J. Phycol. **37,** 86–94 (2001)

ZYGOSPORE GERMINATION IN *CHLAMYDOMONAS MONOICA* (CHLOROPHYTA): TIMING AND PATTERN OF SECONDARY ZYGOSPORE WALL DEGRADATION IN RELATION TO CYTOPLASMIC EVENTS[1]

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The *Chlamydomonas monoica* Strehlow zygospore is a dormant heavily walled spore adapted to survive extreme environmental conditions. The zygospore wall is multilayered and includes an acetolysis-resistant component related to the general class of compounds referred to as sporopollenin. Germination of the zygospore requires induction and completion of nuclear meiotic divisions, cytokineses to produce the four vegetative progeny cells, and breakdown of the zygospore wall to allow progeny release. Analysis of zygospore wall breakdown by transmission electron microscopy of synchronously germinating zygospores revealed differences in the timing and nature of disintegration of the various wall layers. Breakdown of the outer trilamellar sheath occurred within 6 h after light induction, concomitant with the onset of prophase I. At the same time, stored lipid bodies were consumed and replaced by large cytoplasmic vacuoles. Degradation of the inner more massive wall layer was initiated several hours later at about the time of the second meiotic division. In areas beneath breaks in the trilamellar sheath, a fibrous electron opaque bridge of wall material was retained whereas degradation of the remainder of the inner layer progressed. Finally, disintegration of this bridge material, after the completion of the meiotic divisions and synthesis of progeny cell walls, resulted in the opening of large slits in the trilamellar sheath, allowing escape of the flagellated vegetative progeny. The chloroform resistance typical of mature zygospores was lost at approximately the same time that the initial breaks in the trilamellar sheath were detected but before disintegration of the inner wall layer(s).

***Key index words:* *Chlamydomonas monoica*; germination; meiosis; sporopollenin; zygospore; zygospore wall**

All living things have evolved mechanisms for adapting to and surviving seasonal—and often radical—changes in their environment. For unicellular organisms, sporulation, which involves abandoning vegetative growth and entering a resting or dormant stage, is a very effective means for surviving environmental extremes. Many organisms, including bacteria, algae, yeasts, and fungi, use sporulation as a survival strategy (Levinson et al. 1977, Coleman 1983, Trainor 1985, Setlow 1992).

In *Chlamydomonas*, as in many other microorganisms, nutrient starvation induces sporulation (Levine and Ebersold 1960, Coleman 1962, Harris 1989). However, rather than stimulating the differentiation of an asexual cell into the resistant spore, nitrogen starvation stimulates the differentiation of asexual vegetative cells into gametes. Zygotes produced by gamete fusion then differentiate into the resting zygospore with acquired resistance to many additional environmental stresses. The *Chlamydomonas* resting state is thus diploid, which is rather unusual for microbial spores. Diploidy could provide additional protection from DNA-damaging agents by increasing the likelihood that at least some spore progeny will inherit an undamaged allele for any given essential gene (van den Ende and VanWinkle-Swift 1994). Diploidy also necessitates that germination of the zygospore involves not only the breaking of dormancy, but also induction of meiosis to produce the new generation of haploid vegetative cells.

Specialized walls protect spores from such environmental insults as starvation, desiccation, UV radiation, mechanical abrasion, and temperature extremes. Not surprisingly, spore walls (whether derived from prokaryotic or eukaryotic microbes) typically differ in both composition and ultrastructure from the walls of vegetative cells of the same organism (Tipper and Gauthier 1971, Fowell 1974, Weber and Hess 1974, Catt 1979, Grief et al. 1987). The walls of *Chlamydomonas* vegetative cells and gametes typically comprise several distinct glycoproteins (Roberts 1974, Catt et al. 1978, Minami and Goodenough 1978, Goodenough et al. 1986, Woessner and Goodenough 1994). Polysaccharide components, including cellulose, appear to be absent (see discussion in Harris 1989). Although the zygospore walls of *Chlamydomonas reinhardtii* are also known to contain glycoprotein (Woessner and Goodenough 1989, 1994), it is not yet clear what other components may be present, although evidence for a β-1,3-glucan is available (Grief et al. 1987). Most studies on the zygospore wall of *C. reinhardtii* have been directed at the identification of specific glycoproteins rather than nonproteinaceous components (e.g. Minami and Goodenough 1978, Woessner and Goodenough 1989). However, based on ultrastructure alone, it is clear that the zygospore walls of *C. reinhardtii* (Cavalier-Smith 1976) and *Chlamydomonas moewusii* (Brown et al. 1968, Triemer and Brown 1976)

[1] Received 30 November 1999. Accepted 19 September 2000.

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differ dramatically from those of vegetative cells of the respective species.

Attempts to extract glycoproteins from mature zygospore walls of *Chlamydomonas monoica* have not been successful (Donald 1996), although such components may be present. Nevertheless, the autofluorescence and acetolysis resistance of the zygospore walls of both *C. monoica* (VanWinkle-Swift and Rickoll 1997, Blokker et al. 1999) and the closely related *Chlamydomonas geitleri* (Kalina et al. 1993) support the hypothesis that one or more wall layers contain sporopollenin, the major component of pollen exines and one of the most resistant biopolymers known (Brooks et al. 1971, Weirmann and Gubatz 1992, Bedinger et al. 1994, Scott 1994). This unusual class of compounds, derived from phenylpropanoid and fatty acid precursors (Guilford et al. 1988, Weirmann and Gubatz 1992, Hemsley et al. 1993), has been found in the walls of other algal cell types as well (Atkinson et al. 1972, Good and Chapman 1978, Honegger and Brunner 1981, Berkaloff et al. 1983, deVries et al. 1983, Puel et al. 1987, Delwiche et al. 1989), and its incorporation may have been an important step in the adaptation of plants to a terrestrial environment (Graham 1993) with the resultant increased exposure to desiccation and temperature extremes. The resistant biopolymers from algal cell walls are referred to by some as algaenans (Blokker et al. 1998, 1999).

