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Connective structures between tapetal cells and spores in Lycophyta and pollen grains in angiosperms — A review

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ABSTRACT

This article is a review of research and accumulated information about connective structures between, respectively, microspores in the locular space in anthers of angiosperms and the spores in the megasporangia of Lycophyta, as well as a comparison between these structures. The most extraordinary conclusion, if our interpretations of the results are correct, is that the reason for the great number of exine components and the great complexity of the micro- and mega-spore walls is that each exine unit structure acts as a plasmodesma and that each spore unit directs a wick-component to the cytoplasm. Pollen grains and spores that are literally covered by plasmodesmata or plasmodesmatal equivalents may be expected to grow and develop rapidly. These connections are believed to be routes of transport for substances between tapetal cells and the plasma membrane and cytoplasm in both pollen grains and spores. In *Selaginella*, "wicks" which are plasmodesmata equivalents extend from tapetal cells to exospore units and further to plasma membrane and megaspore cytoplasm. In angiosperm pollen grains plasmodesmata equivalents extend from the tapetum into and through exine and into the cytoplasm passing through the core of exine units (tufts), but in spores (e.g., *Selaginella*) the strand-like wicks traverse the space between the components of the exospore and the megaspore cytoplasm. These structures were successfully fixed with ruthenium red and Alcian blue, which tend to stabilize polysaccharides and glycoprotein as well as contrasting the components of the exine and exospore transfer systems.

Contents

1. Introduction

Megaspores and pollen grains depend on transport of nutrients from tapetal cells during their development and both are dependent on a very precise and organized transport system. For example, the volume of a *Poa annua* microspore increases 34 times to pollen grain maturity in a few days (Rowley, 1964). The increase in volume during development of a megaspore is difficult to calculate and was not attempted, but a measure of rapid growth in megaspores may be appreciated by observing the

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enlargement of exospore units from 5–10 nm to over 200 nm in only a few days (Rowley and Morbelli, 1995; Morbelli and Rowley, 1999).

Species of *Selaginella* were studied extensively with respect to megaspore development by Morbelli and Rowley (1992, 1993, 1996, 1999) and Rowley and Morbelli (1995). Wicks were recorded in the sporangia of *S. argentea*, *S. kraussiana* and *S. convoluta* by Morbelli and Rowley (1992, 1993) and Morbelli (1992, 1995). According to El-Ghazaly et al. (2000) and Rowley et al. (2003), it is probable that most plants use a transport system consisting of strands (strands are a substitute for viscin threads) between tapetum and microspores at some time during pollen development. Strands were stabilized by El-Ghazaly by cryoscanning methods and the results indicated that transport from the tapetum through the loculus and the exine is comparable to transport by a strand system of plasmodesmata between plant cells.

The aim of this review is to discuss the data and interpretations about structures that involve the transport between tapetal cells and microspores in anthers of angiosperms and, between tapetal cells and the spores in the megasporangia of Lycophyta.

2. Interpretations of the results and discussion

2.1. Microchannels

Microchannels were among the first substructural components recognized in pollen grains and fern spores. They were observed by Afzelius (1956) and Gullvåg (1966) in *Lycopodium clavatum*. Pettitt (1976) illustrated channels across the exospore of *Lycopodium* spores with his experiments using iron and lanthanum tracers. Morbelli et al. (2001) found conduits traversing the distal and proximal walls of microspores in species of *Selaginella* that grow in Argentina. These authors illustrated them using TEM, and showed microchannels in the outer exospore of *S. sellowii* and many transversal conduits across the exospore in *S. sulcata* (Morbelli et al., 2001, p. 360, fig. 35C; p. 366, figs. 41A, B). Radial canals or conduits have also been reported and extensively described using TEM in Lycophyta (*Selaginella*) by Tryon and Lugardon (1978). Similarly, Tryon and Lugardon (1991) showed abundant canals in the exospore of *S. kraussiana* and *S. rupincula* microspores.

According to Rowley et al. (2003) each microchannel forms the core zone of a tuft. Each tuft is considered to be the unit structure of exines. In mature exines the core zones (microchannels) of tufts are commonly about 25 nm in diameter whereas in microspores the core zone of tufts may reach 40–50 nm in diameter in both the ectexine and endexine (Rowley et al., 2003 figs. 6–10). In their study on the structure and nature of the microspore wall in *S. kraussiana*, Rowley et al. (2002) suggested that the great amount of material in the microspore cytoplasm indicates that there had been extensive transport across the inner and outer exospore through the canals which they called "conduits". These conduits could explain transport of molecules from the tapetum through the loculus to the microspore protoplast. Moreover, Rowley et al. (2002) found that the openings of sectioned conduits were bypassed by the rods that constitute the conspicuous ornamentation of *S. kraussiana* microspores.

