

CHAPTER 14. Ultrastructure: the Asexual Reproductive System

Knowledge of the sequence of morphogenetic changes in the development of sporangia and spores in the watermolds was first arrived at by traditional -- and sometimes rather imprecise -- cytological methodology (Chapter 12) or by simple observational means (Chapters 7, 8). In either case, the resulting information was limited in scope, but it is now possible by analyzing elements of the subcellular structure of the asexual apparatus to confirm, modify, or reject previous views on sporangial morphology in these fungi. That certain major events in the morphogenesis of the sporangium need not be reappraised in the light of information from ultrastructural study is testimony to the keen perception and deductive reasoning of some of the pioneer investigators.

The development of the asexual apparatus -- as it was perceived using light microscopy -- proceeds through a sequence of structural changes leading to the release of motile or encysted spores (Fig. 12). The ultrastructure of the various events that cleave a single protoplast into a number of asexual cells, and of the products of that segmentation, is treated in this chapter.

THE SEPTUM AND EXIT PAPILLA

The end point in transformation of a vegetative hyphal apex into an immature sporangium is signaled by the rapid development of a subapical septum. Once a transverse barrier is deposited across the hypha, the incipient wall quickly thickens (Heath and Greenwood, 1970b; Heath *et al.*, 1971; Heath, 1976) as material is accumulated at its two surfaces. As the septum is being processed, lomasomes, ribosomes, and even lipids (Fig. 28) are incorporated into it. Heath and his associates (1971) demonstrated that wall material also is sequestered internally on the lateral face of the hypha in the vicinity of the septum. This deposition, they noted, was coincident with the presence of numerous vesicles in the adjacent cytoplasm, and it is reasonable to suppose that these organelles were in some fashion involved in the manufacture and placement of the septum. In *Saprolegnia* species, at least, there is supportive evidence from birefringence properties of the septum that cellulose fibrils (perhaps of cellulose or of β -1, 3 glucans) are incorporated into the developing transverse wall (Heath, 1969). There is much yet to be learned, however, about the ultrastructural events leading to and accompanying septum formation.

Little is known of the mechanism by which a discharge papilla (usually apical) is formed on a sporangium. Heath *et al.* (1971) reported that the cytoplasm in the extreme tip of the immature sporangium incorporates numerous vesicles in the immediate vicinity of the evagination that is subsequently to function as an exit apparatus. Vesicles are lacking in the papilla once it is fully developed.

SPORE CLEAVAGE

It has long been recognized for members of the Saprolegniaceae that cleavage of the sporangial protoplast into the incipient spores involves evagination of the tonoplast, as if in channels, toward the sporangium wall. Since the sporangium does not expand during enlargement of the central vacuole (Fig. 12), it seems likely that the cytoplasm condenses or becomes compacted as it is divided into segments. In any event, the spore membrane is in part derived from the tonoplast and in part from the plasmalemma. Electron microscopy has since corroborated this pattern of events.

VESICLE ORIENTATION

Cleavage of the sporangial cytoplasm in *Saprolegnia ferax* is accompanied by small vesicles that, Gay and Greenwood (1966) believed, subsequently evolve by fusion into "cleft vesicles" (Fig. 29). Some of the vesicles merge with the vacuolar membrane (Heath, 1976). For cleavage to occur, membrane synthesis is necessary, and Gay and Greenwood (1966) suggested that this was accomplished through the activity of dense-body vesicles. It appears, however, that the plasmalemma does not increase in extent as the cleavage vesicles develop and push toward the periphery of the sporangium to delimit cytoplasmic clumps (Fig. 30). Thus, in the delimitation of the spores of *S. ferax* membrane is added to the tonoplast and not to the plasmalemma. Other ultrastructural features of spore cleavage, Gay and Greenwood found, are single membrane-bound, bar-like organelles (Heath and Greenwood, 1970a, later located such structures also) generally distributed at random on the sporangial cytoplasm. These bar-like elements, it is now known, are involved in spine formation on spore cyst walls, and have no role to play in spore delimitation (*see* section on ultrastructure of the cyst wall). The nucleus in each incipient spore of *S. ferax* is accompanied by a pair of closely peripheral centrioles positioned at nearly a right angle to each other (kinetosomes at this stage).

