Protoplasma (1988) 144: 132–141

PROTOPLASMA

Ultrastructural Analysis of Conidiophore Development in the Fungus *Aspergillus nidulans* Using Freeze-Substitution

C. W. MIMS[1,*], E. A. RICHARDSON[1], and W. E. TIMBERLAKE[2]

[1] Department of Plant Pathology, [2] Departments of Genetics and Plant Pathology, University of Georgia, Athens, Georgia

Received November 28, 1987
Accepted March 7, 1988

Summary

Conidiation in *Aspergillus nidulans* can be divided conveniently into five morphologically distinct stages. These are development of the conidiophore stalk, formation of the conidiophore vesicle, differentiation of metulae, differentiation of phialides, and production of conidia. The results presented here demonstrate that freeze-substitution fixation greatly facilitates the study of most of these stages. Ultrastructural features of vesicles, mitochondria, microtubules and nuclei were more easily resolved in freeze-substituted samples than in chemically fixed samples. In addition, certain structures and events simply not visible in chemically fixed samples were found routinely in freeze-substituted samples. Examples include Golgi bodies and multivesicular bodies and mitotic divisions associated with various stages of conidiation.

Keywords: Conidiophore and conidium development; *Aspergillus nidulans*; freeze-substitution; transmission electron microscopy.

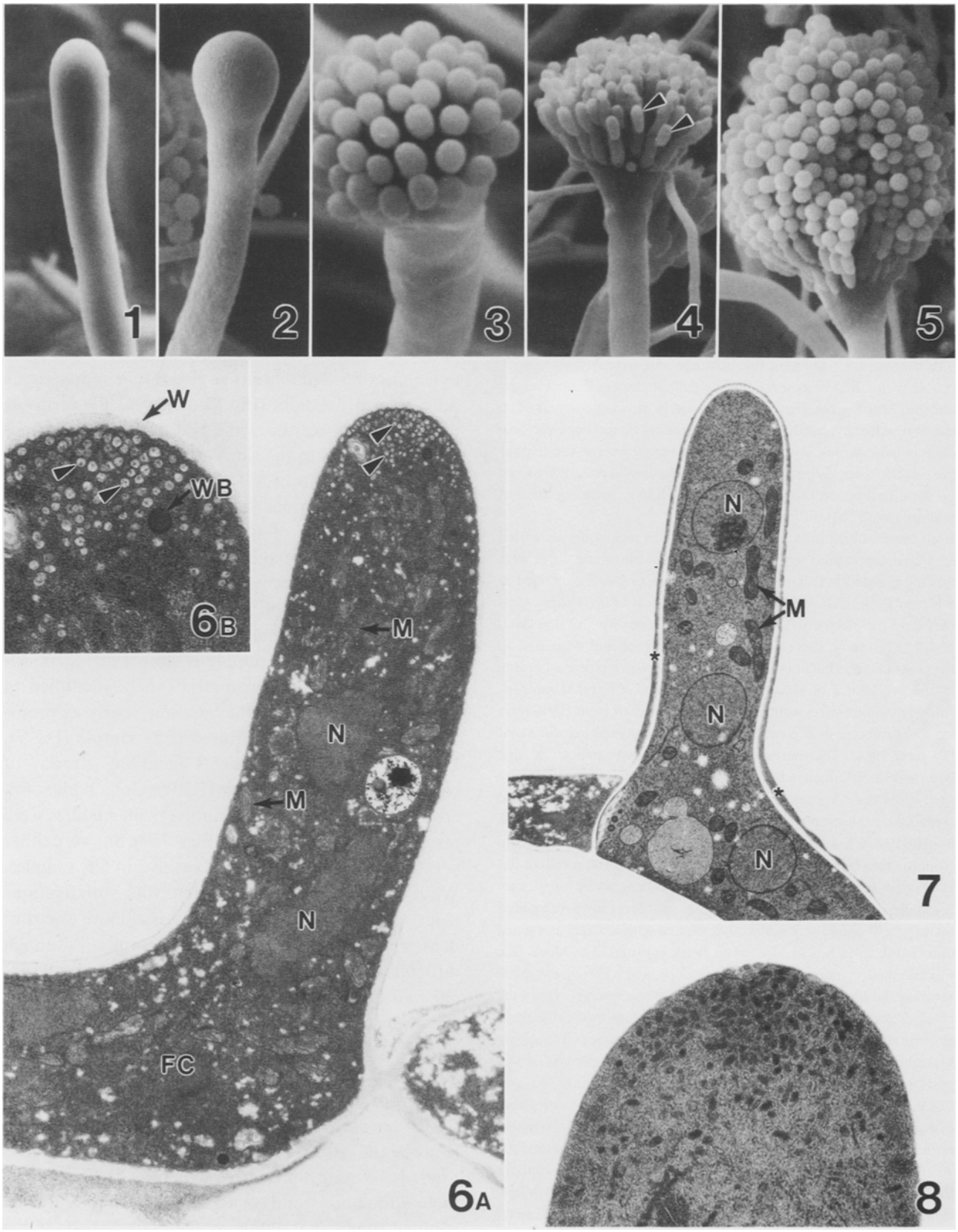
* Correspondence and Reprints: Department of Plant Pathology, University of Georgia, Athens, GA 30602, U.S.A.