Tryon and Lugardon (1991) also reported the presence of numerous canals in the exospore of microspores of heterosporous ferns and other fern families like Blechnaceae (p. 9, fig. 15), Dicksoniaceae (p. 233, figs. 6, 16, p. 229, fig. 6), Hymenophyllopsidaceae (p. 236, fig.5), Cyatheaceae (p. 251, figs. 7, 22, 24), Dennstaedtiaceae (p. 299, fig. 17), Polypodiaceae (p. 320, figs. 11,12), (p. 329, figs. 9, 15, 16), Davallaceae (p. 385, fig. 13) and Dryoteridaceae (p. 488, figs. 21, 22, 24). Similarly, Ramos Giacosa et al. (2004, p. 234, fig. A; p. 235, fig. C) reported radial channels with a darkly contrasted inner structure in the exospore of *Anogramma* species (Pteridaceae). Piñeiro et al. (2006, p.105, fig. 3C, D) showed channels in the exospore of *Adiantopsis* (Pteridaceae). Giudice et al. (2006) also presented abundant records of radial channels in the exospore of various species of Dennstaedtiaceae growing in Argentina. Ramos Giacosa et al. (2007, p. 160, figs. 20–28; p. 162, fig. 34) reported radial channels traversing the exospore of Grammitidaceae. Marquez et al. (2009-this issue) found channels within the exospore and others traversing the deep perispore layer in *Alsophila* (Cyatheaceae).

2.2. Strands originating from tapetal and other anther cells and extending to microspores, megaspores and their cytoplasms

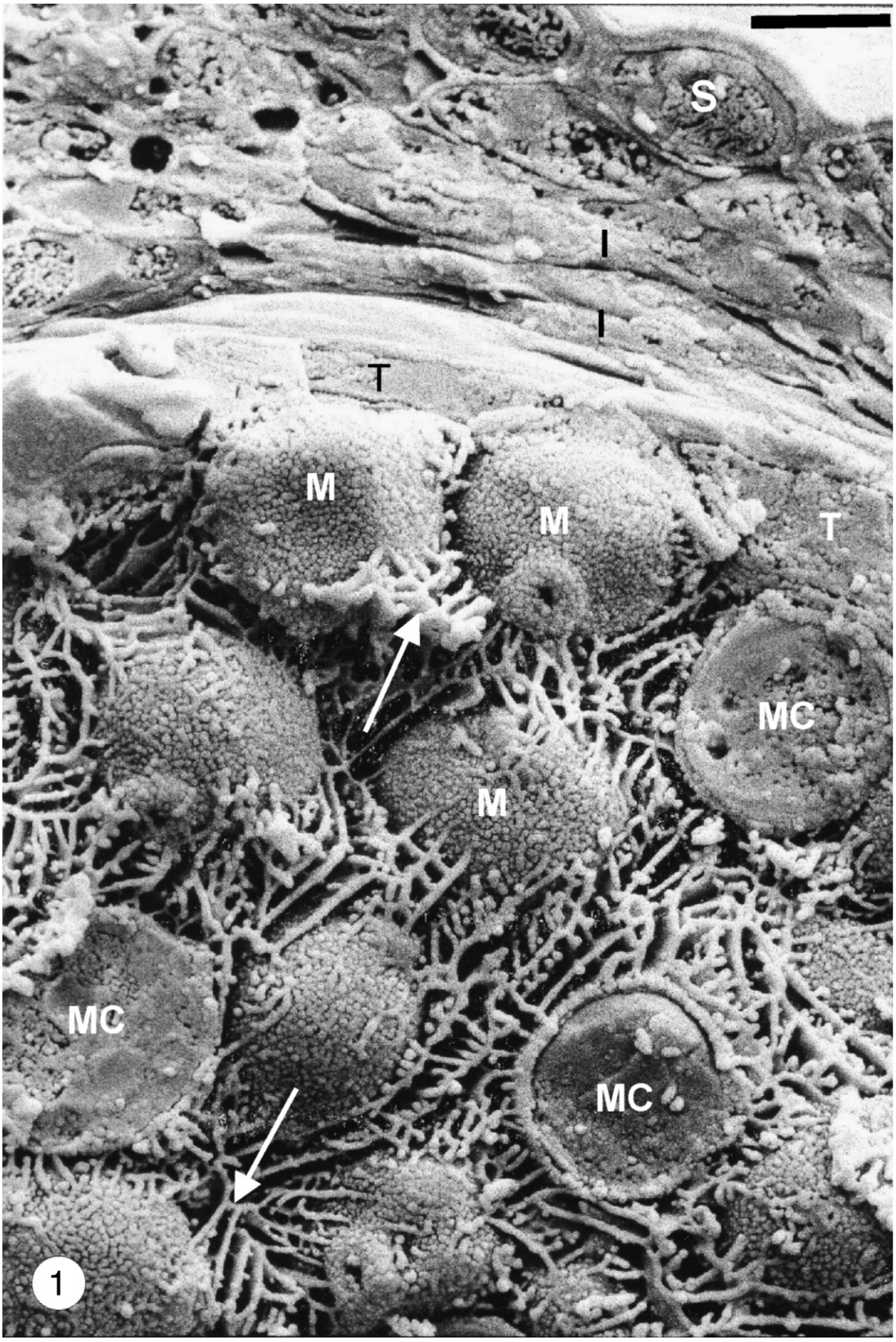
According to the present authors, it is likely that most plants use a transport system of some kind of strands between tapetum and respectively, microspores and megaspores at some time during their development. Perhaps the most widely recognized strand system between tapetum and pollen grains consists of viscin threads (i.e. strands connecting tapetal cells and pollen grains). Threads of various compositions have been reported to be attached to pollen of a number of families (e.g., Hesse, 1984) but viscin threads are positively known in only two families, the Onagraceae and the Ericaceae. However, even in the Onagraceae and in some taxa of the Ericaceae there is little or no positive evidence for transport by these threads or even connections from the tapetal cells to the microspores, as far as we know. The strands we propose as possible connectors between tapetum and microspore exine and cytoplasm are perhaps even more open to question than the viscin threads mentioned above.