DENSE-BODY VESICLES

The suspected involvement of dense-body vesicles in sequestering materials that ultimately enter into membrane production during sporogenesis (Gay and Greenwood, 1966) and oosporogenesis (Gay *et al.*, 1971) warrants special attention. In the sporangia of *Saprolegnia ferax*, the dense-body vesicles (Fig. 31) are sometimes surrounded by parallel bandings resembling a myelin-like lamellation. Gay and Greenwood suggested that this configuration is indicative of the presence of polar lipids. Gay *et al.* (1971) found dense-body vesicles in the hyphae and reproductive apparatus of *S. ferax*, *S. furcata*, and *Dictyuchus sterile*[†] when isolates were grown in water culture. When *S. ferax* was cultured in a weak aqueous nutrient extract, however, such vesicles were absent until the onset of sporangium formation. Chromatographic analyses of mycelium at this point in the fungus' development indicates the specific synthesis of

phosphatidyl choline (a phospholipid) at the time the dense bodies formed. Ultrastructural analysis confirms that the dense bodies decrease in size (or disappear) as vesicles involved in cytoplasmic cleavage enlarge and fuse. Accordingly, these organelles are suspected of participating in three events (Gay *et al.*, 1971): (1) production of additional membrane material destined for incorporation into the vacuolar tonoplasts; (2) supplying phospholipids to be utilized in morphogenesis, and (3) moving fluid into the expanding vesicles (by increasing water potential). The origin of dense-body vesicles and their precise mode of participation in morphogenesis is yet to be discovered. In any event, these organelles also are associated with various stages of development (including some of those in the sexual apparatus in some cases) in *Achlya imperfecta*[†] and *A. ambisexualis* (Tontz, 1969), *Aphanomyces euteiches* (Shatla *et al.*, 1966), and *S. terrestris* (Howard and Moore, 1970).

Two aspects of the alleged role of dense-body vesicles in genesis of the sporangium (and oogonium, for that matter) should be emphasized at this point. First, it is possible that these vesicles only contain phospholipid in the membranes. Moreover, the reported increase in phospholipid (Gay *et al.*, 1971) could be due to a substantial rise in cytoplasmic membrane production during reproductive cell development. Second, additional analyses of dense-body vesicle fractions during cleavage of reproductive units would perhaps yield further information about the putative role of these organelles. To be sure, some dense-body vesicles appear to be involved in cleavage, but there seems not to be direct evidence of a significant reduction of these organelles as would be expected if they are the chief cleavage fractions.

MICROTUBULAR "ROOTS"

Vesicles and cleavage furrows are of course not the only subcellular features characteristic of spore morphogenesis in the relatively few water molds that have been studied. Since the cleavage process in the sporangia of these fungi appears to be rather precise in that it delimits reasonably uniform segments of uninucleate cytoplasm, some subcellular mechanism very likely is operable. Such a mechanism may be seen in *Saprolegnia ferax* where, at the onset of sporogenesis, a complex of microtubular "roots" appears in the cytoplasm of the incipient spores. Heath and Greenwood (1971) suggested that these organelles might mechanically stabilize the uninucleate mass of cytoplasm, while leaving the periphery of the developing spore (an area not permeated by tubules) necessarily weaker. Quite possibly, then, evaginations of the tonoplast might follow such peripheral paths of reduced resistance. These investigators also speculated that the microtubule roots could as well be responsible for the pyriform shape of the primary spores in *S. ferax* and for anchoring the flagella (functioning, perhaps, as a mechanical aid to absorb stress created by flagellar motion).

It is not amiss at this point to recall (Chapter 12) that a few mycologists, limited to traditional cytological methodology, saw in their preparations of developing reproductive cells "astral rays" that were oriented toward the sporangial or oogonial

wall. The parallel between astral ray configuration and the pattern of microtubular roots detected in TEM preparations is at once apparent.

During sporogenesis, the sporangial nuclei that do not degenerate, Heath and Greenwood (1971) noted, change shape from spherical to pyriform. These organelles become positioned radially within the sporangium such that the narrow end is adjacent to the wall, and the wider, basal portion is contiguous to the central axis

KINETOSOMES

In hyphae of *Saprolegnia ferax* prior to sporangium delimitation each nucleus is accompanied by a pair of centrioles situated at 180° to one another. During the early stages of sporangiogenesis prior to cleavage, however, the centrioles elongate and reorient to a $\pm 90^\circ$ angle (this configuration is probably what Gay and Greenwood had reported in 1966 as the position of these organelles adjacent to the nucleus). When the partial rotation of the centrioles and their subsequent elongation has taken place, these organelles are then termed kinetosomes, and are so referred to by Heath (1976). Quite possibly these attenuated centrioles were reported in the early cytological literature on the water molds as basal bodies or blepharoplasts. In any event, kinetosomes eventually develop associated flagella.

Typically an osmiophilic, striate fiber develops to connect the two kinetosomes, and an osmiophilic plate (Fig. 32) appears at the junction of each kinetosome with the spore membrane. At the end of these events, the kinetosomes have formed a V-configuration in the spore apex. Golgi bodies cluster around the nuclear apex (Heath and Greenwood, 1971) in the vicinity of the kinetosomes; perhaps this is evidence of vesicles being contributed to the Golgi by the nuclear membrane (Heath, 1976).