1. Introduction

In preparing to study the effects of selected sporulation-specific genes on conidiation (asexual reproduction) in mutant strains of the fungus *Aspergillus nidulans*, a survey of the literature revealed few ultrastructural studies of conidiation in this species. It therefore was apparent that this process needed to be examined more throughly at the ultrastructural level in a wild-type strain before studying mutants. In an attempt to avoid the fixation problems that have plagued previous TEM studies of the *Aspergilli* (TANAKA and YANAGITA 1963, TRINCI *et al.* 1968, TSUKAHARA 1970, WEISBURG and TURIAN 1971, OLIVER 1972, GHIORSE and EDWARDS 1973, BOJOVIĆ-CVETIĆ and VUYICIC 1974, HANLIN 1976, DEANS *et al.* 1980, COLLINGE and MARKHAM 1982), we decided to try freeze-substitution fixation. This technique has been successful in improving the

Abbreviations Used in Figures: C conidium, *CI* conidium initial, *CV* conidiophore vesicle, *FC* foot cell, *GB* Golgi body, *M* mitochondrion, *ME* metula, *MT* microtubule, *MVB* multivesicular body, *N* nucleus, *PM* plasma membrane, *P* phialide, *RER* rough endoplasmic reticulum, *S* spindle apparatus, *SPB* spindle pole body, *V* vacuole, *W* fungal wall, *WB* Woronin body.

Figs. 1–8. *Aspergillus nidulans*. Figs. 1–5 are scanning electron micrographs of various stages in conidiation. Figs. 6–8 are transmission electron micrographs of very young conidiophores. Fig. 1. Young conidiophore stalk just prior to vesicle formation. × 3,000. Fig. 2. Developing vesicle at tip of conidiophore. × 2,000. Fig. 3. Developing metulae. × 5,000. Fig. 4. Developing phialides (arrowheads). × 3,000. Fig. 5. Tip of mature conidiophore bearing numerous chains of conidia. × 3,000. Fig. 6 *A*. Near-median longitudinal section of a chemically fixed young conidiophore stalk. A portion of the foot cell (*FC*) is visible at the base of the conidiophore. Numerous apical vesicles (arrowheads) are evident at the tip of the stalk. Although nuclei (*N*) and mitochondria (*M*) can be identified, the quality of fixation is poor. × 12,000. Fig. 6 *B*. Higher magnification of a portion of the tip of the stalk shown in Fig. 6 *A*. The fungal wall (*W*) can barely be seen. Apical vesicles (arrowhead) and Woronin body (*WB*) are visible. × 24,000. Fig. 7. Portion of a freeze-substituted stalk. The tip of the stalk containing the apical vesicles was out of the plane of section. Even at low magnification nuclei (*N*) and mitochondria (*M*) are much more prominent than in the chemically fixed stalk shown in Fig. 6 *A*. The dark material (asterisks) covering the stalk was not found on other conidiophores examined and probably represents an artifact of the freezing process. × 6,000. Fig. 8. Apical vesicles in the tip of a freeze-substituted young conidiophore. × 20,000



Figs. 1–8

preservation of ultrastructural details in various fungi (Howard and Aist 1979, Hoch and Howard 1980, Howard 1981, Hoch and Staples 1983, Newhouse *et al.* 1983, Heath and Rethoret 1984, Hoch 1986, Mims *et al.* 1988), but to our knowledge, has not been used in previous studies of conidiation. Results of this study will serve as the basis for future studies of various developmental mutants of *A. nidulans*.

2. Methods

The Glasgow wild-type strain of *A. nidulans* (FGSC 4) used in this study was grown at 22 °C on Czapek's agar plates. Specimens for ultrastructural study were obtained from these plates as follows. Short segments (3–5 mm) of fine platinum wire were flame sterilized, coated with a film of agar by dipping them in molten Czapek's agar and laid near the margins of young, actively growing colonies of *A. nidulans*. After hyphae had grown over the wires and begun to produce conidiophores, a razor blade was used to sever the hyphae around the wires. Wires with adhering fungal material were picked up with forceps and processed for either TEM or scanning electron microscopy (SEM).

Both freeze-substitution and chemical fixation techniques were used to prepare specimens for TEM. For freeze-substitution, wires were quickly plunged into liquid propane in a freezing well contained in a Dewar flask filled with liquid nitrogen (Hoch 1986). Frozen samples were transferred to a substitution fluid consisting of 2% OsO_4 and 0.05% uranyl acetate in anhydrous acetone and processed to the point of embedment in an Epon-Araldite resin mixture according to the procedures of Hoch (1986). Fixed and infiltrated samples, still on platinum wires were then placed in drops of fresh 100% resin on a microscope slide coated with tetrafluorethylene release agent (Taylor 1984). The sharpened end of a wooden application stick was used to dislodge the fungal material from the wires which were then discarded. A coated glass coverslip was used to flatten and spread the resin droplets. Following polymerization of the resin (48 hours at 60 °C), a razor blade was used to separate the coverslip from the thin layer of hardened resin on the slide. The resulting flat embedded samples were examined by light microscopy to locate conidiophores of different ages. Those selected for study were marked by using a diamond scribe objective, excised with a scapel and glued onto blank resin blocks molded in Beem capsules. The block face was trimmed, examined by light microscopy, and a sketch was made showing the location and appearance of the specimen(s) within the block. These sketches were useful for interpretation of subsequent electron micrographs as we knew precisely what the intact specimen looked like. Ultrathin serial sections for TEM were collected on slot grids and poststained with uranyl acetate and lead citrate.