The SEM image in Plate I shows an example of strands between tapetal cells and microspores of *Betula pendula*. At the top of the picture there are surface and endothecial cells of the anther wall above the tapetum and microspores. As in our Plate I, the SEM images of Rowley et al. (2003: figs. 1–3) show many strands in *B. pendula* in contact with tapetal cells, highly magnified strands in contact with the exine outer surface of a microspore, the exine inner surface with columellae below the contact with the strands and strands above the cytoplasm. It is worth noting that these results could be obtained thanks to a protocol described by El-Ghazaly et al. (2000) and Rowley et al. (2003) which consist in plunging fresh anthers of *B. pendula* under vacuum into supercritical nitrogen at −212 °C, then in transferring them under vacuum into a JEOL preparation chamber, in fracturing them with a cold knife and in placing them on the cold stage in the Scanning Electron Microscope (SEM) at −80 °C. After etching, the anther preparation was again moved into the preparation unit, sputter-coated with gold, returned to the cold stage and examined at −125 to −170 °C.

2.3. Plasmodesmata

The primary problem with many interpretations of plasmodesmata fine structure has been that the tubular components are shown to be less than 10 nm in diameter which is too small for a reasonable major transport level. Gamalei et al. (1994) and Rowley (1987) suggested hypothetical models for functional transport states. In that proposed by Gamalei et al. (1994) (our Fig. 1), the ER (endoplasmic reticulum) rod in the centre of a plasmodesma is enlarged with the aid of a contracted stage of the actin/myosin filaments of the cytoskeleton sphincter around the annulus. It shows ER rod in the centre of the plasmodesmata with the ER rod compacted due to a relaxed condition of the actin/myosin filaments surrounding the ER rod. The ER rod