Heath and Greenwood (1971) characterized the transition zone (Fig. 33) between the kinetosomes and the flagella in the post-cleavage stage of spores of *Saprolegnia ferax*. The proximal end of the kinetosome has the typical cartwheel configuration characteristic of the centrioles (Fig. 33 A), but at the junction of the kinetosome with the spore membrane only two members of each set of triplet tubules remain (Fig. 33 B). These doublets (A and B tubules) continue into the body of the flagellum, and are spaced around the central osmiophilic plate (Fig. 33 C) in the transition zone. In the transition region there is a complex of two cylinders and the tubules that will ultimately constitute the central pair of fibrils in the flagellum. The proximal cylinder (Fig. 33 D) is osmiophilic and concertina-like, and is bordered distally by a second cylinder consisting of nine struts connecting each doublet (Fig. 33 E) to the central cylinder. Further along the flagellum base distally, the characteristic 9+2 arrangement of fibrils (Fig. 33 F) is visible (Heath and Greenwood, 1971; Heath, 1976). In the released spore of *S. ferax*, a complex of endogenous microtubules called flagellar roots (Heath, 1976) are to be seen; these are treated in a subsequent section.

MASTIGONEMES

Heath and Greenwood (1971) reported that mastigonemes (“Flimmer” or “tinsel fibers”) were evident on the flagella of spores of *Saprolegnia ferax* prior to release from the sporangium. Mastigonemes likely form as a post-cleavage event, but precisely how they are attached to the sheath is not known. The tapering, solid, basal region of each structure is possibly involved in attachment (Heath, 1976; Heath *et al.*, 1970).

The only structural details of mastigonemes on watermold spores are those provided by Heath and his associates (1970). The proximal end of an individual fiber is tapering, there is a cylindrical body, and at the distal end, one or more hair-like protrusions (Heath, 1976). Available evidence points to the origin of mastigonemes by synthesis in ribosome-studded cisternae of endoplasmic reticulum (Fig. 34). In these vesicle-like cisternae in both *Saprolegnia ferax* and *Dictyuchus sterile*[†], Heath *et al.* (1970) found bundles of fibrils having the diameter, length, and tapered ends characteristic of mastigonemes. These osmiophilic but colchicine-resistant fibrils evidently are not present in the endoplasmic reticulum of the vegetative hyphae. Mastigonemes contained in vesicles also have been detected by Hoch and Mitchell (1972a) in spores of *Aphanomyces euteiches*.

VARIATIONS IN SPORE CLEAVAGE

Because endogenous spore delimitation has been followed ultrastructurally in only a very few representatives of the family (Tontz, 1969, for example, described cleavage by vesicles in *Achlya ambisexualis* and *A. imperfecta*[†]), one is uncomfortable in concluding that there is a particular pattern common to species of the family. Indeed, even among the few isolates studied, variations in the ultrastructure of spore cleavage have been noted. In *Thraustotheca clavata* Heath (1976) described one slight variation in the general process of spore delimitation, namely, the fusing of a network of small vesicles to delimit spore initials. The origin of these vesicles (smaller and less conspicuous than the cleft vesicles reported by Gay and Greenwood, 1966) is not known. The observations by Hoch and Mitchell (1972a) certainly point to cleavage in *Aphanomyces euteiches* as a much simpler series of events than the progression of changes encountered in *Saprolegnia ferax* (Gay and Greenwood, 1966). In *A. euteiches*, vesicles evidently have not been seen to be involved in cleavage; rather the plasmalemma migrates toward the vacuolar tonoplast, and coalesces with it.

According to Shatla and his colleagues (1966) an amorphous layer lines the periphery of the sporangial cytoplasm in *Aphanomyces euteiches*. This layer persists throughout the events in spore differentiation, and, they suggested, might serve as a low-friction barrier during spore release.

OTHER ORGANELLES DURING SPOROGENESIS

Hagedorn and Weinert (1972) gave considerable attention to changes in the various subcellular organelles during sporogenesis in *Saprolegnia monoica*[†]. As spore delimitation takes place in this species, the endoplasmic reticulum increases 50- to 100-

fold; vesicle and dictyosome production also is reinforced at the level of the endoplasmic reticulum and the nuclear envelope. Thus, there is maximum endomembrane production such that all membrane systems are judged to be interconnected by the endoplasmic reticulum. In a later paper (1974), Hagedorn and Weinert extended their observations on quantitative supplementation in membrane-bound organelles during sporogenesis. The most pronounced increase takes place with respect to the endoplasmic reticulum, but when spore maturation is completed, the amount of ER has dropped sharply. Dictyosomes in the sporangia of *S. monoica*[†] are physically connected to the nuclear membrane during sporogenesis. Vesicles -- sparse in undifferentiated hyphae -- increase in the sporangial cytoplasm (Hagedorn and Weinert, 1974) as the basal septum is formed, then drop appreciably in abundance. Lipid-containing vesicles likewise shift in quantity during the formation of the sporangium crosswall. Hagedorn and Weinert concluded that some organelles obviously changed function at various stages in the progression of the sporogenetic process.