Given the presence of a sporopollenin-like component in the *C. monoica* zygospore wall (VanWinkle-Swift and Rickoll 1997, Blokker et al. 1999) and its highly resistant nature, the breakdown of the *Chlamydomonas* zygospore wall during germination presents an intriguing problem and implies the existence of a "sporopolleninase." Earlier studies on meiosis in *C. reinhardtii* (Triemer and Brown 1976) used a trypsin treatment of zygospores to improve fixative penetration of the wall. Thus, an evaluation of wall breakdown *per se* was not possible. Brown et al. (1968) noted that the permeability of the zygospore wall of *C. moewusii* increases at the time of germination, allowing limited studies on late events associated with germination. To evaluate the stages in zygospore wall breakdown and to provide the needed background for identification of the responsible degradative enzyme(s), we undertook an ultrastructural analysis of zygospore germination in *C. monoica*. We found that the timing, patterns, and rates of degradation of the various zygospore wall layers are unique. Our data are compatible with a hypothesis that the outer trilamellar sheath comprises sporopollenin.

MATERIALS AND METHODS

The wild-type strain (WT 22b) of *C. monoica* used in this study was derived from a cross between WT15c (VanWinkle-Swift and Burrascano 1983) and a zygote maturation mutant *spr zym 40* (VanWinkle-Swift et al. 1998). This phenotypically wild-type tetrad product (WT 22b) has been maintained and used because of its stable and high mating efficiency relative to other strains. Conditions for maintaining vegetative cultures and for the induction of gametogenesis and mating are as described previously (VanWinkle-Swift and Burrascano 1983, VanWinkle-Swift and Rickoll 1997). Mature zygospores produced within 7 days after induction of gametogenesis were streaked onto agar-solidified Bold's basal medium (BM) (Bischoff and Bold 1963) and were stored in darkness for 7 days before induction of germination.

To assay for the degree of synchrony in zygospore germination, 50–100 zygospores were micromanipulated away from the background of unmated cells on the BM storage plate. The plate was then inverted over chloroform to kill unmated cells and placed under continuous illumination to induce germination as described previously (VanWinkle-Swift and Burrascano 1983). The plates were examined under a dissection microscope every hour beginning 12 h after induction and continuing until the germination levels were maximal.

Zygospores were sampled for transmission electron microscopy at the end of the 7-day gametogenesis and mating period (before dark storage), at the end of the dark storage period before induction of germination, and at 2-h intervals after induction of germination by light. The last sampling was at 24 h after light induction when germination had reached maximal levels. At each time point, 1 mL of 2% glutaraldehyde was added to the BM plates. A sterile loop was then used to loosen the zygospores and unmated cells from the plates. The liquid containing the cells was then transferred into microcentrifuge tubes and held overnight at 4° C. Procedures for further fixation and embedding were as described by VanWinkle-Swift and Rickoll (1997), with the modification that nitrogen-free BM liquid medium (−NMB) was used in place of phosphate buffer for diluting fixatives and for washing samples between fixation steps. Thin sections were prepared and stained as described previously (VanWinkle-Swift and Rickoll 1997) and were viewed with a transmission electron microscope (model 1200 EX, JEOL USA, Peabody, MA) operated at 60 kV.

For analysis of germination by scanning electron microscopy, mature zygospores were first purified by layering a concentrated suspension of zygospores and unmated cells over a 40% sucrose cushion in a 15-mL sterile centrifuge tube. After centrifugation for 5 min at 3000 rpm, the purified zygospores were collected from the layer just above the sucrose cushion and were then washed in sterile −NBM before plating on BM agar. At the end of the 7-day storage period in darkness, the plates were removed from the dark, inverted over chloroform, and transferred to the light to induce germination. The plates were sampled before dark incubation; after dark incubation with no illumination; and at 12, 14, 16, 18, and 20 h after light induction as described above. After fixation in 2% glutaraldehyde overnight at 4° C, the zygospores were washed for 20 min in −NBM four times at 4° C, followed by two washes in distilled water. The zygospores were then transferred to filter paper and plunged into liquid nitrogen until frozen. The filter paper disks were then transferred into brass containers and freeze dried for 19 h in an Emitech K750 freeze dryer (Emitech Products, Inc., Houston, TX), gold coated, and examined at 15 kV on a LEO 435VP scanning electron microscope (LEO Electron Microscopy, Inc., Thornwood, NY).

To determine if zygospore wall breakdown as observed by transmission electron microscopy affected the chloroform resistance of zygospores, the spores were assayed for acquired chloroform sensitivity as follows. Mature zygospores and unmated cells were plated onto 12 BM plates and stored in the dark for 7 days. At the end of the storage period, 50–100 zygospores were micromanipulated away from the background of unmated cells on each of the 12 plates. All plates were placed in the light to induce germination. Individual plates were then inverted over chloroform vapors at periodic intervals after light induction. Subsequently, each plate was scored for the percentage of zygospores that had retained chloroform resistance as indicated by germination and colony formation.