Plate I. Scanning Electron Micrograph (SEM) showing an anther of *Betula pendula* (Betulaceae) in cross section. Note the numerous connective structures (=strands, viscin-thread-like strands, arrows) between tapetal cells (T) and microspores (M) were put in evidence by fixation at low temperatures. At the top of the figure there is the anther wall consisting of surface (S) and endothecial (I) cells. The anther loculus contains tapetum cells (T) and microspores (M). Three of the microspores have been cross sectioned (MC). The connectors are present between tapetal cells and each microspore. Scale bar = 10 μm.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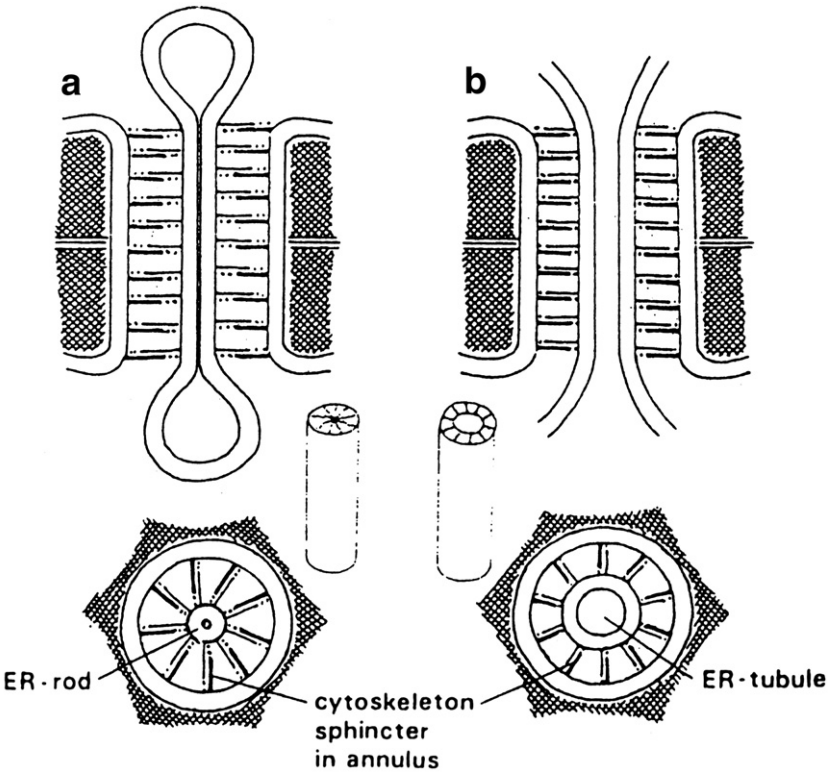

Fig. 1. Hypothetical model of Gamalei et al. (1994) showing the structure of the plasmodesmata in a non-functional (a) state and a functional (b) state. a. Non-functional state. Note that the endoplasmatic reticulum (ER) appears in the form of a central rod: actin/myosin filaments in relaxed state. b. Functional state. Note that the ER appears in the form of a tubule: actin/myosin filaments in a contracted state.

becomes an ER tubule due to contraction of the actin/myosin filaments.

In contrast, the interpretation of Rowley (1987, text fig. 1C) differs with respect to the basic structure of plasmodesmata and enlargement of transporting/conducting (functionaling) and enlarged tubules (see our Fig. 2). A plasmodesma in the tapetum and exine of the Onagraceae consists of a thin boundary layer composed of 10–15 nm tubules coiled into circular plasmodesmata 60–100 nm in diameter. The central part of the plasmodesma consists of five, ca 10 nm diameter tubules that are usually coiled and when in a non-functional state (for example, following fixation), the coils are interdigitated and fill the binder tube. In a transversally sectioned plasmodesma, the middle thin section will show a pentahedral image (Fig. 2c).

Plasmodesmata have a great capacity for expansion. Rowley (1986, 1987) noted that they may increase from 60–70 nm to ca 100 nm and that their central space may pass from very few nanometers to 40 nm. Moreover, according to Rowley et al. (1999), Rowley and Claugher (1996), El-Ghazaly and Rowley (1998), each exine unit (a tuft) in the tectum, the columellae and the endexine is a plasmodesmata equivalent.

2.4. Sporoderm structure in transport routes

Exine units, both ectexine and endexine form on the plasma membrane and are reported as part of the transport system between tapetum and microspore cells (Rowley et al., 2003). Uptake of exine units by procolumellae (developmental precursors of columellae) as plasmodesmata equivalents is one of their functions in the development processes.

In his early research on pollen ontogeny, Heslop-Harrison (1963, 1964) reported that there is a correspondence between cisterns of the endoplasmic reticulum under the plasma membrane and a connection with sites at the base of procolumellae. According to this author, procolumellae served in basic way at the beginning of developmental processes.

