Überreicht vom Verfasser · Nicht einzeln im Buchhandel!
Sonderdruck aus «Zeitschrift für Pflanzenphysiologie», Band 92, Heft 5, Seite 417–430 (1979)
Gustav Fischer Verlag Stuttgart

Institut für Botanik und Mikrobiologie der Technischen Universität München, München, Federal Republic of Germany

Welwitschia mirabilis: Fine Structure of the Germinating Seed VIII. Interface of the Developing Feeder

CHRIS H. BORNMAN, VALERIE BUTLER and WILLIAM A. JENSEN[1])

With 12 figures

Received September 7, 1978 · Accepted December 6, 1978

Summary

Within 36 h of imbibition nucleoli increase in size, long profiles of rough ER form, polysome configurations appear and protein bodies fuse; starch reserves reappear, and dictyosomes and microbodies increase in number. By 48 h mitochondria increase and microbodies decline in number; smooth ER inflates and rough ER blebs; dictyosomes are seemingly active. By day 4 reserve materials have largely disappeared; wall convolutions of the transfer type form in the cells of the feeder's ventral surface; cell walls are mucilaginous and there is strong adherence between feeder and gametophyte. By day 6 the plumule emerges; the feeder is maximally extended and many ventral cells senesce giving a strongly positive acid phosphatase reaction; except for mitochondria, organelles are rare. The anatomy of the feeder suggests its participation in nutrient transfer from gametophyte to embryo.

Key words: Welwitschia, seed, embryo-gametophyte interface, feeder.

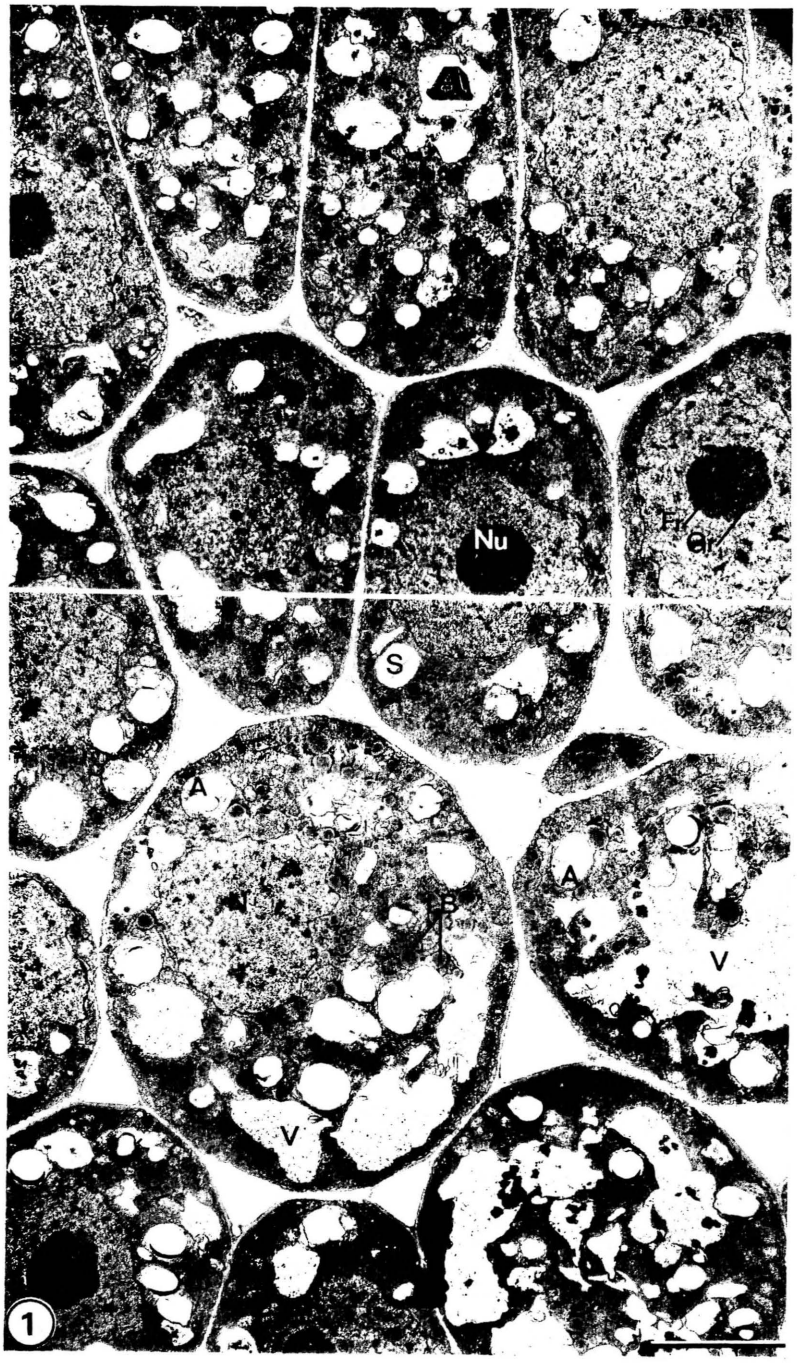
Introduction

In the previous paper (BUTLER et al., 1979 b) we discussed the subcellular changes that occur in the cells of the embryo collar during the first 24 h of imbibition of the *Welwitschia mirabilis* seed. Approximately 36 h following imbibition this collar gives rise to a lateral wedge-shaped, footlike process which eventually will extend to about 5 mm and remain closely attached to the nutritive megagametophyte (see BORNMAN et al., 1979). This lateral process is rightly or wrongly termed a feeder and we report in this paper on the fine structural changes that occur in those of its cells in the immediate feeder-gametophyte interface zone.