RESULTS

Ultrastructural events occurring during zygospore germination can be interpreted effectively only if pop-

ulations of zygospores can be induced to germinate with a high degree of synchrony. In *C. monoica* this was accomplished by storing mature zygospores in darkness but in the presence of a nitrogen source for 1–7 days. In the absence of this dark storage period, zygospores germinated asynchronously under continuous illumination if provided with a suitable nitrogen source and showed reduced maximal germination efficiencies relative to dark-stored zygospores. Although longer dark storage periods (i.e. greater than 7 days) promoted more rapid germination, premature germination in the dark also occurred and was particularly problematic if zygospores were stored on a medium containing ammonium rather than nitrate as the nitrogen source (data not shown). The highest degree of synchrony in germination that we achieved followed a 7-day dark-storage period on BM (nitrate-containing) medium (Fig. 1).

Mating and zygote formation initially occur within approximately 2 days after transfer of vegetative cells to a nitrogen-limited liquid medium (VanWinkle-Swift and Bauer 1982). During the next 2 days the zygotes mature, assemble the secondary zygospore wall, and release the transient primary zygote wall (VanWinkle-Swift and Rickoll 1997). The present study began with zygospores derived from 7-day-old mating cultures (i.e. zygotes that were approximately 5 days old and that had matured fully into zygospores).

As described by VanWinkle-Swift and Rickoll (1997) and shown here in Figure 2, the mature zygospore of *C. monoica* was surrounded by a thick reticulate secondary wall made up of at least two layers. The outer trilamellar sheath included a surface fuzzy coat and an electron-translucent underlying layer. The innermost secondary wall layer was electron opaque, several times thicker than the trilamellar sheath, and uneven in thickness. In thin section, the wall thus appeared as a pattern of peaks and valleys (Fig. 2, a and b). Within the electron-opaque inner layer, electron-transparent regions with a honeycomb appearance were frequently found at the base of a peak in the wall (arrowhead, Fig. 2b) or filling the entire peak (data not shown). At the mature zygospore stage, all wall layers were intact and fully encased the zygospore. The mature zygospore contained ample storage reserves in the form of large globular lipid bodies and starch granules (Fig. 2a) (VanWinkle-Swift and Rickoll 1997).

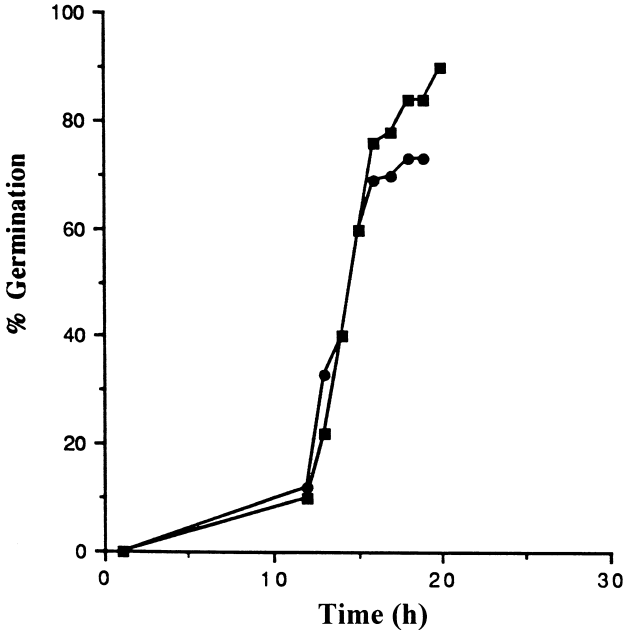

FIG. 1. Synchrony of zygospore germination. Shaded squares and circles represent data from two independent experiments. A total of 50–100 zygospores was scored for germination at each time point. See Materials and Methods for details of procedures for zygote maturation, zygospore storage, and induction of germination.

Within 2 h after dark-stored zygospores were returned to the light to induce germination, the zygospores became hydrated and swelled, resulting in an apparent stretching of the zygospore wall. The surface became smoother, and the peaks and valleys became less distinct. However, all wall layers remained intact (data not shown).

As germination progressed, more dramatic changes occurred both within the cytoplasm and within the zygospore wall. Within 6 h after light induction, large vacuoles appeared in the cytoplasm, apparently involved in the mobilization of the lipid storage reserves (Fig. 2c). Individual condensed chromosomes appeared as the nucleus prepared for the first meiotic division (Fig. 2c). At the same time, the first evidence of secondary wall breakdown appeared. Breaks in the trilamellar sheath were seen at discrete locations around the zygospore, exposing the electron-opaque inner layer to the external environment (Fig. 2d).

The cytoplasmic vacuoles continued to enlarge as the number of lipid bodies decreased inside the cell. The vacuoles then collapsed in on themselves and retracted away from the zygospore wall (Fig. 2e). At the same time the cytoplasm of the cell pulled away from the zygospore wall, producing a greatly expanded periplasmic space. Breaks in the trilamellar sheath were easily detected (Fig. 2, e and f), and the electron opacity of the inner layer began to decrease. Before the first nuclear division, numerous small vacuoles appeared within the nucleus (Fig. 2g).

Within 16 h after light induction, progression of cytokinesis resulted in the formation of two daughter cells, the products of meiosis I (Fig. 3a). These first daughter cells were naked, lacking the glycoprotein walls typical of vegetative cells. Degradation of the zygospore wall progressed further. Electron-opaque fibrillar bands, apparently part of the inner wall layer, continued to connect the edges of the broken trilamellar sheath (Fig. 3b). However, regions showing more extensive degradation of the inner wall layer were also observed (Fig. 3c).