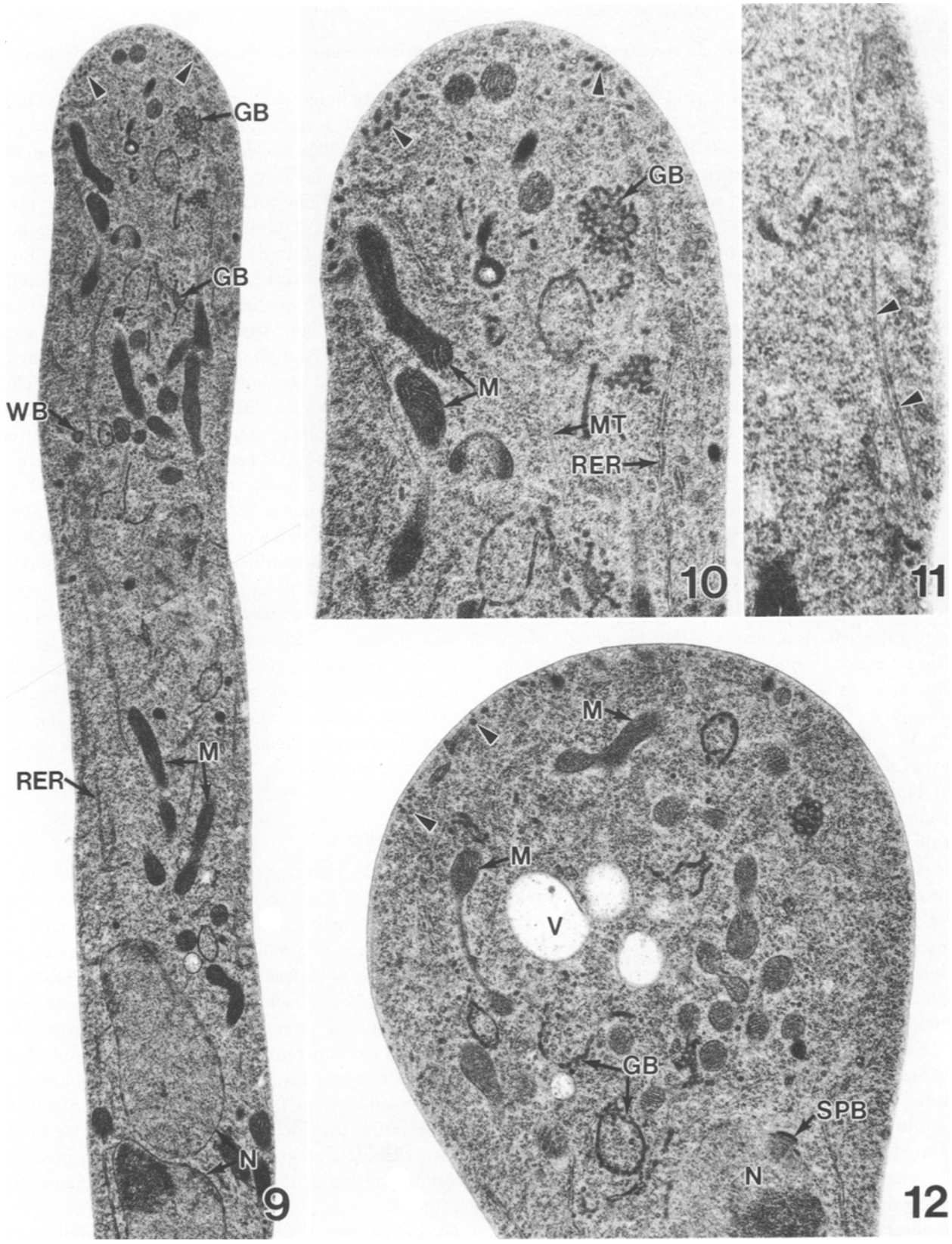
For chemical fixation samples were placed in a solution consisting of equal parts 5% glutaraldehyde and 0.1 M potassium phosphate buffer, pH 6.8. After 1–2 hours at 4 °C the samples were washed for 30 minutes with buffer and postfixed for 2 hours at 4 °C in similarly buffered 1% OsO_4. Samples were then washed in distilled water, stained overnight in aqueous uranyl acetate, washed in distilled water, dehydrated in a graded ethanol series and either infiltrated and prepared for TEM according to the procedures described above or processed for SEM according to Mims (1981).

3. Results

Conidiation in *A. nidulans* can be divided conveniently into five morphologically distinct stages. These are illustrated in the Figs. 1–5. The process begins with the formation of the aerial hypha that constitutes the conidiophore stalk. The stalk (Fig. 1) arises as a vertical outgrowth from a hyphal compartment known as the foot cell. Developing stalks can usually be distinguished from somatic hyphae because of their greater diameter and thickened walls. The stalk grows until it reaches a length of about 100 µm and then swells at its tip to form the conidiophore vesicle (Fig. 2) which is about 10 µm in diameter. From this vesicle numerous small outgrowths termed metulae develop (Fig. 3) and grow to lengths of 5–7 µm. Metulae give rise to sporogenous cells, termed phialides (Fig. 4). One phialide forms at the tip of each metula while additional phialides develop below and to the sides of the first phialide. Phialides reach lengths of 5–7 µm and then begin to form asexual spores termed conidia that accumulate in chains at the ends of the phialides (Fig. 5). Mature conidia are spherical and about 3 µm in diameter.

Freeze-substitution proved to be superior to conventional chemical fixation for the study of most aspects of conidiation in *A. nidulans*. A typical example of a chemically fixed (CF) sample is shown in Fig. 6. Although nuclei and mitochondria can be identified in this conidiophore stalk, they are not nearly as prominent as in a freeze-substituted (FS) sample (Fig. 7). Likewise, cellular components including strands of rough endoplasmic reticulum (RER), Golgi bodies, microtubules, ribosomes and multivesicular bodies were resolved easily in FS samples (Figs. 7–9), but were either very difficult or impossible to resolve in CF samples. While apical vesicles were apparent with both fixations (Figs. 6 and 8), their contents appeared more electron-dense in FS samples than in CF samples. Although difficult to detect using either fixation procedure, fungal wall material was usually more prominent in CF samples than in FS samples when sections were poststained for similar lengths of time with uranyl acetate and lead citrate.