ULTRASTRUCTURE OF THE SPORE

More attention has been given to the subcellular structure of the spores of water molds than to any other element in the general morphology of these fungi. Sporulation in *Saprolegnia* species -- the ones most extensively examined ultrastructurally -- is uniquely complex, with two motile stages, and an encystment and excystment phase in addition to germination. Ultrastructural studies fully reflect this morphological complexity. For convenience, we consider first the general features of the subcellular structure of the primary and secondary planonts, then turn to encystment and excystment, and finally, chronicle the structural aspects of spore germination.

GENERAL ULTRASTRUCTURAL CHARACTERISTICS

In 1977(b) Holloway and Heath published a detailed account of spore ultrastructure in a species of *Saprolegnia* (possibly *S. ferax*) and demonstrated that the constituent organelles in the motile stages of the asexual reproductive units are generally distributed in the cytoplasmic matrix in a random fashion. Moreover, they postulated that subcellular changes were not necessarily associated with microtubules. The general ultrastructural features of the primary spore are shown in Figure 36, those of the secondary one in Figure 37. Table 23 compares the nature and distribution of organelles in the two spore types (data from Holloway and Heath, 1977b; reviewed by Heath, 1976).

Mitochondria are structurally similar in both primary and secondary planonts of *Saprolegnia* species. Much of the rough endoplasmic reticulum in the pyriform spores (Holloway and Heath, 1977b) consists of stacked cisternae, in contrast to the condition in the reniform spores. Water expulsion vacuoles are present in both spore types

(Table 23), and also have been reported (Hoch and Mitchell, 1972b) in the spores of *Aphanomyces euteiches*. In this latter species these particular vacuoles are surrounded by a zone containing vesicles characterized by the absence of a sharply defined membrane (“fuzzy” vesicles).

Gay and Greenwood (1966) first described bar-like structures arranged randomly in the cytoplasmic matrix of the sporangia and spores of *Saprolegnia ferax*. Similar elements (called primary bars) were seen by Heath and Greenwood (1970a). Bar units are also present in the secondary motile spore (Heath and Greenwood, 1970a) and are designated as secondary bars. Because the size and configuration of the inclusions in primary bars resemble the cyst spines (*see* section on cyst structure) it has been suggested that the latter originate in these bar-like organelles, and that the amorphous layer around both the primary and secondary bars is released to the outside during cyst formation. Although Heath and Greenwood (1970a) thought that perhaps spherical structures in the bars gave rise to the “boat hooks” (ornamentations or “appendages”) on the cyst wall, Holloway and Heath (1977b) postulated that they did not. The osmiophilic cortex of the secondary bars, the latter suggested, could contain the boat hook appendages that appear on the secondary cyst wall. In any case, the bars seem to have an exogenous secretory function.

Two types of kinetosome-associated bodies also are evident in the primary and secondary planonts (Table 23) of *Saprolegnia* sp. The K₁ body is seen in the differentiating sporangium (Holloway and Heath, 1977b) as a sub-cellular structure with a granular matrix, an osmiophilic cortex, and a crenulated membrane. What is presumably the same type of organelle occurs in the primary spore, but with a smooth membrane. A loose, fibrillar matrix, and an osmiophilic core and cortex are the structural elements of K₂ bodies.

Other spore structures of *Saprolegnia* sp. include free ribosomes and ones bound to the endoplasmic reticulum (Heath, 1976), and Golgi bodies. The latter, in the primary spore, are reported (Heath, 1976; Heath and Greenwood, 1971) to lie near the apex of the pyriform nucleus of the primary spore in a position near the kinetosomes, suggesting that a functional relationship might exist among these elements. Heath (1976) reported that the nuclear envelope may bud off vesicles that evidently contribute to Golgi formation.

The description by Hoch and Mitchell (1972a, b) of the ultrastructure of preemergence primary spores and the secondary ones in *Aphanomyces euteiches* indicates that the subcellular configuration of the spores of this plant pathogen do not differ markedly from those of other water molds. One subcellular element, designated simply as an “unidentified body”, has been found only in the spores of *A. euteiches*. This organelle located near the two kinetosomes (130° angle) on the side of the pyriform nucleus closest to the contractile vacuole, contains helical fibers (Fig. 35). Its function is unknown, but it persists into the encystment stage of the spore.

Gross Morphology of Encystment and Excystment: -- The cytoplasmic microtubular system in both primary and secondary planonts of *Saprolegnia ferax* has

been studied in detail and interpreted by Holloway and Heath (1977a). It will be recalled that the anchoring system in the spore initials during their delimitation in the sporangium consists primarily of two kinetosomes connected (presumably to strengthen the basal apparatus against the beating of the flagella) by a striate fiber. The proximity of Golgi-derived elements to the kinetosomes has also been mentioned. By means of temperature shock treatment, Holloway and Heath induced synchrony in spore encystment and excystment in *S. ferax* then followed the subcellular morphological events accompanying the development of these stages.

