Fine Structure of the Wall and Appendage Formation in Ascospores of *Podospira anserina*

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SUMMARY

The ascospores of *Podospira anserina* (Ces.) Rehm are delimited by a double membrane system. The primary spore wall develops within this, the outer part of the double membrane being pushed out to form the spore membrane and the inner part forming the plasmalemma of the spore. Starting in the middle of the matrix of the expanding primary wall, a secondary wall is laid down and gradually extends to the outer periphery of the spore wall. Later, a thick tertiary wall is formed at the inner side of the secondary wall by blocks of electron-dense material between which channels of the primary wall matrix remain. This is the pigmented layer of the spore wall. On the innermost side of the spore wall, a part of the original primary wall remains.

The primary appendage at the base of the spore arises as part of the spore initial, but, after it has been cut off by a septum, its contents degenerate and it is bounded only by the primary and secondary wall layers. The secondary appendages, formed at the apex of the spore and at the bottom of the primary appendage, are considered to be actively growing processes bounded by the spore membrane.

INTRODUCTION

In this study the electron microscope has been used to extend the optical microscope observations by Beckett & Wilson (1968) on the development of the spore wall and the primary and secondary appendages (Fig. 1 F) of *Podospira anserina*.

METHODS

Perithecia of *Podospira anserina* were removed from cultures (Beckett & Wilson, 1968) and the contents dissected out into a drop of phosphate buffer on a glass slide. Asci so obtained were then transferred to a 2% (w/v) potassium permanganate solution maintained at pH 7.2 with 0.1 M-phosphate buffer and fixed for 45 min. to 1 hr at room temperature. The material was then washed in distilled water, dehydrated in a graded ethanol series, soaked in propylene oxide and finally embedded in either Araldite or Epon. Various staining procedures were employed involving both saturated aqueous uranyl acetate solution and lead citrate (Reynolds, 1963). Details of these are given in the explanation of the plates.

Sections were cut with glass knives on either a Huxley ultramicrotome or an LKB Ultratome II. Observations and photographs were made using AEI EM 6 and AEI EM 6 B electron microscopes. Correlative observations of fresh material were made on a Baker phase-contrast light microscope.

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RESULTS

Spore wall development

Delimitation of the spore initial in the ascus of *Podospora anserina* is by a double membrane system as in *Pustularia cupularis* (Schantz, 1967). Soon after the completion of this process the primary spore wall may be detected as a conspicuous electron-

Fig. 1. Semi-diagrammatic representation of spore wall development and appendage formation in *Podospora anserina*. Nuclei omitted. A, spore initial with primary wall. B–D, Stages in formation of secondary appendages and secondary-wall layer and early stages in differentiation of spore head and primary appendage. E–G, Stages in formation of tertiary-wall layer in spore head and septum, not in primary appendage.

transparent layer between the two unit membranes, the outer of which becomes the spore membrane and the inner the plasmalemma of the spore. With the gradual widening of the primary wall new electron-dense material is deposited within it. This material appears as discrete blocks measuring about $120 \text{ \AA} \times 250 \text{ \AA}$ (Pl. 1, fig. 2)

which later merge to form a continuous layer (Pl. 1, fig. 3, 4; Pl. 2 fig. 9-11). Prominent at this stage are numerous membrane-bound vesicles, often grouped together, resembling the lomasome structures described by Moore & McAlear (1961). The electron-transparent primary wall then widens considerably and more of the electron-dense secondary material is deposited towards the outer side of the wall until it fills the whole space extending to the spore membrane (Pl. 1, fig. 3, 4; Fig. 1 D). These primary and secondary wall layers are laid down round the whole spore initial, the expanding spore head and the narrower tail portion.