Numerous Golgi bodies, mitochondria and strands of RER were present in the developing conidiophore stalk (Figs. 9 and 10). Golgi bodies were more numerous toward the stalk tip than elsewhere in the stalk. Mitochondria and strands of RER were more evenly distributed along the length of the stalk. Most of the strands of RER lay parallel to the long axis of the stalk (Fig. 9). Microtubules were commonly found just be-



Figs. 9–12. Freeze-substituted samples of *Aspergillus nidulans*. Fig. 9. Near median longitudinal section of an almost fully elongated stalk just prior to vesicle formation. Portions of two nuclei (*N*) are visible some distance below the tip of the stalk. Various cellular components including apical vesicles (arrowheads), mitochondria (*M*), Golgi bodies (*GB*), strands of rough endoplasmic reticulum (*RER*) and a Woronin body (*WB*) are also visible. × 13,000. Fig. 10. Higher magnification of the tip of the stalk from Fig. 9. Visible are mitochondria (*M*), Golgi bodies (*GB*) in various planes of section, strands of rough endoplasmic reticulum (*RER*) and a short segment of a microtubule (*MT*). Apical vesicles (arrowheads) are less numerous than in a young stalk (Fig. 8) and appear around the margin of the stalk apex. × 27,000. Fig. 11. Section near the surface of a stalk showing microtubules (arrowheads). × 50,000. Fig. 12. Developing conidiophore vesicle with numerous mitochondria (*M*) and Golgi bodies (*GB*). Some apical vesicles (arrowheads) are visible around the periphery of the vesicle. Portions of a vacuole (*V*) and a nucleus (*N*) with its spindle pole body (*SPB*) are also visible. × 20,000

neath the plasma membrane (Fig. 11). Apical vesicles were also present near the tip of the elongating stalk (Figs. 6 and 8), but once the stalk reached its maximum length, the number of vesicles declined significantly. Those remaining appeared to be dispersed around the periphery of the tip (Figs. 9 and 10).

More than one nucleus appeared to enter the young stalk from its foot cell (Figs. 6 and 7). Although mitotic divisions were not observed in the elongating stalk, it rapidly became multinucleated. Nuclei remained some distance below the tip of the elongating stalk (Fig. 6) until the conidiophore vesicle formed (Fig. 12). By the time metulae began to differentiate (Figs. 13 and 14) numerous nuclei had moved into the vesicle (Fig. 15).

Metulae arose synchronously from the conidiophore vesicle and initially appeared as small protrusions containing primarily ribosomes and cytoplasmic vesicles (Fig. 13). Soon Golgi bodies entered the metulae (Fig. 14) followed by mitochondria, strands of RER, vacuoles and multivesicular bodies (Figs. 15 and 16). As metulae enlarged, nuclei within the conidiophore vesicle became positioned below the metulae and divided mitotically (Fig. 17). The orientation of division was such that one daughter nucleus entered the metula (Fig. 18) while the other remained in the vesicle. Nuclei further down in the vesicle and stalk also divided at this time (Fig. 19). Following mitosis, a centripetally developing septum formed at the base of the uninucleate metula, delimiting it from the conidiophore vesicle (Fig. 20). This septum possessed a central pore on either side of which Woronin bodies were present (Fig. 21). These darkly-staining, spherical bodies were present in earlier stages of conidiation (Figs. 6 and 7), but became much more numerous as septa formed.