Holloway and Heath (1977a) reported that the shape change of the primary spore at encystment from pyriform to spherical takes place in approximately one second. At excystment, a localized bulge develops in the cyst wall, and enlarges to a short tube. The cyst protoplast emerges slowly through a terminal opening in this tube until the nucleus pushes through; the entire content of the cyst then quickly emerges from the confining wall with two or three pulsations lasting some 2-10 seconds. The emerged spore is oblate, lacks flagella, and characteristically contains a rhythmically contracting and expanding water expulsion vacuole. The cyst does not expand during emergence of its contents. Flagella appear either before or after the emerged cell develops the lateral groove characteristic of the secondary planont. The flagella are synthesized (Holloway and Heath, 1977a) at a rate of about 2 μm per minute. When the spore ceases swimming each flagellum develops a "bead", the cell rounds up, and the lateral groove disappears; these successive events take place in a time span (Holloway and Heath, 1977a) of about two seconds. The water expulsion vacuole disappears quickly after the flagella are lost. Hoch and Mitchell (1972a) have shown that the discharged primary spores in *Aphanomyces euteiches* lack flagella.

Microtubular Flagellar Roots: -- The primary spore of *Saprolegnia* sp. contains (Holloway and Heath, 1977a) two sets of perinuclear microtubular fibrils -- each set appearing to be embedded in an osmiophilic matrix, and prescribing a conical configuration -- in the vicinity of the kinetosomes. One cone of fibrils extends into the spore cytoplasm adjacent to the nucleus; the fibrils of the second cone are positioned near the plasmalemma (Fig. 38). Holloway and Heath have suggested that these perinuclear cones of microfibrils act as an exoskeleton for the nuclear apex in both the primary and secondary planonts.

The "root system" associated with the bases of the flagella in the secondary planonts of *Saprolegnia* sp. is much more intricate (Fig. 39) than that of the primary ones.

Holloway and Heath (1977a: fig. 37) have shown that the elements of the microtubular root system (except for the rib-like groupings) are interconnected by means of a complex osmiophilic skeletal matrix. From this matrix five series of microfibrils (Fig. 39) project. Anterior and posterior doublets pass through the cytoplasmic matrix toward the opposite ends of the reniform spore body. The base of each of these bundles (microfibrils) is embedded in osmiophilic material at the same level (Holloway and Heath, 1977a) as the intermediate bundle, a structure that projects laterally over the mid portion of the spore and opposite the nucleus. An octet

(sometimes 6 or 9 elements) of fibrils projects toward the posterior end of the spore (Fig. 39), and at the proximal end is attached to a fibrous buttress anchored in the osmiophilic skeleton connecting the various microtubular elements located in the vicinity of the nucleus. A triplet of fibers (Fig. 39), buttressed by osmiophilic material in a ring-like configuration at the base of the kinetosomes bearing the tinsel flagellum, extends toward the anterior end of the spore. From this triplet a series of microtubular "ribs" extends into the spore cytoplasm (Fig. 39). Striated fibers (they are electron dense in preparations of the motile spores of *Aphanomyces euteiches* according to Hoch and Mitchell, 1972b) connect the bases of the kinetosomes. In a reconstruction of the physical relationships among the various organelles and microtubules making up the endogenous "root" system of the flagella, Holloway and Heath (1977a: fig. 37) show the fibers attached to a striated strap adjacent to the nuclear envelope.

Except for the ribs and the intermediate bundles, the arrangement of organelles in the secondary planont of *Saprolegnia* sp. is symmetrical. The distribution of the microtubular systems, particularly, in the spores of water molds, suggested to Holloway and Heath (1977a) that these elements have a dual function: to provide a skeletal framework (which may serve to maintain cell shape) and to anchor the flagella.

The microtubular system in the secondary (reniform) planont of *Aphanomyces euteiches* has been examined and interpreted by Hoch and Mitchell (1972b). In spores of this species (*see* Chapter 8 for a discussion of the terminology applied by Hoch and Mitchell for these asexual units) two kinetosomes are anchored in the cytoplasmic matrix by two rows of microtubules, with the outer row consisting of as many as 12 elements. Secondary microtubules are attached at right angles and at regular intervals along the rootlets. Holloway and Heath (1977a) regard the twelve-microtubule root complex in *A. euteiches* as the functional equivalent of the octet of fibrils in the secondary spores of *Saprolenia ferax*.

The Flagella: -- Little needs to be added to what has already been recorded for flagellar structure in the Saprolegniaceae (Chapter 8), save for a few points of historical background. The first ultrastructural study of the locomotory organelles in the water molds was that by Manton and her coworkers (1951, 1952). They found the anterior flagellum of the primary and secondary planonts of *Saprolegnia ferax* to be constructed of an electron-transparent sheath enclosing two central fibril strands, and having attached to the sheath two rows of pointed hairs 2-3 μm long (mastigonemes). Flagellar fragments showed that there were nine strands within the sheath in addition to the two central ones. Manton and her colleagues were unable to determine if two of the 11 strands in the posterior flagellum were centrally located as were those in the anterior one, but the trailing organelle was without mastigonemes. The two flagella of the primary spore of *S. ferax* were of equal length (Manton *et al.*, 1951) in some secondary planonts, on the contrary, the posterior one was often twice the length of the anterior (tinsel) one. The report by Nagai and Takahashi (1962) of the fine structure of the flagella (and cysts) in spores of *S. diclina* does not differ in any respect from the characteristics determined for *S. ferax* by Manton and her colleagues (1951, 1952).

