed in liquid medium of the same composition but without agar. This was left to grow for about 17 h, then CFDA was pipetted into the growth medium to a final concentration of  $10 \mu\text{g ml}^{-1}$  and incubated for 10 min without disturbance. Following three partial rinses over 10 min in fresh growth medium without CFDA, the preparation was mounted in medium on a slide using teflon spacers to support the coverslip (S. Jackson, pers. comm.) for observation.

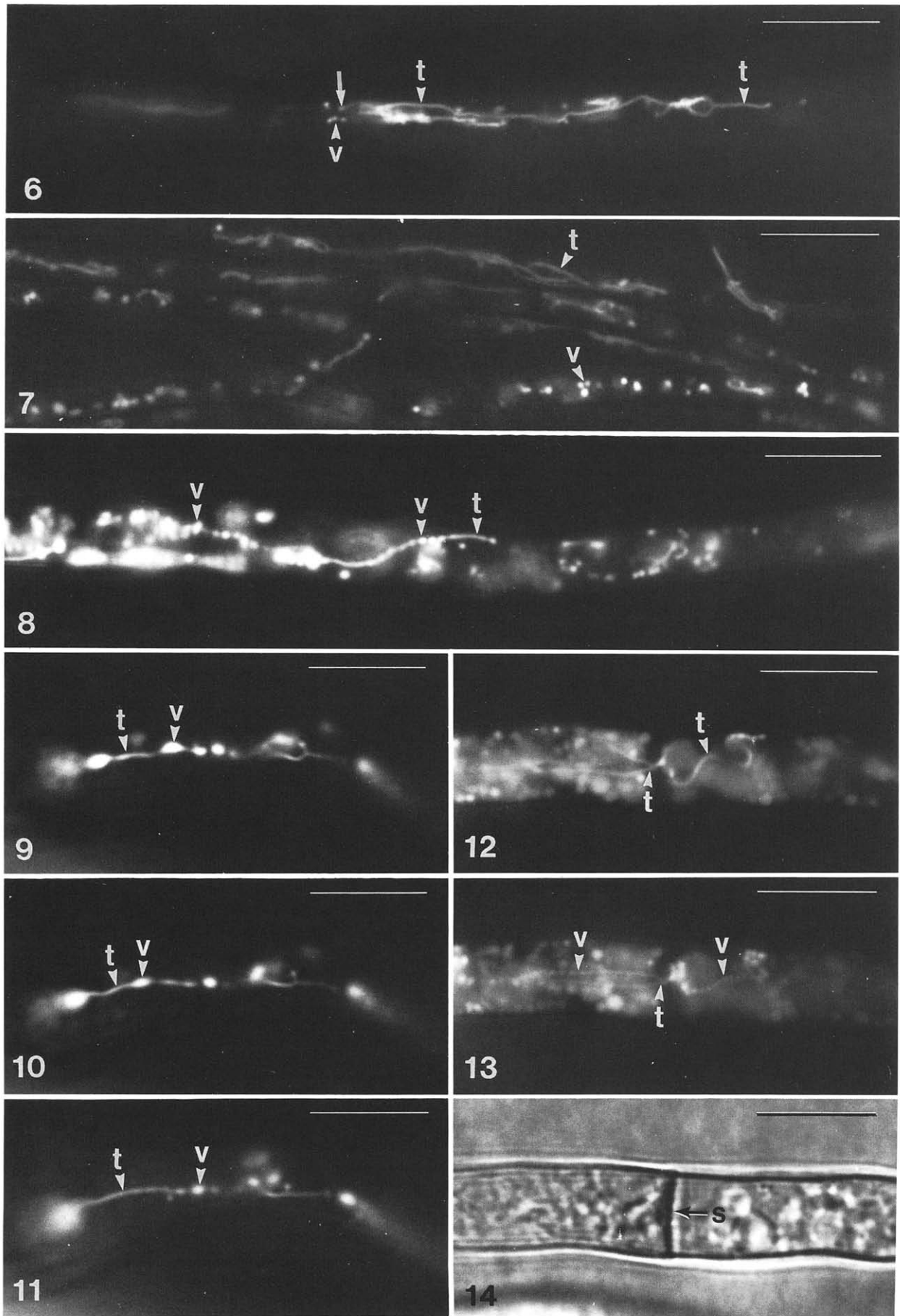
Observations were made predominantly on the tip-cells and penultimate cells. Viability of the cells was confirmed by the presence of cytoplasmic streaming, motility and appearance of the tubules and vacuoles, and the general appearance of the hyphae. Preparations were viewed by

fluorescence microscopy on a Zeiss Axiophot microscope with the filter combination BP450-490, FT510 and LP520. Photomicrographs were taken on Kodak Technical Pan film rated at 400 ISO and developed with Technidol developer.