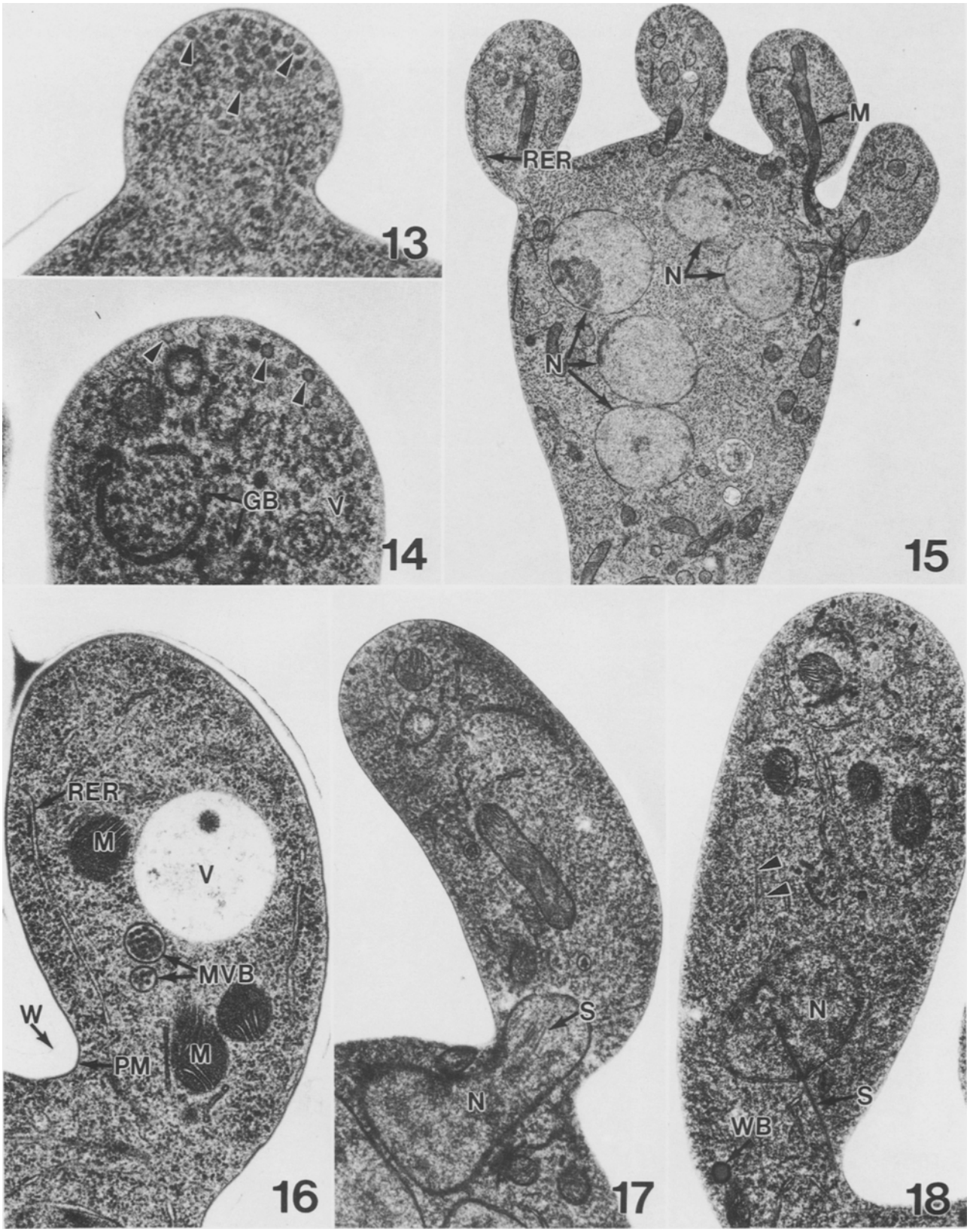
Phialide development began shortly after metulae were delimited from the conidiophore vesicle (Fig. 22). Tips of developing phialides (Fig. 23) were characterized by the presence of Golgi bodies and numerous vesicles similar to those present in metulae (Figs. 13 and 14). Microtubules were common in metulae (Fig. 24) and often extended into developing phialides. Phialide elongation continued as additional cellular components including a single nucleus entered each phialide. We did not observe the mitotic divisions associated with phialide development. A septum formed at the base of the phialide delimiting it from its uninucleate metula (Fig. 25). Septum development was similar to that observed at the base of the metula. Once this septum formed, an additional phialide developed from the side of the metula just below the first phialide (Fig. 25).

A conidium initial developed as a protrusion from the phialide neck as materials moved into it from the phialide. The phialide nucleus entered the neck region and divided mitotically (Fig. 26). One daughter nucleus entered the developing conidium (Fig. 27) while the other moved down out of the neck. A centripetally developing septum (Fig. 27) formed at the base of each conidium. Although in undisturbed specimens a chain of conidia formed at the tip of each phialide, preparatory techniques used in both freeze-substitution and conventional chemical fixation disrupted these chains leaving only one or two developing conidia on each phialide (Fig. 28). It was also difficult to obtain good freeze-substituted samples of these latter stages of conidiation. Apparently air trapped between metulae, phialides and chains of conidia interferred with the freezing. Typically we found only a few adequately fixed phialides and developing conidia on any one conidiophore. Latter stages of conidium maturation were not observed. As evident in Figs. 19 and 22, vacuoles began to appear in the conidiophore vesicle early in the conidiation process. However, older vesicles were not completely vacuolated and numerous cellular components including nuclei were still present in conidiophore vesicles bearing phialides with maturing conidia (Fig. 28). Metulae and phialides became vacuolated only very late in the conidiation process.