Examples of hollow columellae with a central core of about 40 nm in diameter were shown in chemically fixed anthers during initial stages of development in *Leontodon autumnalis* (Asteraceae) by El-Ghazaly (1982) and at the end of the tetrad period in *Borago officinalis* (Boraginaceae) by Gabarayeva et al. (1998). Blackmore and Claugher (1987) and Blackmore (1990) noted that the early exine in *Echinops sphaerocephala* and *Scorzonera hispanica* (Asteraceae) consists of a system of hollow tubes. Rowley and Dahl (1982) referred to the 10–15 nm diameter tubules as described in Section 2.3 on Plasmodesmata (see above) and in our Fig. 2 where they are called "core subunits" of tufts. Blackmore and Claugher (1987) termed the zone around the hollow tubes a "boundary zone". Blackmore (1990) considered that after accumulation of sporopollenin in the hollow tubular structures, they become solid as they are usually found to be in mature exines. It seemed interesting to see if hollow core subunits could be reopened. After considerable exploration of potentially useful methods we found that exposure of mature pollen to 4-methylmorpholine N-oxide monohydrate (MMNO H_sO) resulted in satisfactory results (Rowley et al., 2001). Following MMNO exposure, the tectum of *Betula pendula* had many regularly positioned ca 40 nm holes in thin sections of the tectum and holes of various sizes in the centre of each columella and much erosion in the endexine. There were holes in exines of *Calluna vulgaris* and *Fagus sylvatica* exines and some erosion in *Lilium*, *Lycopodium* and *Pinus*. MMNO actually removes polysaccharides and, according to us, it is this that affected the change in exine morphology. Moreover, we think that it is unlikely for sporopollenin to be directly eroded or removed by MMNO H_sO.

2.5. Wicks

Whereas the megaspores were believed to develop within the locular space of the megasporangia without any contact with the

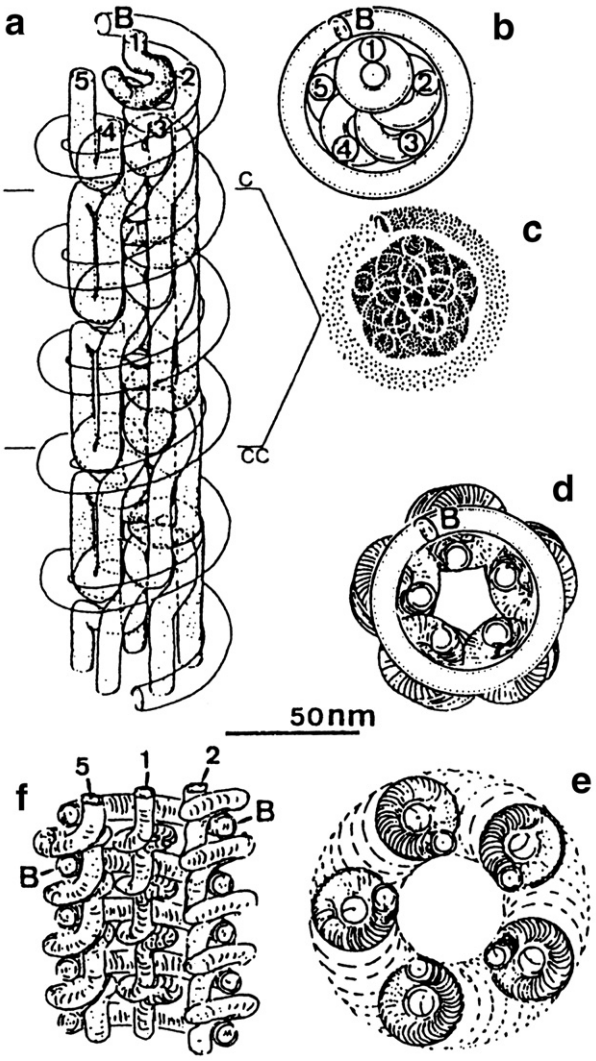

Fig. 2. Interpretative diagram of Rowley (1987) about the processes in tapetal cell surface (= plasmodesmata). Scale bar = ca 50 nm. a. Basic structure of a plasmodesma showing a spiral of boundary subunits, ca 30 nm in diameter, that surround five core tubules (Nos 1–5), most of which are longitudinally orientated. b. Detail of the five coiled core tubules stacked within the ca 60 nm diameter confinements of the boundary subunits. c. Aspect of a TEM image of a ca 70 nm thick plasmodesma cross-sectioned between c and cc, in a. Note that the central dark pentagon which is a common feature in TEM images, is due to cross-over area of the five core tubules. The structures shown in a–c occur in a non-functional condition with respect to transfer of nutrients in a Plasmodesmatal-mode and Plasmodesmata in a non- active mode, the core subunits are staked within the confinements of the boundary subunits structures in a non-functional condition with respect to the transfer of nutrients. In contrast, the diagrams in d–f suggest plasmodesmatal active structures with their 30 nm wide coiled core subunits. In a plasmodesmatal active mode, the core subunits are interdigitated between the boundary components (d) leaving the central part of the tuft, ca 40 nm wide, open (d–e) and suitable for transfer of nutrients.

tapetal cells, Morbelli and Rowley (1993) found rod-shaped structures between them. However, previously Taylor (1991) described irregular fibrillar material at the outer surface of exospores in *Selaginella galeottii*. This author also described a continuous sheath of "weft-like material" at the locular-facing surface of tapetal cells and beaded wefts in the loculus between the tapetal cells and the megaspores. Pettitt (1976) determined that anastomosing channels within the *Lycopodium* exospore were filled or lined with fibrillar glycoprotein.