The second meiotic division occurred soon after the first division was completed, within 18 h after light induction. As the second meiotic division began, vege-

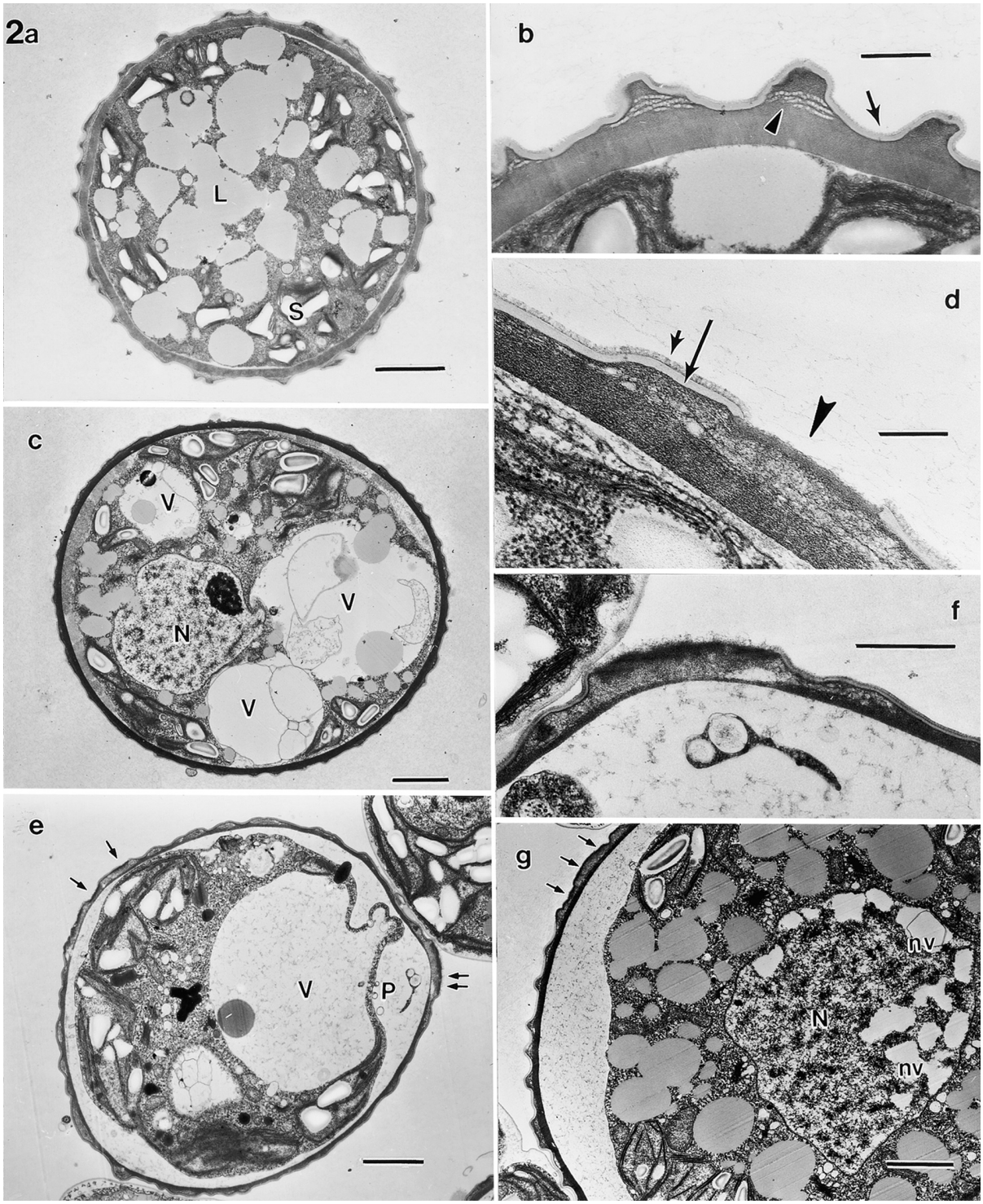

FIG. 2. Changes in the zygospore wall before completion of the first meiotic division. (a) Mature dormant zygospore with stored lipid (L) bodies and starch granules (S) and a fully formed reticulate secondary zygospore wall. Scale bar, 2 μm. (b) Detail of secondary zygospore wall showing surface trilamellar sheath (arrow) covering the electron-opaque inner layer. The inner layer includes electron-translucent areas ("honeycombs"; arrowhead) at the base of several of the peaks within the wall. Scale bar, 500 nm. (c) At the time of chromosome condensation within the nucleus (N), large cytoplasmic vacuoles (V) appear that include lipid bodies within them. Scale bar, 2 μm. (d) Breaks in the trilamellar sheath appear at this stage of germination. Both the surface fuzzy coat (small arrow) and the underlying electron-translucent band (large arrow) of the trilamellar sheath have been removed in a limited region (arrowhead) of the zygospore wall. Scale bar, 250 nm. (e) Enlargement and retraction of the cytoplasmic vacuole (V) leads to expansion of the periplasmic space (P) separating the zygospore cytoplasm from the zygospore wall. Breaks in the wall are readily detected (small arrows). Scale bar, 2 μm. (f) Higher magnification of the wall regions marked by double arrows in (e). Scale bar, 1 μm. (g) Preceding completion of the first nuclear division, numerous vacuoles (nv) appear within the nucleus (N). Small arrows indicate an extended region where the outer trilamellar sheath layer of the secondary zygospore wall has been removed. Scale bar, 2 μm.