## RESULTS

### *Eumycota*

**Basidiomycotina.** A motile tubular vacuole system similar to that described by Shepherd *et al.* (1993a, b) in *Pisolithus tinctorius* (Fig. 1) was present in cultures of all species examined from this sub-phylum. The various fungi differed in their ability to accumulate CF and there were subtle differences in the appearance and stability of the system. Slow growing, hydrophobic cultures such as *Fistulinella mollis* (Fig. 2), *Suillus granulatus* and *Paxillus involutus* (Fig. 3) all accumulated CF into a system of tubules and vacuoles after about 15 min exposure to CF followed by a 15 min rinse. The system consisted of motile vacuoles and interconnecting tubules which varied from 240 to 480 nm in diameter (estimated from fluorescence micrographs) and was stable for several hours



Figs 6-14. For captions see facing page.

following CF accumulation. The system showed a zonation in the tip-cells similar to that found in *Pisolithus tinctorius*. Tubules extended and retracted, and dilated and contracted, propelling fluorescent material in both directions along the cells as in *P. tinctorius* (Shepherd *et al.*, 1993a). In the more wettable fungi, such as *Agaricus carneus* (Fig. 4) and *Armillaria mellea* (Fig. 5), a CF-accumulating tubule and vacuole system with similar properties to that in *P. tinctorius* was also found but was harder to demonstrate. Rhizomorphs of *Armillaria mellea* accumulated fluorochrome in the fine surface hyphae only.

In fast growing cultures of hydrophobic fungi such as *Sphaerobolus* sp., *Nidularia* sp. and *Sclerotium rolfsii* the hyphae were very easily damaged and the system was difficult to demonstrate. The time of exposure to CFDA was reduced to 1–3 min and preparations were viewed immediately after a brief rinse. The system was seen to consist of tubules and vacuoles as in *Pisolithus tinctorius*. The tubules on exposure to the exciting light very rapidly rounded up into chains of vesicles and stopped moving.

**Ascomycotina.** A motile tubular vacuole system similar to that in *P. tinctorius* was present in all members of the Ascomycotina examined. Initially, some fluorescence was seen in the cytoplasm and this was followed by a rapid accumulation of CF into a characteristic system of motile tubules and vacuoles (Figs 6–7). Once again this was best demonstrated in slow growing hydrophobic cultures, such as *Claviceps* sp. (Fig. 6) and *Hymenoscyphus ericae* (Fig. 7), where tubules and vacuoles could be seen in most tip-cells. Some background fluorescence was retained in the cytoplasm, especially in the hyphal tip region, but in all respects the system in these two species closely resembled that in *Pisolithus tinctorius*. In the two faster growing species, *Monilinia fructicola* and *Peziza whitei*, CF accumulated in a tubule and vacuole system when cultures taken directly from refrigerated slope cultures were briefly exposed to CFDA, but was not so easily demonstrated in hyphae of subsequent faster growing subcultures. In these latter two species even in the best preparations the system was only demonstrated in a small number of tip-cells.

**Deuteromycotina.** Slide cultures gave the best results with fungi selected from this subphylum and again a system similar to that in *Pisolithus tinctorius* was demonstrated. In *Aspergillus niger* a system of tubules and small vacuoles was apparent in

the hyphal tip-cells (Fig. 8) and in one cell it was possible to record the movement of dilations containing fluorochrome along a tubule which could be seen to be confluent with larger vacuoles (Figs 9–11). The fluorochrome faded rapidly and the system was very fragile under the excitation. There were many very small fluorescent vacuoles that moved with the cytoplasmic streams, and there was fluorescence throughout the cytoplasm. In *Penicillium expansum* there was also fluorescence throughout the cytoplasm (Figs 12–13), and a tubule and vacuole system could be seen in cells that had accumulated fluorochrome. In one of these, a branched tubule system was seen across the simple pore in the septum between the apical and penultimate cells (compare Figs 12–14). Some parts of the tubules rounded up into strings of small interconnected vacuoles during observation, exactly as seen in *Pisolithus tinctorius* (Figs 12–13). Larger vacuoles were found at the base of the tip-cell and in more basal cells, again as in *P. tinctorius*. Cells of *Candida albicans* under the growth conditions used here did not accumulate CF and so the system could not be detected.