Indeed, Morbelli and Rowley (1993) clearly demonstrated that there are rod-shaped structures between the tapetal cells and the megaspores in the locular space of the megasporangia in *Selaginella. argentea* and *S. kraussiana* which are large structures, as illustrated in Plate II, 1. These authors called them "wicks". Indeed, according to the TEM images in Plate II, 2–3 and Plate III, 1–2, it is clear that the wicks which are derived from cells of the megasporangium and especially the tapetum, extend without interruption across the fluid of the locular space. Moreover, the TEM images of Plate II, 3 and Plate III, 1–2 show that the wicks entering the exospore look like tubular rods which are circular in cross section. The illustrations by Morbelli and Rowley (1993) show in detail the passage of the wicks through the exospore, from the tapetum to the plasma membrane and cytoplasm of the megaspore. Morbelli and Rowley (1993) interpreted the wicks as probable routes of transport of materials between tapetal cells and the developing megaspores. Wicks in the Lycophyta were shown to pass between the exospore components (Plate III, 1–2).

Moreover, Morbelli and Rowley (1993) have shown that wicks were well preserved in megasporangia fixed with mixtures of lanthanum/osmium, ruthenium red/osmium and Alcian blue/osmium. The contrast staining in wicks was greater in Alcian blue than in the other mixtures. With lanthanum fixation and stained with UA and Pb the positive reaction indicated the presence of glycoprotein and or mucopolysaccharides.

2.6. Possible wick-like strands

Pettitt (1976) described anastomosing channels filled with fibrillar glycoprotein in *Lycopodium*. Similarly, Taylor (1991) described irregular fibrillar material next to the megaspore exospore in *Selaginella galeottii*. Moreover, in his studies on megaspore development in *Isoetes melanopoda*, Taylor (1992) cited the presence of darkly stained wefts of material in the sporangium locular space in the vicinity of the megaspore siliceous surface at various stages. Taylor (1992, pl. III, fig. 10) also documented wefts and irregular material at the outer surface of the developing siliceous deposit. Moreover, it is worth noting that in sections of the megaspore wall in *Isoetes setaceum*, Tryon and Lugardon (1991, p.18, fig. 54; p. 631, fig. 232.50) showed a detail of an early stage of exospore including "strands of silica" strikingly similar to other connective structures. Similarly, in *Blechnum spicant*, Tryon and Lugardon (1991, p. 6 figs. 7–14; p. 19, fig. 62) showed strands (especially evident in fig. 11) and groups of globules associated with "tapetal wall remnants" and "rodlets partly fused into strands" at the sporangium edge.

Plate II. A young stage of development in a megasporangiun of *Selaginella argentea* with TEM details of the connective structures (i.e. the wicks) between the tapetal cells and the megaspores. (see on page 162)

1. LM photomicrograph showing a megasporangium sectioned adaxially with respect to the megasporophyll and the ligule. Scale bar = 50 μm. Note that the wicks are not visible at this low magnification but that, in fact, they cross the locular space between the tapetum (T) and the exospore of the three large megaspores visible in the middle. Ep = Epidermis; I = intermediate layer.
2. Ultra thin section in TEM showing wicks (W) in the locular space between the tapetal cells (T) and the exospore (E) of a megaspore. Scale bar = 1 μm.
3. Detail of the wicks (W) in TEM. Note their radial and parallel orientations near the exospore (E). Scale bar = 1 μm.

Plate III. TEM images showing connective structures (= wicks) in the locular space and within the exospore of young megaspores in *Selaginella argentea*. (see on page 163)

1. Wicks (W) entering the exospore (E) of the megaspore (arrows) and spreading between its unit structures. Note that the wicks divide into several slender strands and spread throughout the exospore; this is especially well seen at the lower right. Scale bar = 0.5 μm.
2. Detail of the wicks (W). Note that they are rod-like as it is visible in those which are cross-sectioned (circled area) and in those which are inside the exospore (E) (white arrowheads). Moreover note that the wicks not only divide into several components but they are larger within the exospore. Scale bar = 0.1 μm.