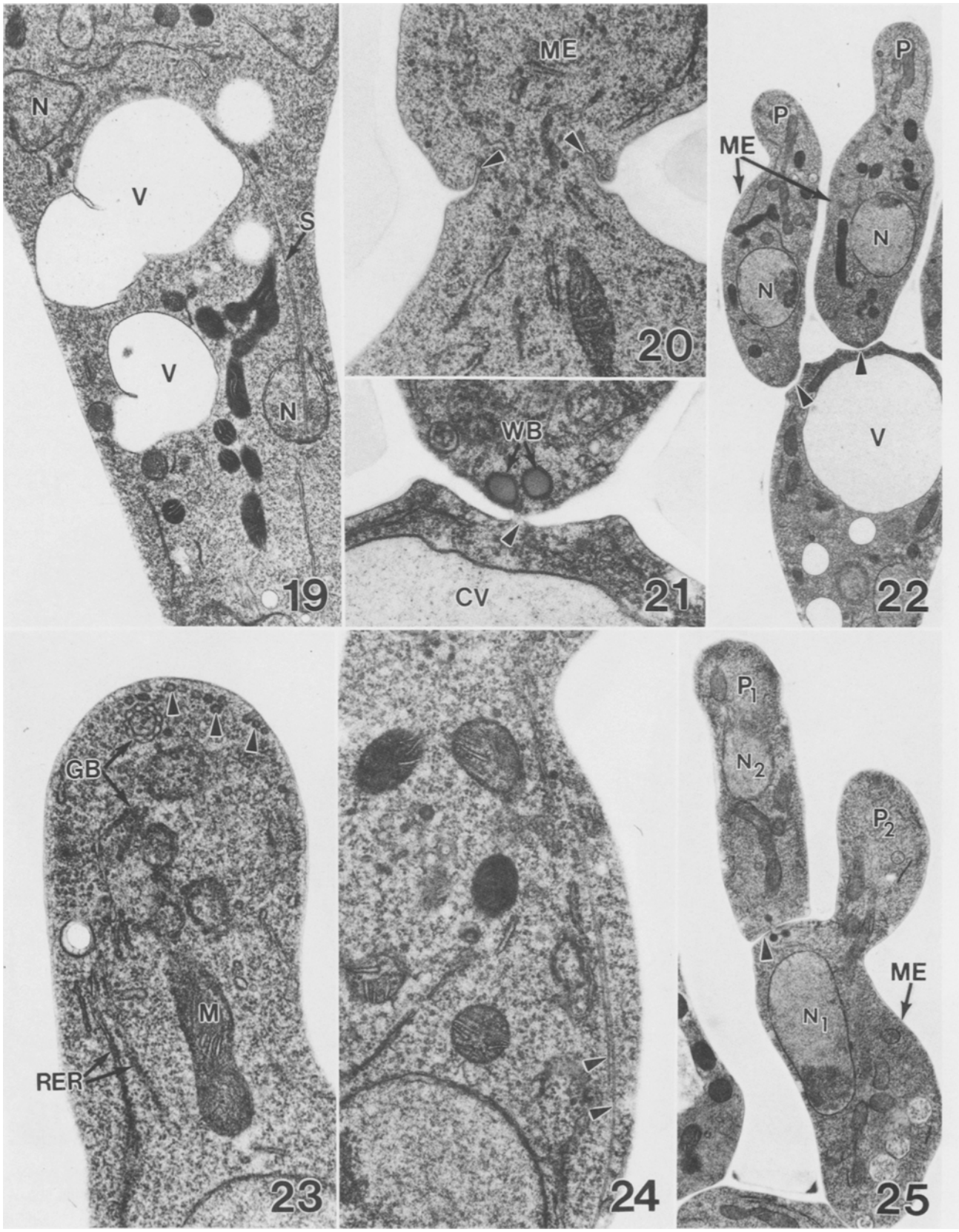
4. Discussion

Data on the ultrastructural aspects of conidiation in the *Aspergilli* have been summarized by SMITH *et al.* (1977). Although general information is available on various stages of conidiation in a few species, detailed data on the ultrastructural aspects of conidiation in the *Aspergilli* are lacking primarily because of poor preservation of samples obtained with conventional chemical fixation protocols. The results of this study demonstrate that many of these fixation problems can be avoided by using freeze-substitution. The quality of preservation provided by this technique yielded data not available from CF samples and also eliminated certain fixation artifacts resulting from chemical fixation.

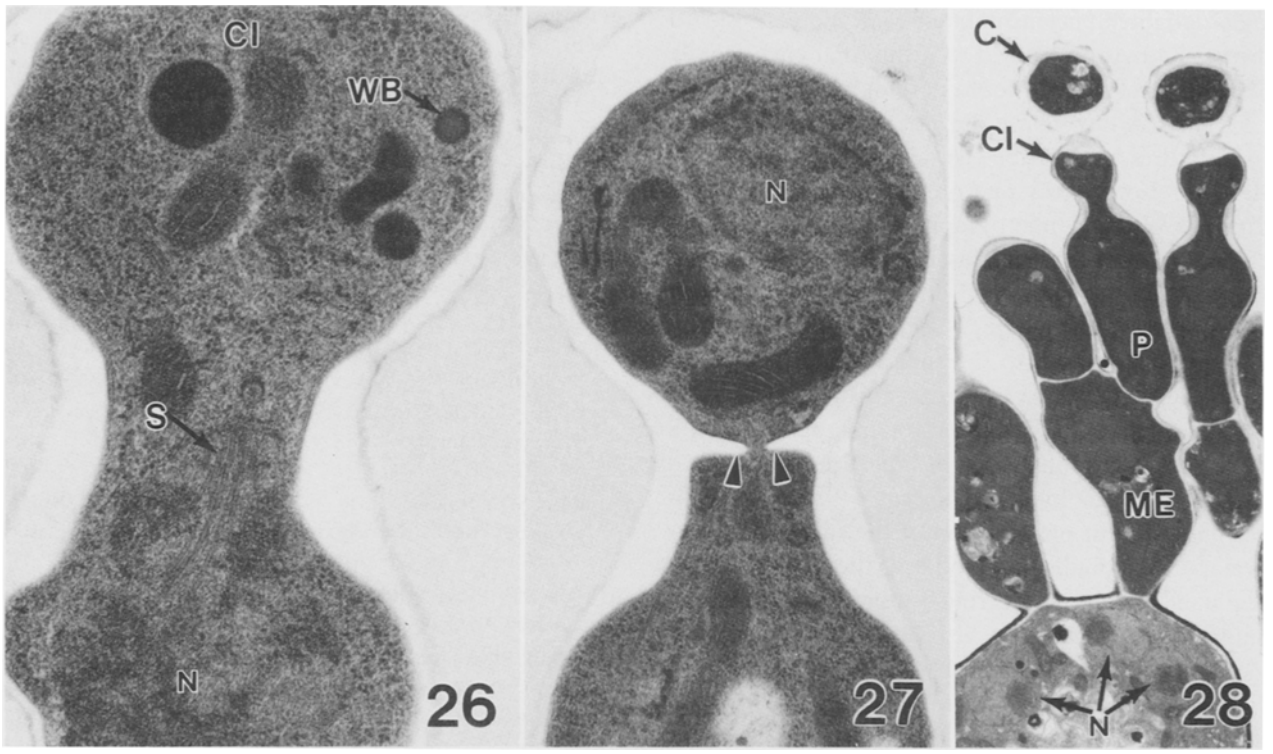
The cytoplasmic vesicles we observed in conidiophore stalks and vesicles and young metulae and phialides were also noted by OLIVER (1972) and appear to be characteristically present in both CF and FS samples of fungal hyphal tips. However, neither the Golgi bodies nor the multivesicular bodies so conspicuous in our FS samples were mentioned by OLIVER (1972). Con-