### Zygomycota

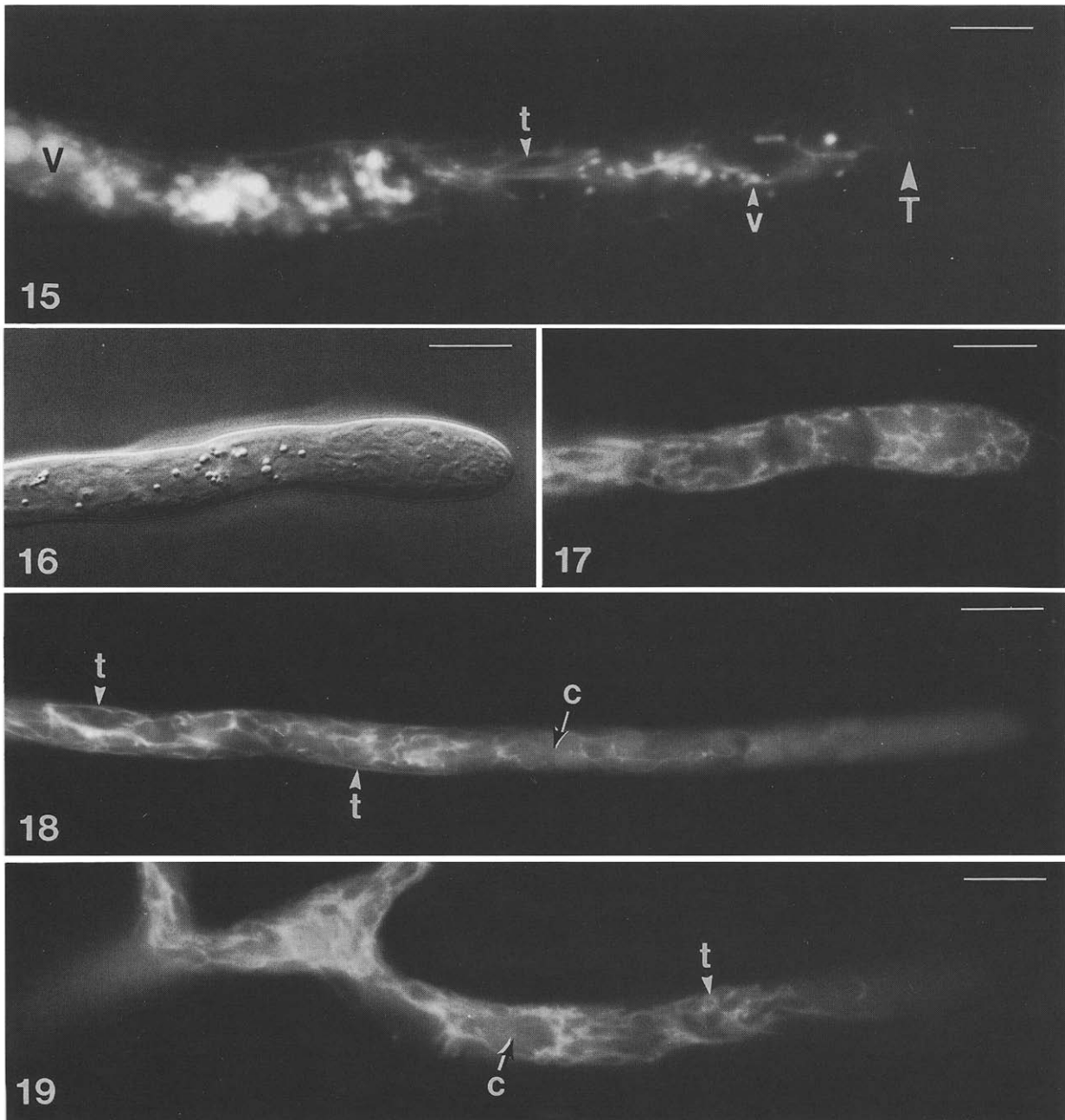
All the organisms examined from this group also contained a system which accumulated CF and under favourable conditions was seen to consist of tubules variously associated with vacuoles. Again, there was a high level of background fluorescence in the cytoplasm of many cells and the system was fragile. In *Rhizopus stolonifer* the system was seen to consist of short branched tubules connected with small vacuoles in the hyphal tip region (Fig. 15). Tubules were occasionally seen to extend into the extreme tip of the cell and retract again. In more basal regions the vacuoles were larger, more frequent and clustered and there were a few short tubules associated with them. Similarly, in *Zygorhynchus moelleri* and *Thamnidium elegans* an extensive motile tubule system interacting with a few small vacuoles was seen.