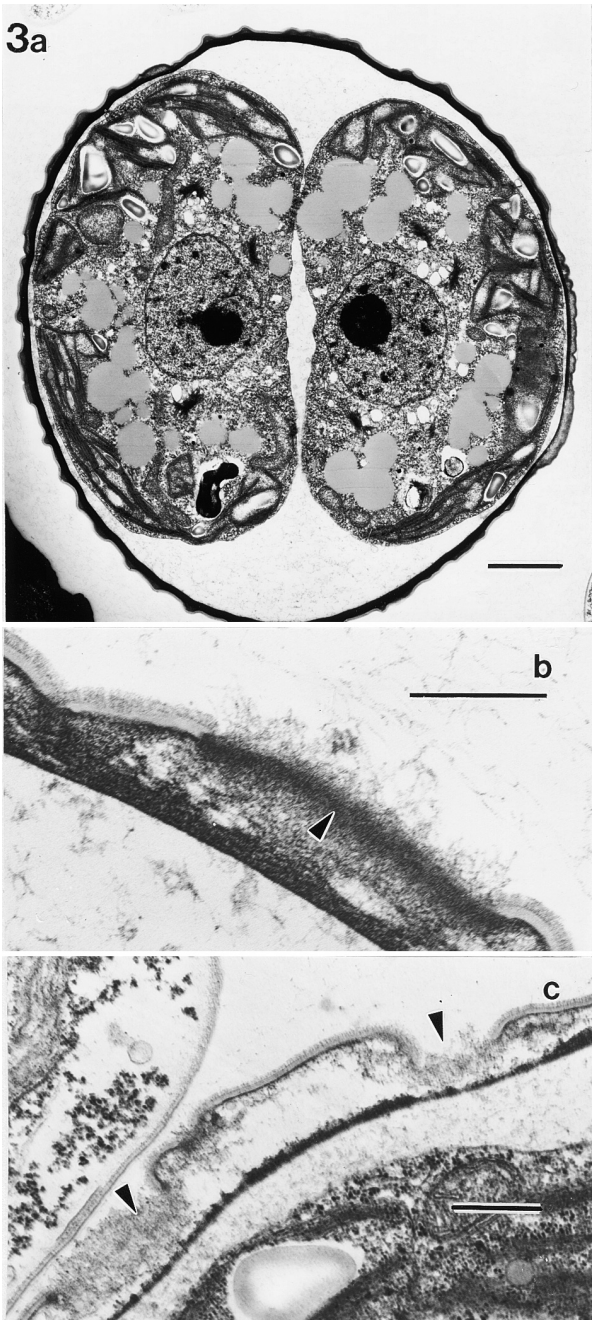

FIG. 3. Zygospore wall changes associated with the completion of the first meiotic division. (a) At the completion of meiosis I and the associated cell division, two wall-less progeny are produced. Scale bar, 2 μm. (b and c) Wall detail from additional zygospores nearing completion of the first meiotic division. (b) Note the electron-opaque band of fibrillar material (arrowhead) underlying the region where the trilamellar sheath has been removed. Scale bar, 250 nm. (c) Similar fibrillar regions (arrowheads) are disintegrating, and much of the inner wall layer has been degraded. Scale bar, 500 nm.

tative cell walls were absent (Fig. 4a). However, the final four haploid meiotic products, held within a partially degraded secondary zygospore wall, were walled and flagellated (Fig. 4, b–e). Little of the electron-opaque material of the inner secondary zygospore wall remained, although the extent of degradation varied considerably among zygospores that had completed the meiotic divisions. Further degradation of the trilamellar sheath did not occur. The inner wall material directly beneath the breaks in the trilamellar sheath was the last region to be degraded. At the time of progeny release, the inner zygospore wall was completely degraded, and the trilamellar sheath remained intact except in those discrete regions where wall breakdown had been initiated (Fig. 4c).

Figure 5 shows the progress of zygospore wall breakdown as viewed by scanning electron microscopy. Using this approach, only the fate of the trilamellar sheath can be clearly addressed. Upon induction, the mature zygospores swelled, and their surfaces became less reticulate (Fig. 5a). Isolated deep ridges then formed (data not shown) and appeared to define where the slits in the sheath would occur (Fig. 5b). Although several long slits were produced through the sheath, providing the route for progeny escape, the trilamellar sheath appeared to be largely resistant to degradation and remained as an empty casing after progeny release (Fig. 5, c and d).

Analysis of changes in the resistance of zygospores to chloroform vapors during the course of germination indicated that chloroform resistance was lost at about 4–6 h after induction (Fig. 6). At this time, the first breaks in the trilamellar sheath had been produced, but the inner zygospore wall layer appeared to be intact (Fig. 2, c and d).

DISCUSSION

Zygospore germination, which entails a change from dormancy to active metabolism and growth, comprises several distinctive events, including (a) the mobilization of stored energy reserves, (b) induction of meiotic nuclear division and cytokinesis, and (c) degradation of the zygospore wall to allow release of the vegetative progeny cells. Although this study has focused on the latter process, unique cytoplasmic events have also been documented.

Mobilization of stored energy reserves appears to be associated with vacuole formation and has been observed in other plant systems during the transition from a resting to an active state. Mobilization of protein bodies (which include lipids and RNA in addition to stored proteins) occurs during the germination of many seeds and provides the energy needed for increased metabolism (Ashton 1976). In the cotyledons of *Vicia faba* (broadbean), hydrolysis of the protein bodies leaves behind small vacuoles that coalesce to form a large central vacuole (Jacobsen 1984). Lipid metabolism in pollen also leads to vacuole formation. During pollen tube elongation, lipid granules are digested and vacuoles form in the pollen tube (Noguchi 1990). In *C. monoica* the large globular lipid bodies in the mature zygospore are broken down into smaller lipid bodies that can be seen in transit into the vacuoles and fully contained within them (Fig. 2c).