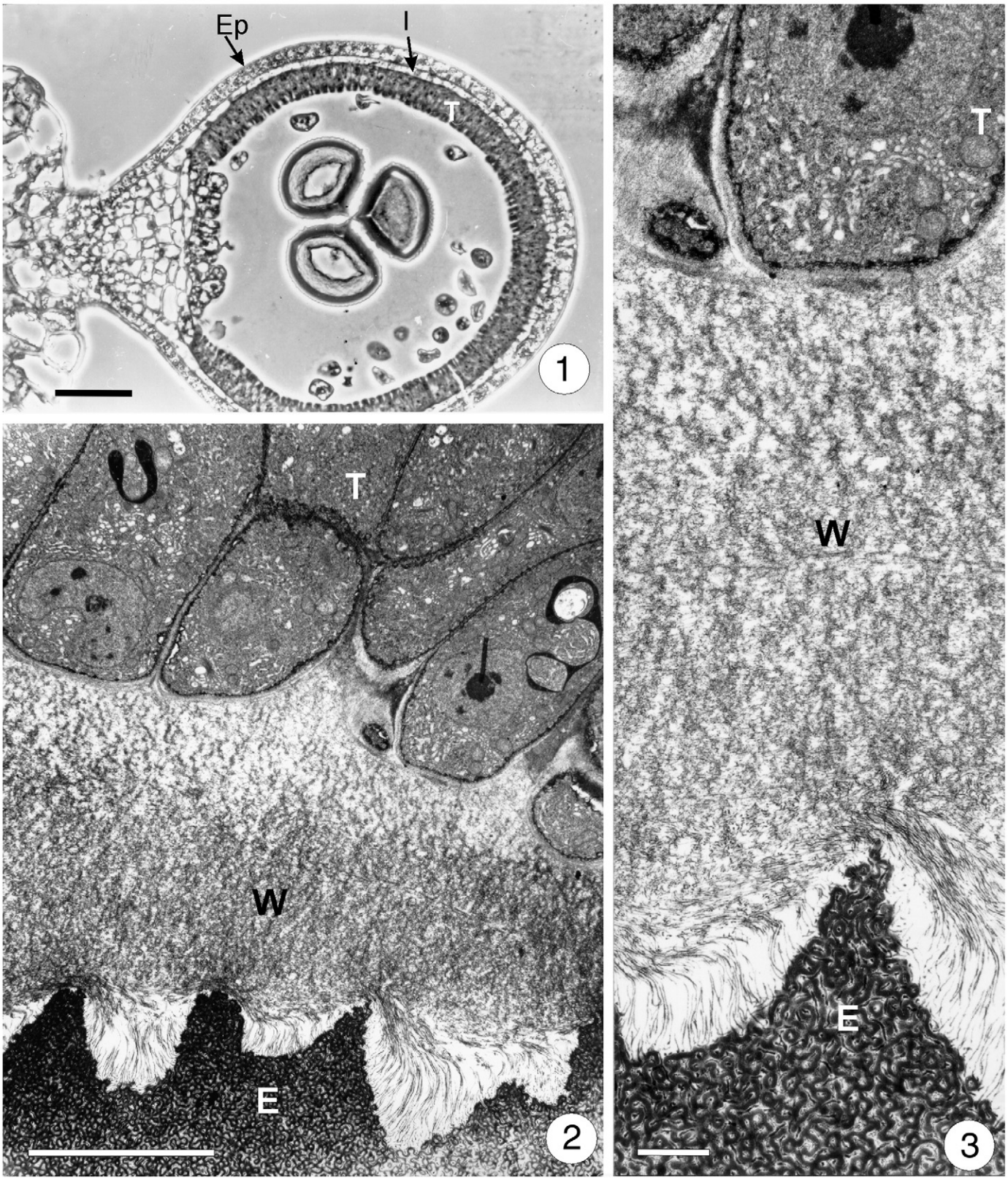

Plate II.

In Angiosperms too, and particularly in *Oenothera biennis*, Takahashi and Skvarla (1990) described interwoven fibrous strings connecting the surface of tapetal cells with the developing ectexine and showed that later those strings developed into viscin threads.