### Oomycota

Hyphal tips of members of the Oomycota were very easily disrupted by the treatment. For this reason hyphal tips of *Saprolegnia parasitica* were first examined with Nomarski DIC optics (Fig. 16) and those with characteristic organelle zonation and the appearance of growing, viable hyphae were selected

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**Figs 6–14.** The tubular vacuole system in the Ascomycotina and Deuteromycotina. Bars, 10  $\mu$ m. **Fig. 6.** A tubular reticulum (t) is shown throughout this hypha of *Claviceps* sp., with few vacuoles. One cluster of very small vacuoles (v) occurs in association with the tubules and one of the tubules near this cluster has a dilation at its end (arrow). **Fig. 7.** In *Hymenoscyphus ericae* some hyphae are seen to contain a reticulum of tubules (t) with few to no spherical vacuoles while others contain a series of spherical vacuoles (v) with few to no tubules. The hyphae are narrower than those in other genera examined in this subphylum, but the tubules remain fairly constant in diameter. **Fig. 8.** In this hypha of *Aspergillus niger* CF has accumulated into both tubules (t) and vacuoles (v). Tubules were often seen to form long arrays of small connected vacuoles like strings of beads. **Figs 9–11.** A sequence of fluorescence micrographs at 8 s intervals in *Aspergillus niger* demonstrates a succession of peristalsis-like movements of a tubule (t), such that the tubule dilations (v) are captured in various positions. **Figs 12–14.** Fluorescence (Figs 12–13) and bright field (Fig. 14) micrographs of the same field of *Penicillium expansum* showing two adjacent cells in which a tubule (t) is seen across the simple septum (s) characteristic of this subphylum. Branching of the tubule can be seen at point (t) near this septum. Fig. 13 was taken about 8 s after Fig. 12. During this period, the tubule system vesiculated into chains of small vacuoles (v) of varying sizes. Other somewhat larger fluorescent vacuoles, not apparently connected to tubules occur throughout this region of the hypha. The septum (s) is clearly visible in Fig. 14.



**Figs 15–19.** CF accumulating systems within the Zygomycota (Fig. 15) and the Oomycota (Figs 16–19). Bars, 10  $\mu$ m. **Fig. 15.** Hypha of *Rhizopus stolonifer* showing a tubular vacuole system which accumulates CF. Tubules (t) are generally shorter than those in *P. tinctorius* and do not form an extensive reticulum. They are associated with small vacuoles (v) near the tip (T) of the hypha and some tubules have dilations at their ends. There are larger vacuoles (V) further back and some fine fluorescent tubules are also present in this region. **Figs 16–17.** The same hyphal tip of *Saprolegnia parasitica* (Saprolegniales) viewed firstly with DIC optics (Fig. 16) to demonstrate organelle zonation, the position of vesicles and other structural features indicative of viable hyphae and then with blue excitation (Fig. 17), showing a fluorescent reticulum in the hyphal tip. **Fig. 18.** hyphal tip region of *Pythium irregulare* (Pythiales) showing a similar reticulum to that in *S. parasitica*. The CF is accumulated initially in the cytoplasm (c) which retains its fluorescence. A fine, branched, extensive reticulum of tubules (t) subsequently accumulates CF. No fluorescent spherical vacuoles are present. **Fig. 19.** Hyphal tip region of *Phytophthora cinnamomi* (Pythiales) also showing an extensive reticulum of fluorescent tubules (t), with no spherical vacuoles. Again the cytoplasm (c) is fluorescent.

(Heath & Kaminskyj, 1989; Yuan & Heath, 1991a, b). These were then irradiated with blue excitation and one or more fluorescence micrographs were taken (Fig. 17). Fluorescence disappeared from this reticulum rapidly as the hypha was irradiated and it could only be demonstrated in hyphae with

an organelle zonation characteristic of growing hyphal tips. (Note that this definition includes the sub-apical region as well as the extreme hyphal tip). The reticulum showed very limited motility and did not exhibit the rapid dilations and contractions which characterized that in the Eumycota. It was confluent

with larger vacuoles in sub-apical regions and these also accumulated CF under the same conditions. In hyphae which we believe had been perturbed (i.e. where growth or cytoplasmic streaming had stopped or where there was obvious structural damage) this reticulate system was rarely seen and fluorochrome was distributed throughout the whole cell or was predominantly in the cytoplasm. In this case, structures which were of various shapes and sizes were seen as unstained areas against this fluorescent background. These presumably represent various organelles.