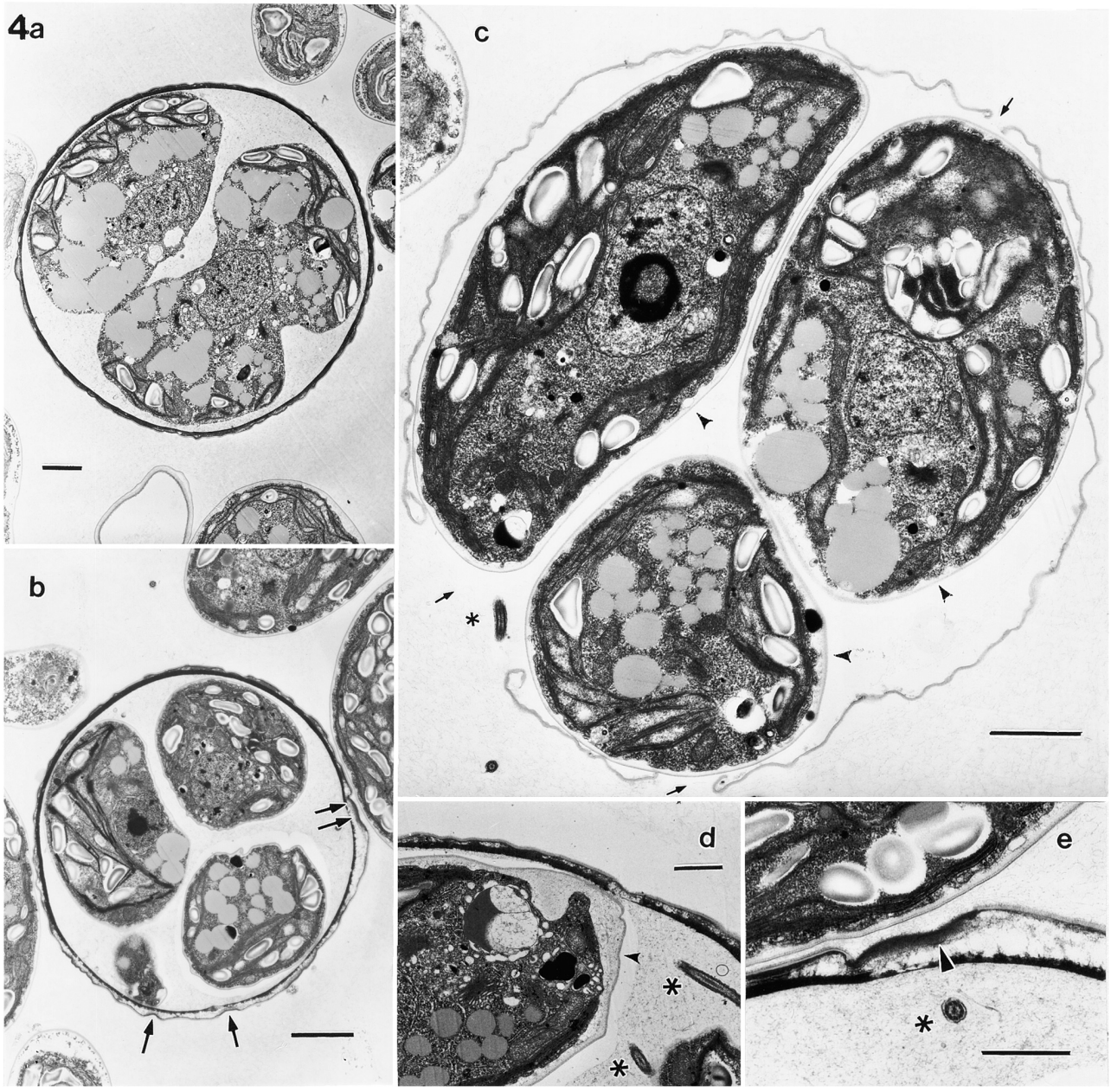

FIG. 4. Zygospore wall changes associated with the second meiotic division. (a) Early cleavage stages in which progeny cells remain wall-less. Scale bar, 2 μm. (b) As cell divisions are completed, progeny cell walls are formed and degradation of the inner zygospore wall layer progresses. Arrows indicate regions of trilamellar sheath breakdown. Scale bar, 2 μm. (c) At the completion of meiosis II and associated cell divisions, the walled (small arrowheads) and flagellated (*) progeny cells can escape through the openings (small arrows) in the trilamellar sheath. Degradation of the inner wall layer is complete. Scale bar, 2 μm. (d) Progeny produced at the completion of meiosis II are flagellated (*) and walled (marked by small arrowhead). Scale bar, 2 μm. (e) Higher magnification of the wall region marked by the small double arrows in b. Note the electron-opaque band (arrowhead) marking the region where the trilamellar sheath is absent. Scale bar, 1 μm.

Many lipid bodies that are found in pollen tubes are used for energy needed in tube elongation (Dorne et al. 1988). The lipid bodies in pollen grains and tubes may also serve as a source of precursors needed for membrane synthesis during elongation, growth, and division (Dorne et al. 1988). It is likely that the lipid bodies of the *C. monoica* zygospore are used both as a source of membrane precursors and, if fully metabolized, as a source of energy.