Materials and Methods

The procedures were outlined earlier (BORNMAN et al., 1979).

[1]) Department of Instruction in Biology, University of California, Berkeley, California, U.S.A.



Results

36-Hour-Imbibed Collar Cells

Figure 1 is a montage showing surface and deeply-situated collar cells at the 36-hour-imbibed stage. With increased hydration inner or deeply-situated (area B) and outer (area A) collar cells no longer display the initial diversity of the one-day-imbibed embryo noted earlier (Figs. 1 and 2 in BUTLER et al., 1979 b). There has been

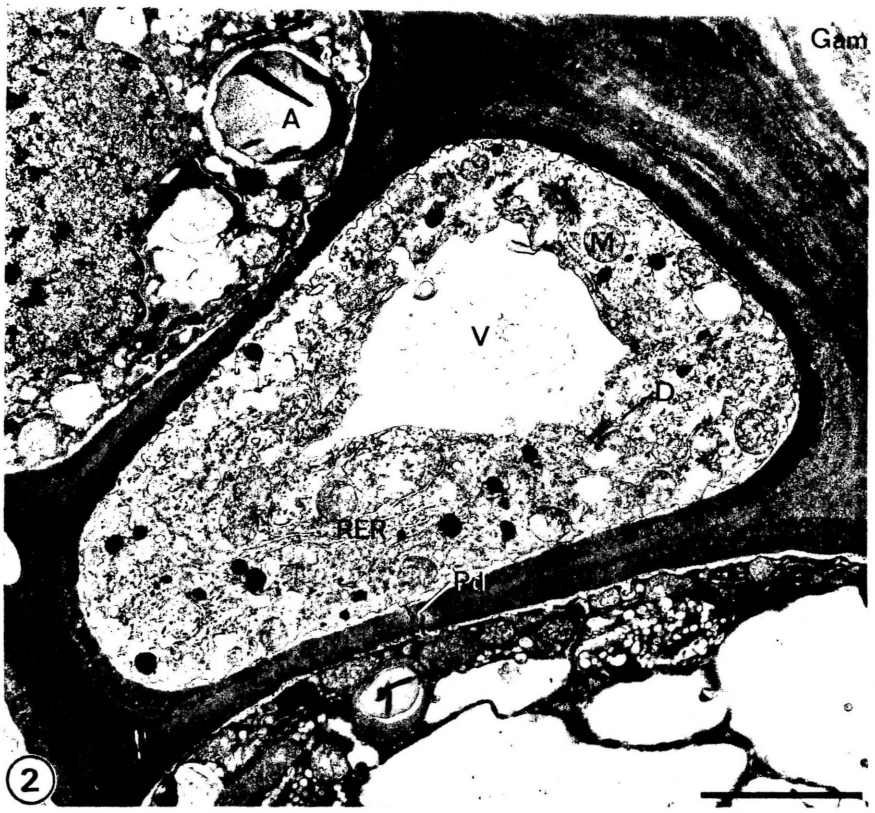


Fig. 2: Surface cells of collar (now referred to as feeder interface cells) in direct contact with gametophyte (Gam). A = amyloplast; D = dictyosome; M = mitochondrion; Pd = plasmodesmata; RER = rough ER; V = vacuole. Bar represents 5 μm (Fig. 2).

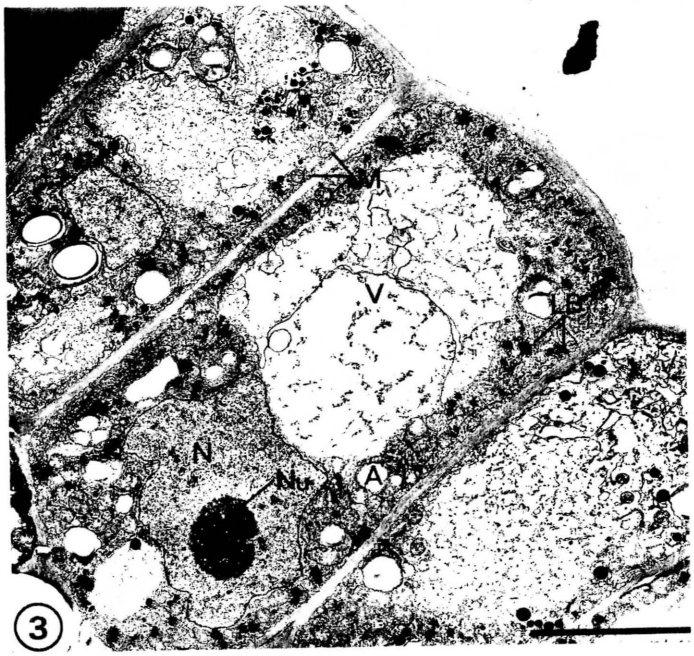
Fig. 1: Montage showing surface (A) and deeply-situated (B) collar cells of the developing feeder 36 h after germination. Cellular activity has increased greatly. Nuclei (N) become irregular. Nucleoli (Nu) have enlarged and the peripheral granular zone (Gr) becomes interspersed in the central fibrillar region (Fr). Fusion of protein vacuoles (V) continues while lipid bodies (LB) decline in number. Starch (S) reserves reappear in the amyloplasts (A). Bar represents 5 μm (Fig. 1).