A system that accumulated CF very similar to that in *Saprolegnia parasitica* (Fig. 17) was also demonstrated in the two other members of the Oomycota examined *Pythium irregulare* (Fig. 18) and *Phytophthora cinnamomi* (Fig. 19). Again it consisted of a branched reticulum which was quite different in both appearance and motility from the tubular vacuole, system in all the other subphyla.

## DISCUSSION

When assessing the results it should be borne in mind that appearance of fluorescence in the vacuole system is dependent on a multistep process involving uptake of CFDA across the plasma membrane, its subsequent transport across the tonoplast, and its hydrolysis by esterases that are probably located in the vacuole (Rotman & Papermaster, 1966; Klionsky, Herman & Emr, 1990; Oparka, 1991). The resulting compound, CF, is fluorescent, membrane impermeant and accumulates inside the vacuole. In order to demonstrate the system by this method all of these criteria must be satisfied. In addition the hyphae must be viable and both fungal structure and membrane integrity must be intact (Yuan & Heath, 1991*a, b*). There were many instances where we suspected that one or more of these criteria had not been met. This resulted in fluorescence patterns which could not be distinguished from artifacts. These have not been described and will not be discussed here.

A pleiomorphic motile tubular vacuole system similar to that described by Shepherd *et al.* (1993*a, b*) was present in all members of the Basidiomycotina and Ascomycotina examined. The tubule diameter was remarkably constant in all organisms accumulating CF, but the ratio of tubule to hyphal diameter varied from one species to another and, as a result, the system appeared finer in organisms with wider hyphae. The system is not restricted to mycorrhizal or parasitic organisms, nor to those organisms which are hydrophobic or slow growing in culture. It is, however, most easily demonstrable in slow-growing cultures of hydrophobic organisms, many of which are mycorrhiza formers. This may be because the system in faster growing organisms is more fragile under the observation conditions imposed.

The system in *Pisolithus tinctorius* is capable of transferring material along hyphae by alternate dilation and contraction of the tubular elements. Shepherd *et al.* (1993*b*) showed that tubules transferred material not only along cells but from cell to cell across the intervening dolipore septa. Data here show tubules similarly across the more simple septum of the Ascomycotina indicating that the system has potential for cell-to-cell transport in members of that sub-phylum also. The similarity between systems in the Basidiomycotina and

Ascomycotina supports the general view that members of the two groups have a common ancestry (Bartnicki-Garcia, 1970). In the only mycelium-forming yeast examined this system could not be found, presumably because the vacuoles did not accumulate CF under the conditions used. It is interesting to note, however, that using fluorescent markers intervacuolar transport has been seen to occur in other yeasts during zygote formation (Weisman & Wickner, 1988). The fluorescent tracks connecting parent vacuoles, interpreted by the authors as tracks of vesicles and thought to be involved in equilibration of vacuole contents, bear a remarkable resemblance to the tubules seen here in the other groups.

Members of the Zygomycota examined also appear to possess a system that consists of a motile tubular reticulum interacting with vacuoles. They appear to differ in that the vacuoles are smaller and the tubules appear shorter and do not form an extensive reticulum as they do in the *P. tinctorius* system. Vacuoles in the apical and penultimate cells of *P. tinctorius* contain polyphosphate and it has been proposed that the tubular elements may be involved in phosphorus transfer between clusters of vacuoles (Orlovich & Ashford, 1993; Ashford *et al.*, 1994). The findings that a similar system exists in members of the Zygomycota has implications for phosphorus uptake and transfer in vesicular arbuscular mycorrhizae, although it has not been demonstrated in those members of the subphylum that are mycorrhizal.

Not surprisingly, the protoctistan Oomycota, no longer regarded by many as fungi, but which exhibit a mycelial style of growth, display accumulation of CF in a reticulum of quite different appearance but which nevertheless is still connected to large vacuoles and is therefore apparently part of a vacuole system. Most importantly this was found in hyphae where zonation of organelles was as described for healthy growing cells by Heath and colleagues (Heath & Kaminskyj, 1989; Yuan & Heath, 1991*a, b*), and the fluorescence disappeared rapidly as the cells were irradiated or showed signs of damage or rearrangement of organelles. The reticulum was remarkably similar in members of the two subgroups examined. In conclusion, although it was not always the same in appearance and motility, CF has enabled us to visualise a reticulate vacuole system in a wide range of fungi from most taxa.

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