Regression of the large vacuole during zygospore germination is similar to events observed during pollen maturation. For example, in *Datura* pollen, the large central vacuole that is present in the uninucleate microspore starts to invaginate or retreat at the bi-

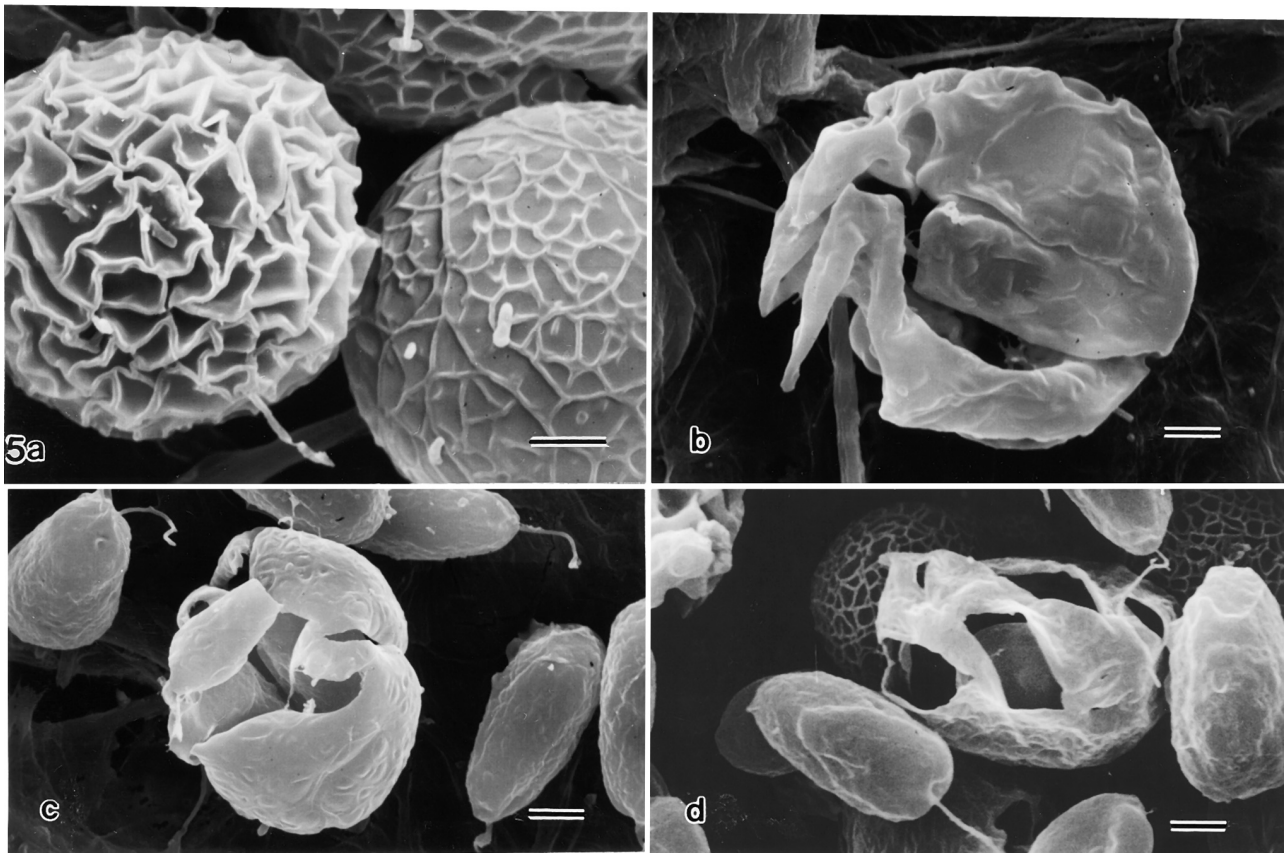

FIG. 5. Scanning electron microscopy of zygospore germination. (a) Upon light induction, zygospores hydrate and swell, and reticulation of the surface becomes less pronounced (compare surfaces of the two zygospores shown). (b) As the surface pattern changes and its reticulation lessens, slits appear in the wall. (c and d) As the progeny escape, the torn remnants of the trilamellar sheath are left behind. Scale bars, 2 μm.

nucleate stage (Sangwan and Sangwan-Norreel 1987). The invagination and retreat of the vacuole draws the cytoplasm into the free space, much like the regression of the cytoplasm away from the zygospore wall in *C. monoica*.

Vacuoles within nuclei, as observed in the *C. monoica* zygospore during germination, also have been observed in many plant species during meiosis. In *Datura* microsporogenesis, as the chromosomes condense during early meiotic prophase, vacuoles are produced by invagination of the inner membrane of the nuclear envelope (Sangwan 1986). Additional vacuoles, formed later in prophase via the invagination of both inner and outer membranes, expand and move further into the nuclear space before disappearing at metaphase (Sangwan 1986).

Nuclear vacuole formation has also been seen in the ferns *Pteridium, Dryopteris*, and *Mansilea* and in the higher plants *Pinus* and *Lycopersica* (Sheffield et al. 1979). In the ferns, vacuole formation coincides with early stages of chromosome condensation, whereas in the higher plants vacuole formation correlates more closely with synaptonemal complex formation (Sheffield et al. 1979).