Figs. 13–18. Freeze-substituted samples of *Aspergillus nidulans*. Fig. 13. Early stage in the development of a metula. Many small vesicles (arrowheads) are visible in the metula. × 34,000. Fig. 14. Tip of a slightly older metula than that shown in Fig. 13. Numerous Golgi bodies (*GB*) and vesicles (arrowheads) are present in the metula. × 40,000. Fig. 15. Portion of a vesicle showing four young metulae. Five nuclei (*N*) are visible in this section of the vesicle. Mitochondria (*M*) and strands of rough endoplasmic reticulum (*RER*) extend into the metulae. × 9,000. Fig. 16. Higher magnification of a developing metula. Some wall material (*W*) is visible. The plasma membrane (*PM*) of the metula is visible as well as multivesicular bodies (*MVB*), a vacuole (*V*), strands of rough endoplasmic reticulum (*RER*) and mitochondria (*M*). × 30,000. Fig. 17. Mitotic nucleus (*N*) extending from the conidiophore vesicle into a metula. A portion of the spindle apparatus (*S*) is visible. × 18,000. Fig. 18. Late telophase nucleus (*N*) in a metula. A portion of the spindle apparatus (*S*) protrudes from the nucleus. Portions of microtubules (arrowheads) and a Woronin body are visible. × 18,000



Figs. 19–25



Figs. 26–28. Freeze-substituted (Figs. 26 and 27) and chemically fixed (Fig. 28) samples of *Aspergillus nidulans*. Fig. 26. Mitotic nucleus (*N*) in a phialide neck below a conidium initial (*CI*). A portion of the spindle apparatus (*S*) is visible. A Woronin body (*WB*) is present in the conidium initial. × 30,000. Fig. 27. Developing septum (arrowheads) at the base of a conidium (*C*). The conidium nucleus is shown at *N*. × 20,000. Fig. 28. Section showing a maturing conidium (*C*), conidium initial (*CI*), phialide (*P*), metula (*ME*) and a portion of the conidiophore vesicle in which three nuclei (*N*) are visible. × 4,000

versely, the prominent plasmalemmasomes evident in his micrographs were not found in our FS samples. Although we did find a few examples of structures that might be interpreted as lomasomes, these structures were not as numerous as in CF samples of either *A. nidulans* (Oliver 1972) or *A. clavatus* (Hanlin 1976). The possible presence of lomasomes in FS samples needs additional study as it has been suggested (Hoch and Staples 1983, Hoch 1986) that these structures, as well as plasmalemmasomes, are artifacts of chemical fixation.

Our observations regarding the sequence of nuclear events occurring during metula formation do not agree with those of Clutterbuck (1969) and Oliver (1972). These authors reported that numerous mitotic divisions occurred in the conidiophore vesicle as metula formed. Following these divisions a single nucleus was reported to migrate into each fully developed metula. We, on the other hand, found what appeared to be a single, more or less synchronous mitotic division within the vesicle. The orientation of the division of nuclei located at the bases of developing metulae was such that a

Figs. 19–25. Freeze-substituted samples of *Aspergillus nidulans*. Fig. 19. Portion of a conidiophore at the base of a vesicle that was forming metulae. Late telophase nucleus (*N*) and a portion of its spindle apparatus (*S*) are visible. The large electron transparent areas (*V*) are vacuoles. × 15,500. Fig. 20. Developing septum (arrowheads) at the base of a metula (*ME*). × 34,000. Fig. 21. Mature septum at the base of a metula. Visible are the central pore (arrowhead) and Woronin bodies (*WB*). The Woronin bodies on the conidiophore vesicle (*CV*) side of the septum were out of the plane of section. × 34,000. Fig. 22. Early stage in phialide development. Developing phialides (*P*) are present at the ends of the metulae (*ME*). A single nucleus (*N*) is visible in each metula. Septa (arrowheads) are visible at the bases of the metulae. A large vacuole (*V*) is present in the conidiophore vesicle. × 8,500. Fig. 23. Tip of a developing phialide showing numerous vesicles (arrowheads) and Golgi bodies (*GB*). A mitochondrion (*M*) and strands of rough endoplasmic reticulum (*RER*) are visible. × 34,000. Fig. 24. Microtubules (arrowhead) in a metula that was forming a phialide. × 30,000. Fig. 25. Metula (*ME*) bearing one phialide (P_1) that had been delimited by a septum (arrowhead) and a second developing phialide (P_2). The metula nucleus is visible at N_1 while a portion of the older phialide nucleus is visible at N_2. × 9,500

single daughter nucleus entered each metula. Such a "directed" division seemed to insure that each metula received a nucleus prior to the development of a septum at its base. Although we did not observe enough division figures to describe the details of mitosis accurately, it is noteworthy that ours appear to be the first published electron micrographs of mitotic nuclei in conidiophores of any fungus. This is surprising considering the large number of ultrastructural studies of conidiation in various fungi (see Cole 1984 for a review), but may relate to the superior preservation of mitotic nuclei provided by freeze-substitution (Heath and Rethoret 1984). Two electron micrographs of FS mitotic nuclei in hyphae of *A. nidulans* are also included in a recent paper by Asmani *et al.* (1984) and demonstrate excellent preservation of the spindle, nuclear envelope and spindle pole bodies. Mitotic divisions of the phialide nucleus occurring during the formation of conidia by various fungi have been also difficult to find by using either light or electron microscopic techniques. The few mitotic figures we found in phialides of *A. nidulans* were always in the neck region of phialides bearing almost fully expanded conidium initials. Although we did not observe telophase, we suspect that one end of the telophase phialide nucleus extends into the conidium initial. Phase contrast observations by Kozakiewicz (1978) clearly demonstrate that this occurs in *A. flavus*.