During maturation the ascospores undergo a gradual pigmentation, the main spore head changing from hyaline to green to dark brown. Observations on sections of spores at the green stage show, in the wall around the spore head, the deposition of a tertiary wall layer to the inside of the secondary layer, consisting of blocks of electron-dense material separated by regions in which the primary wall material remains continuous (Pl. 1, fig. 6; Fig. 1 E-G). The channels may be homologous with the ectodesmata described by Kirk (1966) in *Ceriosporopsis halima*. The tertiary wall represents the pigmented layer of the main spore head and in the completely mature spore is seen in section as a wide band of electron-dense material running between the primary wall layer on the inside and the secondary wall layer on the outside (Pl. 1, fig. 7; Fig. 1 G). The dense granules seen at the outer edge of the primary wall of the mature spore closely resemble the pigment granules observed by Delay (1966) in the spore wall of *Ascobolus immersus*.

At the point at which the spore head joins the cylindrical primary appendage a septum is formed by the inward growth of the primary wall and the intercalary tertiary wall (Pl. 1, fig. 8; Fig. 1 E, F). This septum limits the growth of the pigmented tertiary wall of the spore head, while the wall of the primary appendage remains hyaline and two-layered (Pl. 1, fig. 5, 6, 8; Fig. 1 G). No median sections were obtained of this septum and it was not determined whether a pore was present in it. A single germ pore does exist at the extreme apical tip of the main spore head and passes through all the wall layers (Pl. 1, fig. 8).

Appendage formation

Two types of spore appendages are formed on the ascospores of *Podospora anserina* (Pl. 1, fig. 1; Fig. 1 B-F). The primary appendage is initially continuous with the main spore head and contains all the organelles common to the latter except the nuclei (Pl. 1, fig. 1; Fig. 1 A-D). After nuclear division in the spore initial (Moreau & Moreau, 1951; Beckett & Wilson, 1968) and the formation of the septum at the base of the spore head, the cytoplasm and the one daughter-nucleus which migrated into the appendage degenerate (Pl. 1, fig. 8; Fig. 1 E, F).

The second type of appendage present on the ascospore is the secondary appendage. Normally one of these is formed near the apex of the spore, to one side of the germ pore, and another at the basal tip of the primary appendage (Pl. 1, fig. 1; Fig. 1 B-F). These secondary appendages are long, hyaline structures as seen with the light microscope and often become folded within the ascus.

The first indications of the formation of secondary appendages are seen with the electron microscope as out-pushings of the spore membrane, normally at each end of the spore initial, but sometimes along the sides of the spore also (Pl. 2, fig. 9). Later the lateral out-pushings are usually lost and a concentration of dense material can be seen

accumulating at the spore apices between the rudimentary secondary wall and the spore membrane (Pl. 2, fig. 10). Further development produces a distinct cylindrical appendage, completely bounded by the spore membrane (Pl. 2, fig. 11) and situated at each end of the spore.

Very rarely, small lateral appendages persist during development of the spore and may be seen enclosed by the spore membrane, on both sides of the spore where the spore head joins the primary appendages (Pl. 3, fig. 12). Similar appendages were first reported by Moreau (1953) as a result of optical-microscope observations.

DISCUSSION

Earlier investigators of the spore wall have used such terms as epispore, mesospore and endospore, terms which indicate the relative positions of the various layers making up the wall. Recently Kirk (1966) has attempted to establish homologies between the epispore, mesospore and endospore of different species within a family by cytochemical methods. In this study it has seemed more logical to designate the wall layers according to the order in which they develop, especially since both secondary and tertiary layers are laid down within the expanding matrix of the primary wall and are not completely separate entities.

Delay (1966) considered that the pigment granules which colour the spore coat of *Ascobolus immersus* originate in the epiplasmic vacuoles outside the spore. The position of similar, electron-dense granules seen in the primary wall layer of *Podospira anserina* suggests an origin within the spore. Carroll (1966) has demonstrated the presence of small electron-dense vesicles within the spore which pass out through the primary wall and deposit material on the outside of the spore in *Ascodesmis sphaerospora*.