Although the function of these nuclear vacuoles is unknown, Dickinson and Heslop-Harrison (1977) have proposed that the nuclear vacuoles are related to the change from the sporophytic to gametophytic phase and serve to eliminate mRNA and polysome complexes so that the gametophytic phase can be established. Others have suggested that the nuclear vacuoles may establish an inner nuclear membrane flow that subsequently moves the chromosomal telomeres over the nuclear envelope (Rasmussen 1976). Whatever the function of these vacuoles, they apparently are associated only with meiotic nuclear division, and perhaps specifically with meiosis I, both in *C. monoica* and in higher plants.

Germination of *C. monoica* zygospores includes wall breakdown. As shown in this study, the timing and nature of wall degradation is different for the different wall layers. The first ruptures in the zygospore wall occur in the outer layer long before viable progeny cells are present. The discrete breaks in the outer trilamellar sheath expose the inner wall layer to the environment. At the same time as the breaks appear in the outer trilamellar sheath, zygospore resistance to chloroform vapors is lost (Fig. 6), although the inner wall

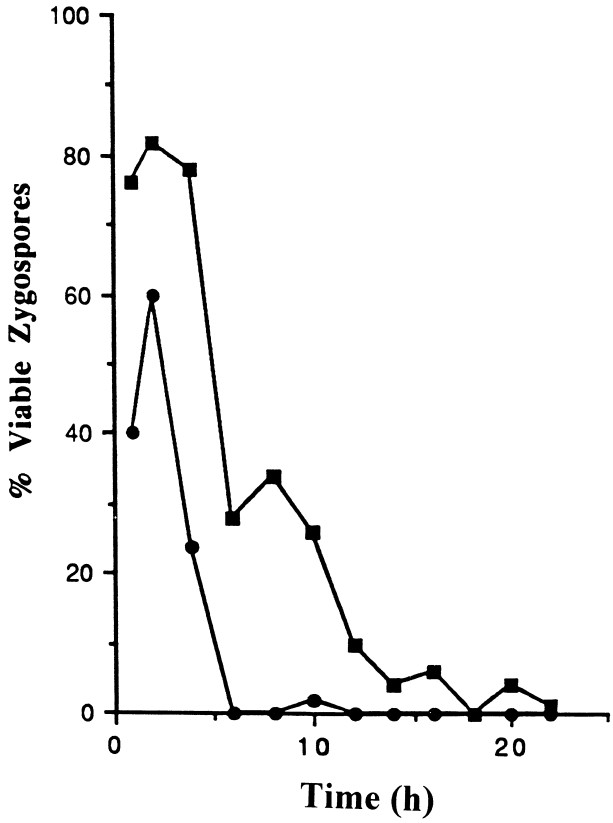

FIG. 6. Timing of the onset of sensitivity to chloroform vapors during zygospore germination. Zygospores were exposed to chloroform vapors at periodic time points after light induction of germination and were scored subsequently for release of viable progeny (germination). Shaded squares and circles represent data from two independent experiments. See Materials and Methods for experimental details.

layer remains intact. This suggests that the outer trilamellar sheath is responsible for the chloroform resistance of mature zygospores.

A number of lines of evidence, although circumstantial, suggest that it is the outer trilamellar sheath that contains sporopollenin: (a) the residue remaining after acetolysis of mature zygospores includes some ultrastructural features reminiscent of the trilamellar sheath (VanWinkle-Swift and Rickoll 1997), (b) mature viable zygospores display autofluorescent surfaces (VanWinkle-Swift and Rickoll 1997), and (c) as shown in this study, the trilamellar sheath is more resistant to degradation during germination than is the underlying more massive electron-opaque wall layer. Furthermore, according to Kalina et al. (1993), only the outermost wall layer of the zygospores of the closely related species *C. geitleri* is acetolysis resistant.

Gherardini and Healey (1969) suggested that the exine (outer surface layer) of the pollen grain is degraded enzymatically and that in order for pollen tube growth to occur the sporopollenin must be degraded. However, the pollen grains of many higher plants include preformed apertures in the exine through which pollen tube elongation occurs, thus making the need for a "sporopolleninase" less obvious (Cresti and Tiezzi 1990). Although no enzymes are known that degrade sporopollenin, modification of "immature sporopollenin" in the vicinity of pores through the activity of an esterase has been suggested (Ahokas 1975, reviewed by Southworth 1990). Clearly, in the case of the *C. monoica* zygospore, no such preexisting pores or discontinuities in the spore wall are available to promote wall breakdown and progeny release. Thus, lysis of the trilamellar sheath is an absolute requirement.

The inner wall continues to be degraded until all that remains are remnants and the fibrillar electron-opaque connections between the broken edges of the trilamellar sheath. This suggests that these connections differ in composition from that of other regions of the inner wall. Ultimately these regions must also be degraded to allow progeny cells to escape through the broken sheath. A mechanism apparently exists to delay this last step until the progeny cells are fully developed (e.g. walled and flagellated).

Storage of enzymes within the inner wall layer (intine) of mature pollen, including acid phosphotase, ribonuclease, esterase, and amylase, has been demonstrated (Knox and Heslop-Harrison 1969, Knox 1971). It remains to be seen whether the enzymes involved in zygospore wall breakdown in *C. monoica* are synthesized during zygote maturation and are incorporated within the wall layers or whether they are synthesized *de novo* upon the induction of zygospore germination.

Support from the National Science Foundation through grant DCB- 9728461 is gratefully acknowledged. We thank Dr. Lawrence Fritz, Dr. Wayne Rickoll, and Marilee Sellers for technical assistance and helpful discussions, and we acknowledge the support provided by the Northern Arizona University Electron Microscope Facility.

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