3. Conclusions

During the development of their spores, the megasporangia of *Selaginella* have structures between tapetal cells and the megaspore

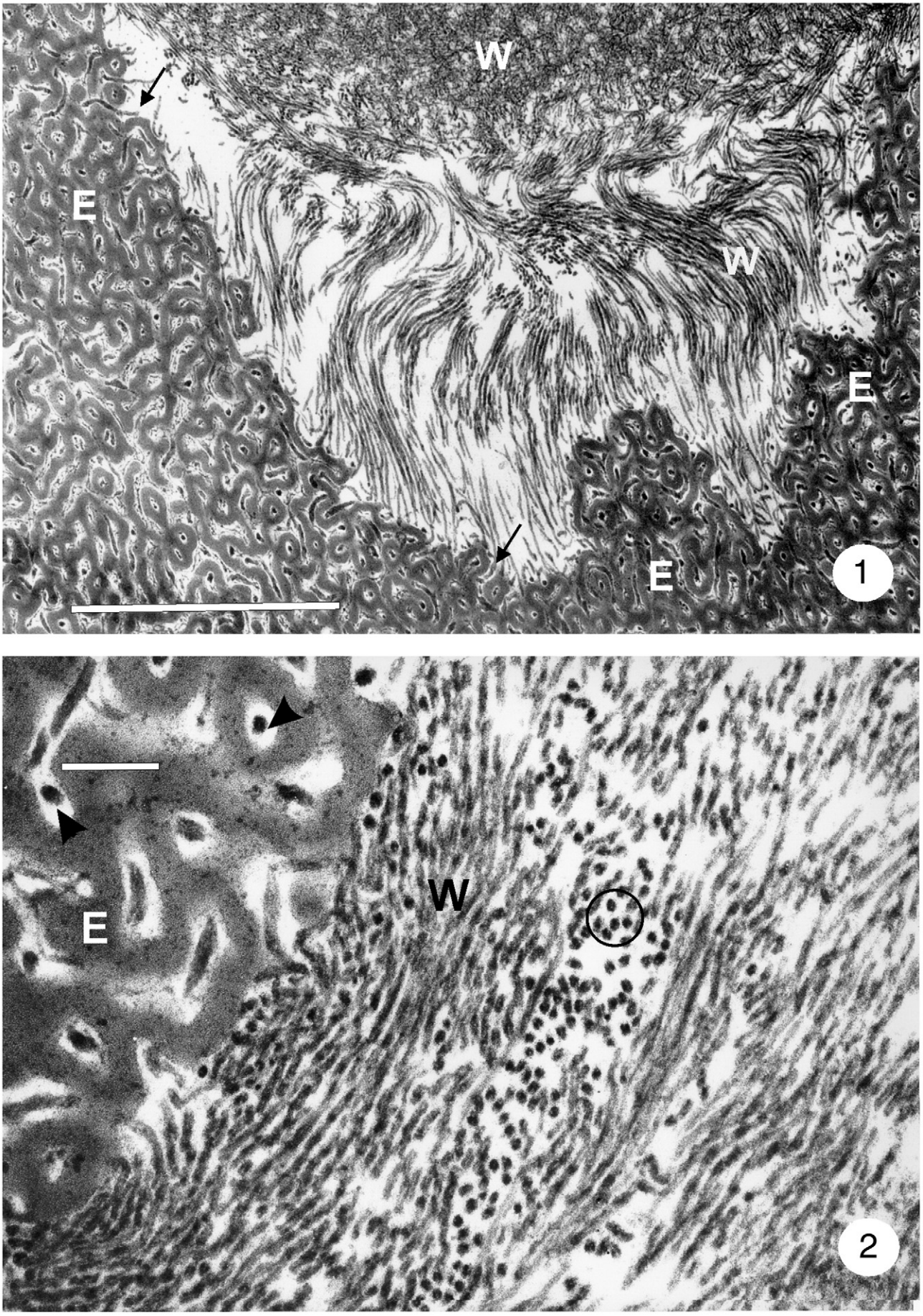

Plate III (*see caption on page 161*).

contents that we called "wicks". These wicks extend from the tapetal cells to and through the megaspore wall (exospore and mesospore), to the plasma membrane and protoplast of the megaspores. We suppose that "wicks" are involved in the transfer of material during megaspore development and especially that of the protoplast. Rowley and collaborators have found plasmodesmatal substructures to be the basic components of the pollen exine columellae, tectum and foot layer.

It is our theory that all or most components of the exine, not just the columellae, consist of the same substructures as plasmodesmata. Thus the well appreciated rapid development and packed contents of pollen grains are due to a vast number of plasmodesmata that literally surround, and actually constitute the exine of each grain.

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