Mitosis: -- Chapter 13 chronicles the events in mitosis in the hyphae of certain water molds. Save for a very brief reference to mitosis in a germinating spore of *Aphanomyces euteiches*, the only detailed account of nuclear replication in spores appears in Slifkin's report of 1967(a), on *Saprolegnia delicat*[†]. The early phases of mitosis as she described it occurring in the spores of this species are unusual and differ sufficiently from those of nuclear divisions in the hyphae to require that some attention be given to her account.

According to Slifkin (1967a) the chromatin network in the nucleus of the spore of *Saprolegnia delicat*[†] gathers into a single mass as division commences. Subsequently, a pore develops in the nuclear envelope, and a centriole migrates out through the opening. This is a most unusual occurrence in light of what is known of subcellular events in mitosis in nuclei of the vegetative hyphae, and is in need of confirmation. From vesicles clustered near the nucleus microtubules radiate outward. Once in position outside the nucleus the centriole divides, and restricted migration in pockets of the nuclear envelope takes place. As the elongating nucleus constricts equatorially (in division) a mitochondrion appears in the resulting narrowed isthmus-like region. The remainder of the mitotic stages in *S. delicat*[†] (there is no metaphase plate configuration) as Slifkin described them are essentially the same as those taking place in somatic division. The ultrastructural features of mitosis in the spores of other water mold species must be studied thoroughly to confirm whether or not the early divisional events in *S. delicat*[†] are the rule or an exception.

THE ULTRASTRUCTURE OF ENCYSTMENT

Eventually in the behavior sequence of primary and secondary planonts of water molds there is an encystment stage in which subcellular organelle reorganizations are effected. Heath (1976) suggested that the transition from, a motile to an encysted stage involves a sequence of three events, namely, loss of the flagella, elaboration of the cyst wall, and a reversion of organelles to a vegetative structure and function. Germination would constitute a fourth process in the transition.

Flagellum Loss: -- As has been mentioned in a previous chapter, observations of spore behavior on a gross morphological level led to discordant opinions about the fate of the flagella possessed by the primary and secondary planonts at the onset of encystment. To what extent has ultrastructural methodology provided an insight into flagellum loss?

The first account of changes in the fine structure of retracting planont flagella seems to be that of H. Meier and Webster (1954). They noted that when the locomotory organelles of the primary spores of *Saprolegnia ferax* and *S. parasitica*[†] had disappeared, tufts of minute hair-like fibrils were visible on the spore wall where the flagella had projected. This was evidence, they wrote, of loss by retraction. It was later shown (Heath and Greenwood, 1971) in spore preparations of *S. ferax* that when the tinsel

flagellum disappears, its mastigonemes are left attached to the developing cyst wall. Once inside the encysting spore the axonemes shorten and the remnant of the flagellar apparatus remaining are kinetosome-like bodies having the same configuration as the precleavage organelles. Holloway and Heath (1977a) described a somewhat different series of events, also in the primary spores of what was probably *S. ferax*. At spore encystment the flagellar axonemes disintegrated in a step-wise fashion, beginning with the formation of a "bead", within the filament matrix, resulting from the coiling of the axonemes. Occasionally in the planonts of this *Saprolegnia* one flagellum would wrap about the spore body, where its axoneme was then absorbed into the cytoplasmic matrix to become a part of the plasmalemma. Holloway and Heath (1977a) were not able to follow axonemal depolymerization during the absorption process.

A different pathway of flagellar loss in the primary planonts of *Saprolegnia terrestris* was reported in 1974 by Holloway and Heath. As we have already recorded (Chapter 8), flagellum loss in this species may take place with or without the formation of "beads" (coiled axonemes). Holloway and Heath described the sequence as a shortening of the two flagella at different rates until about 3 μm of each flagellum remained. These short remnants suddenly pivoted to rest upon the developing cyst surface and then disappeared, leaving only a minute raised area at the site of contact. Incorporation of the flagellar remnants could occur (Holloway and Heath, 1974: fig. 7) whether or not they remained attached to the base plate (in the transition zone at the flagellum/spore interface). Without a doubt, a substantial quantity of membrane material must be absorbed, changed structurally, and be dispersed within the spore matrix during and after flagellar loss.

The emerging primary spores of *Aphanomyces euteiches* lack flagella (Hoch and Mitchell, 1972a), and those on the secondary planonts (Hoch and Mitchell, 1972b) are shed just prior to encystment. At the site (visible on the planont surface) where each flagellum is disarticulated, a membrane-bound granular "knob" remains attached to the distal face of the kinetosome terminal plate.