Overall differentiation of conidia from phialides of *A. nidulans* appears to be similar to that described in various other phialidic fungi including *A. clavatus* (Hanlin 1976). A detailed discussion of what is known of the ultrastructural aspects of conidium development in phialidic species has been provided by Cole (1986). Currently we know little about the ultrastructural aspects of conidium maturation in *A. nidulans*, as older spores were very difficult to fix using either conventional chemical fixation or freeze-substitution. Currently we are working on techniques that will allow us to study more effectively these latter stages of the conidiation process.

Acknowledgements

This work was supported by NIH and USDA grants to W. E. Timberlake.

References

Asmani SA, May GS, Morris NR (1987) Regulation of the mRNA levels of *nimA*, a gene required for the G 2-M transition in *Aspergillus nidulans*. J Cell Biol 4: 1495–1504

Bojović-Cvetić D, Vujicic J (1980) Membranous aggregations in hyphal tips of *Aspergillus flavus*. Arch Microbiol 126: 245–249

Cole GT (1986) Models of cell differentiation in conidial fungi. Microbiol Reviews 50: 95–132

Collinge AJ, Markham P (1982) Hyphal tip ultrastructure of *Aspergillus nidulans* and *Aspergillus giganteus* and possible implications of Woronin bodies to the hyphal apex of the latter species. Protoplasma 113: 209–213

Clutterbuck AJ (1969) Cell volume per nucleus in haploid and diploid strains of *Aspergillus nidulans*. J Gen Micro 55: 291–299

— (1977) The genetics of conidiation in *Aspergillus nidulans*. In: Smith JE, Pateman JA (eds) Genetics and physiology of *Aspergillus*. Academic Press, New York, pp 305–317

Deans SJ, Gull K, Smith JE (1980) Ultrastructural changes during microcycle conidiation of *Aspergillus niger*. Trans Br Mycol Soc 74: 493–499

Ghiorse WC, Edwards MR (1973) Ultrastructure of *Aspergillus fumigatus* conidia. Development and maturation. Protoplasma 76: 49–59

Hanlin RT (1976) Phialide and conidium development in *Aspergillus clavatus*. Am J Bot 63: 144–155

Heath IB, Rethoret K (1984) The ultrastructure of mitotic spindles from conventionally fixed and freeze-substituted nuclei of the fungus *Saprolegnia*. Eur J Cell Biol 35: 384–395

Hoch HC (1986) Freeze-substitution of fungi. In: Aldrich HC, Todd WJ (eds) Ultrastructure techniques for microorganisms. Plenum Pub Corp, pp 183–212

— Howard RJ (1980) Ultrastructure of freeze-substituted hyphae of the basidiomycete *Laetisaria arvalis*. Protoplasma 103: 281–297

— (1981) Conventional chemical fixations induce artificial swellings of dolipore septa. J Exp Mycol 5: 167–172

— Staples RC (1983) Ultrastructural organization of the differentiated uredospore germling of *Uromyces phaseoli* variety *typica*. Mycologia 75: 795–824

Howard RJ (1981) Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörper, cytoskeleton and endomembranes after freeze-substitution. J Cell Sci 48: 89–103

— Aist JR (1979) Hyphal tip cell ultrastructure of the fungus *Fusarium*: Improved preservation by freeze-substitution. J Ultrastruct Res 66: 224–234

Kozekiewicz Z (1978) Phialide and conidium development in the *Aspergilli*. Trans Br Mycol Soc 70: 175–186

Mims CW (1981) SEM of aeciospore formation in *Puccinia bolleyana*. Scan Elect Micros 3: 299–303

— Roberson RW, Richardson EA (1988) Ultrastructure of freeze-substituted and chemically fixed basidiospores of *Gymnosporangium juniperi-virginianae*. Mycologia (in press)

Newhouse JR, Hoch HC, McDonald WC (1983) The ultrastructure of *Endothia parasitica*. Comparison of a virulent with a hypovirulent isolate. Can J Bot 61: 389–399

Oliver PTP (1972) Conidiophore and spore development in *Aspergillus nidulans*. J Gen Micro 73: 45–54

Smith JE, Anderson JG, Deans SG, Davis B (1977) Asexual development in *Aspergillus*. In: Smith JE, Pateman JA (eds) Genetics and physiology of *Aspergillus*. Academic Press, New York, pp 23–58

TAYLOR JW (1984) Correlative light and electron microscopy with fluorescent stains. Mycologia 76: 462–467

TANAKA K, YANAGITA T (1963) Electron microscopy on ultrathin sections of *Aspergillus niger*. II. Fine structure of conidia-bearing apparatus. J Gen Appl Microbiol 9: 189–204

TRINCI APJ, PEAT A, BANBURY GH (1968) Fine structure of phialide and conidiophore development in *Aspergillus giganteus* Wehmer. Ann Bot 32: 241–249

TSUKAHARA T (1970) Electron microscopy of conidiospore formation in *Aspergillus niger*. Sabouraudia 8: 93–97

WEISBURG SH, TURIAN G (1971) Ultrastructure of *Aspergillus nidulans* conidia and conidial lomasomes. Protoplasma 72: 55–67