Comparing the spore appendages of *Podospira anserina* with those of some marine Pyrenomycetes recently investigated with the light microscope (Kirk, 1966), the primary appendage of *P. anserina* and the apical appendages of *Corollospora maritima* begin their development in the same way as parts of the spore initial, but the later stages in their development diverge. The terminal mucilaginous appendages of *Ceriosporopsis halima* and the secondary appendages of *P. anserina* have some superficial resemblances. In the former, Wilson (1965) suggested that the appendages grow as a result of the extrusion of material from spore vacuoles through apical pores into an out-pushed epispore, while Kirk (1966) considered that the appendages develop from the epispore material itself owing to a change-over from chitin to mucilage production at these sites. In *P. anserina* there is no extrusion of material through a pore in the formation of the secondary appendages; they appear to grow *in situ*, but are contained within the spore membrane.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Photomontage showing L.S. of young ascospore with two nuclei (*N*) in spore head (*SH*), primary appendage (*PA*), and apical and basal secondary appendages (*ASA*, *BSA*). KMnO_4 fixation, sections stained with lead citrate and embedded in Araldite. $\times 2250$.

Fig. 2. L.S. of parts of walls of two adjacent young ascospores *AS 1*, *AS 2*, showing electron-dense blocks of secondary-wall material (*SW*) forming towards outer edge of primary wall (*PW*) which lies between the spore membrane (*SM*) and the plasma membrane (*PM*). Technique as for fig. 1. $\times 100000$.

Fig. 3. L.S. of part of ascospore wall showing widening of primary wall (*PW*) and a continuous layer of electron-dense secondary-wall material (*SW*) between the primary wall and spore membrane (*SM*). KMnO_4 fixation, sections stained with uranyl acetate and lead citrate, embedded in Epon. $\times 20,000$.

Fig. 4. L.S. of spore wall at later stage. Continued deposition of secondary-wall material (*SW*) has now filled space between spore membrane (*SM*) and remaining primary wall (*PW*). KMnO_4 fixation, uranyl acetate after fixation, sections stained with lead citrate, embedded in Epon. $\times 20,000$.

Fig. 5. L.S. of part of spore at point where spore head (*SH*) joins primary appendage (*PA*). Part of septum (*S*) is shown and early stages of tertiary-wall development (*TW*). KMnO_4 fixation, sections stained with uranyl acetate and lead citrate, embedded in Epon. $\times 22,500$.

Fig. 6. L.S. of similar part of spore to fig. 5. Tertiary-wall material (*TW*) in electron-dense blocks in septum (*S*) and spore head (*SH*), but not in primary appendage wall (*PAW*). Pore-like regions (*E*) can be seen between blocks of primary-wall matrix. KMnO_4 fixation, sections stained with uranyl acetate and lead citrate, embedded in Epon. $\times 45,000$.

Fig. 7. L.S. of part of wall of nearly mature spore head. Three layers seen: a wide primary-wall layer (*PW*) with numerous electron-dense granules (*G*) along outer edge, a wide, electron-dense tertiary-wall layer (*TW*) with persistent ectodesmata or pores (*E*), and a narrower electron-dense secondary-wall layer (*SW*) on the outside. KMnO_4 fixation, sections stained with lead citrate, embedded in Araldite. $\times 30,000$.

Fig. 8. Light micrograph of ascospore at green pigmented stage showing restriction of pigment to spore head. Germ pore (*GP*) at apex. $\times 900$.

PLATE 2

Fig. 9. L.S. of spore initial showing the pushing out of the spore membrane (*SM*) during early stage of secondary appendage formation. Lomasomes (*LO*) can be seen associated with developing spore wall. KMnO_4 fixation, uranyl acetate staining after fixation, embedded in Epon. $\times 11,500$.

Fig. 10. L.S. of part of spore head apex showing concentration of secondary appendage material (*SA*) beneath spore membrane (*SM*). An almost continuous line of secondary-wall material (*SW*) can be seen outside the electron-transparent primary wall (*PW*). Fixation, staining and embedding as for fig. 9. $\times 23,000$.

Fig. 11. L.S. of part of spore head apex showing further development of apical secondary appendage (*ASA*). Fixation staining and embedding as for fig. 9. $\times 23,000$.

PLATE 3

Fig. 12. L.S. of part of spore showing abnormal lateral secondary appendages (*LSA*). KMnO_4 fixation, uranyl acetate staining after fixation, embedded in Epon. $\times 15,000$.

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