Encystment by planonts of the water molds also involves a change in shape from pyriform or reniform to a compact spheroid. Two principal events accompany this alteration, namely organelle reversion and elaboration of a cyst wall. Holloway and Heath (1977a) published an account of the changes taking place in certain microtubular organelles and in the kinetosomes during excystment and encystment by secondary planonts of *Saprolegnia ferax*. For example, the octet and triplet tubules elongate and project well into the cytoplasmic matrix during the excystment process (Holloway and Heath, 1977a: fig. 40 A, B). At encystment these same organelles shorten (Holloway and Heath, 1977a: fig. 40 D, E).

Organelle Reversion: -- Heath (1976) suggested that the kinetosomal/flagellar root complex was one of the first systems to depolymerize as the spore began to become spherical. On the other hand, in fully encysted spores of *Aphanomyces euteiches* there is still evidence of the microfibrillar root system (Hoch and Mitchell, 1972b) suggesting that these tubules may not revert all at once.

The outer core of microtubules in the primary planont (Holloway and Heath, 1977a) disappears coincident with the change in spore shape from elongate to spherical. At the same time, loss of the perinuclear cone of microtubules occurs. During spore encystment in both *Saprolegnia ferax* and *Aphanomyces euteiches* (Heath, 1976; Hoch and Mitchell, 1972b) the mitochondria appear to “migrate” from their position near the nucleus or vacuole into a random distribution within the cytoplasm. In the primary and secondary spore cysts of *S. ferax* dense-body vesicles and lipid droplets become randomly distributed as well, and the water expulsion vacuole fades. Similarly, in *A. euteiches*, the contractile vacuole in the motile spores seems to disintegrate (Hoch and Mitchell, 1972b) during encystment. In *S. ferax* the K₁ bodies within the primary spore cysts are replaced by the K₂ elements. Kinetosomes are present in the reniform planont, but disappear from it (Holloway and Heath, 1977a).

The Cyst Wall: -- The initial wall elaborated around encysting spores of *Saprolegnia ferax* and *Dictyuchus sterile*^t has been shown by Heath and Greenwood (1970a) and Heath (1976) to be a thin, osmiophilic unit. On the outer surface of the cyst wall -- depending upon spore type -- ornamentations are deposited, but precisely how these structures evolve is not known. As to the origin of the wall itself, Heath and Greenwood (1970a) suggested that the amorphous “layer” around the primary and secondary bar bodies was liberated to the outside of the spore in an early phase of encystment, there to contribute to the osmiophilic layer. With time, the spore cysts of *S. ferax* and *D. sterile*^t are invested with an inner wall that is less osmiophilic than the first one to be deposited. This second wall appears to give rigidity to the spore. By means of PASH methodology Heath and Greenwood showed that the vesicles might be derived from dictyosome cisternae.

The observations by Hoch and Mitchell (1972b) on the ultrastructure of encystment in the spores of *Aphanomyces euteiches* point to a sequence of wall deposition at variance with that just described for *Saprolegnia ferax* and the *Dictyuchus* species. Cyst wall formation in *A. euteiches* commences as soon as the spore attains a spherical configuration and is completed in about 30 minutes. The first wall to be laid down is a thin, osmiophilic layer (just as in *S. ferax*), but it is not ornamented with spines (Hoch and Mitchell, 1972b). Subsequently, the enclosing “membrane” of the cyst takes on a tripartite aspect, leaving the impression that the cyst wall could have been deposited below the plasmalemma. This unusual deposition pattern needs corroboration through further study that should certainly include other species of *Aphanomyces*. The ultrastructure of the cyst wall *per se* is treated in a separate section to follow.

Primary Spore Extrusion: -- In Chapter 8 we reviewed in depth the literature treating the gross morphology of planont discharge in the Saprolegniaceae. An account published in 1972(a) by Hoch and Mitchell in which subcellular events in spore release in *Aphanomyces euteiches* are recorded warrants attention at this point. Since the spores of *A. euteiches* are not motile at discharge, only hydrostatic pressure in the sporangium, Hoch and Mitchell suggested, would account for their exit. Further, such a pressure

could exist only if the sporangium were a temporary barrier to water loss. They proposed that inasmuch as there was no plasmalemma in the mature sporangium -- this membrane, together with the tonoplast, having gone into the formation of the spore plasmalemma -- the barrier was obviously the sporangium wall. In addition, new vesicles appeared in the primary spores prior to their release from the sporangium, and these organelles could well have functioned in some fashion in the discharge process. Some experimental work by Hoch (1972) on osmotic characteristics of spore formation and release in *A. euteiches* suggests the existence of a relationship between matric water potential (ψ_m) and discharge. These data are not corroborated because subsequent repetitive experimentation is lacking.

Hoch and Mitchell (1972a) found that the newly discharged spores of *Aphanomyces euteiches* are bounded initially by a single layer. Vesicles are located in the vicinity of this layer, and subsequently the first cyst wall is thickened by the deposit of cellulose (glucans?). The wall of the primary cyst at maturity consists of an osmophilic outer layer, and a less dense inner one.

THE ULTRASTRUCTURE OF GERMINATION

Little is known of the subcellular structural changes accompanying spore germination in the Saprolegniaceae, observations having been made only on *Saprolegnia ferax* and *Dictyuchus sterile*[†] (Gay *et al.*, 1971; Heath *et al.*, 1971), and *Aphanomyces euteiches* (Hoch and Mitchell, 1972b). Germination is a process consisting fundamentally of three events: cyst wall penetration, development of a germ hypha with an adequate external wall, and, subsequently, the growth of that initial filament.

One pregermination event in the spores of *Saprolegnia ferax*, Heath and associates (1971) observed, is the accumulation of vesicles at the inner periphery of the cyst's plasmalemma where breaching of the wall is likely to occur. Gay and his colleagues (1971) found dense-body vesicles in the spores at this stage and reported that these organelles enlarged and fused to produce the first vacuoles in the emerging germ hypha (that these vesicles are simply specialized vacuolar components cannot be ruled out). These observations recall Dargent's (1977) concept of the ultrastructural and chemical continuity of organelles in hyphal tip growth, and, going back into classical cytology, Guilliermond's (1941) theory of the *de novo* origin of vacuoles.

One of the early stages in spore germination in *Aphanomyces euteiches* is likewise marked by the accumulation of vesicles at the bulge that is to become the exit port for the emerging germ tube (Hoch and Mitchell, 1972b). The wall of the protrusion is single-layered, while that of the cyst is bipartite. Hoch and Mitchell found that there is a scattered deposit of amorphous material on the surface of the evagination as it protrudes from the cyst, but no such accumulation on the adjacent cyst wall.

ULTRASTRUCTURE OF THE CYST WALL

The very first elements of a watermold to be examined for subcellular structure were the spores of *Saprolegnia ferax*. Manton, Clarke, and Greenwood (1951:327) described the secondary spore cysts of this species as being invested with stalked double-headed boathook-like structures resembling "... the style and bifid stigma of the Compositae". Nagai and Takahashi (1962) working with *S. diclina* confirmed their observations.

The most broadly based study of the cyst wall configuration in a variety of species of the family was that by H. Meier and Webster (1954). A summary of their determinations is given in Table 24. They were of the opinion that the "boathook" appendages are not chemically identical to the cyst wall since the latter can be dissolved without disintegrating the former. It is pertinent to note that the similarity among species of *Saprolegnia* and *Isoachlya*[†] in cyst wall ornamentation suggested to Meier and Webster a close relationship between these genera; sixteen years later these taxa were combined (Seymour, 1970). On the same ultrastructural basis, they remarked, the genus *Protoachlya* seemed more closely akin to *Achlya* than to *Saprolegnia*.

The fine structure of the cyst wall of secondary spores of watermolds isolated from fish was investigated by Pickering *et al.*, 1979). The cyst wall of spores of some species (and forms of *Saprolegnia diclina*) is provided with bundles of long, hooked, bifurcate hairs or fibrils, other cysts have single, short, fibrillar protrusions, and still others are equipped with short hairs lacking any terminal hook. There is a range of appendage morphologies, among the species examined, with respect to hooked-fibril length, number of fibrils per bundle, and interbundle distance. It was suggested by H. Meier and Webster (1954) that species of *Saprolegnia* having spore cyst walls with long, grouped fibrils or hairs were possibly adapted to a parasitic existence. The observations by Pickering and his associates seem to support this interpretation.

Heath and Greenwood (1970a) found osmiophilic plaques on the cyst wall of secondary spores of *Saprolegnia ferax*; the "boathook" ornamentations are attached at these points. The secondary spore cysts of *Dictyuchus sterile*[†] are provided with a scattering of spines (Heath and Greenwood, 1970a), while those of *Aphanomyces euteiches* are smooth (Hoch and Mitchell, 1972b). The parasitic *Aphanomyces astaci*, however, has randomly-oriented fibrils on the cyst wall of its secondary spores (Nyhlén and Unestam, 1978). Identical fibrils or hairs are positioned on the germ hypha wall, and both in the case of the cyst wall and that of the hypha, the fine "appendages" are embedded in amorphous β -1, 3-glucan.

In a previous section, mention was made briefly of the origin of cyst ornamentations. Heath and Greenwood (1970a) suggested that the spines and hooks are preformed in the cytoplasm and released to the cyst wall from the bar bodies (Gay and Greenwood, 1966). Holloway and Heath (1977b) doubted, however, that the bars containing spherical structures (in the spores of *Saprolegnia ferax*), are the source of the boathook ornamentations on the cysts.