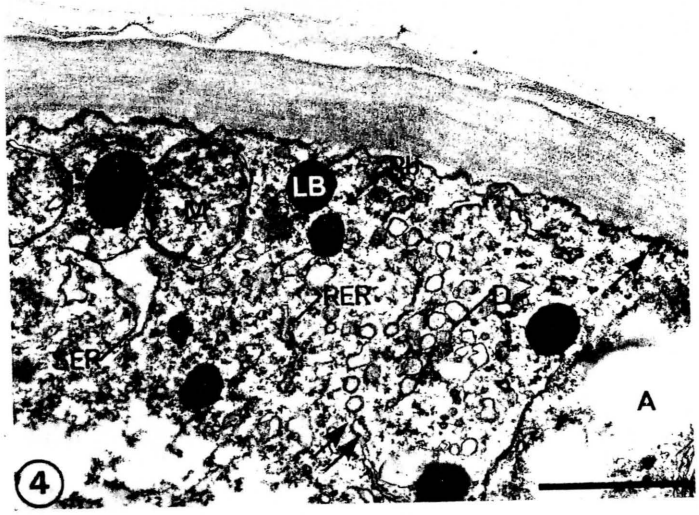
a dramatic increase in cellular activity. While nuclei become slightly irregular, nucleoli increase in size. In each nucleolus the peripheral granular zone enlarges further and appears to become interspersed in the central fibrillar region. Long profiles of rough ER are common and polysome configurations are often identified. Fusion of protein body vacuoles continues and is frequently more advanced in inner as opposed to outer collar cells. Lipid bodies decline in number. They no longer line the plasmalemma and, although some remain in contact with protein body vacuole membranes, the majority occur in the cytoplasm. Concurrent with lipid degradation, starch reserves reappear in the amyloplasts. Mitochondria have lost their original electron-transparency and now contain numerous cristae. Dictyosomes and microbodies have increased in number.

2-Day-Imbibed Feeder Interface Cells

Feeder interface cells (Fig. 2) are usually elongate in transection (ca. 21 × 10 μm). Their nuclei strongly resemble those described for Fig. 1 (36 h) with their irregular contours and large active nucleoli. However, after two days of germination, very little if any chromatin remains apparent in the nucleoplasm. Fairly big vacuoles, formed by the fusion of protein body vacuoles, now occupy large volumes of the cells (Fig. 3). Some lipid persists, but the amount present is much less than that at the 36-hour-stage. The remaining lipid bodies have a scattered distribution in the cytoplasm. Starch grains continue to occupy the amyloplasts. Large numbers of mitochondria, indicative of a high respiration rate, are primarily concentrated along the cell walls (Figs. 3, 4 M). While microbodies decline in number, dictyosomes increase producing vesicles containing fibrillar material which is presumably contributed to the cell walls (Fig. 4). The vesicles migrate towards and fuse with the plasmalemma (Fig. 4, arrow), releasing their contents upon fusion. Occasionally a few vesicles pass through the plasmalemma prior to discharge of their contents (Fig. 5, arrow). Long and short ER profiles are scattered in the cytoplasm. Most are rough but some are smooth. Smooth ER is seen to inflate and rough ER to bleb (Fig. 4, double arrow). Ribosomes exist as mono- or polysomes either free or attached to ER. In the vicinity of lipid bodies, ribosomes stain more darkly (Fig. 4).

Figs. 3, 4: Feeder interface cells after 2 days germination. – Fig. 3. TS of elongate feeder interface cells. Note irregular-shaped nucleus (N) with large active nucleolus (Nu). Vacuoles (V) are characteristically located close to surface cell wall. The remaining lipid bodies (LB) display a scattered distribution and amyloplasts (A) commonly contain starch grains. The numerous mitochondria (M) are primarily concentrated along the cell walls. – Fig. 4: TS of portion of feeder interface cell showing outer cell wall. Mitochondria (M) and dictyosomes (D) are associated with cell wall. Dictyosome – derived vesicles containing fibrillar material migrate towards and fuse with the plasmalemma (arrow). Smooth ER (SER) sometimes inflates and rough ER (RER) may bleb (double arrow). In the vicinity of lipid bodies (LB) ribosomes (Rb) stain more darkly. The outer surface of the cell wall appears to be disintegrating. A = amyloplast. Bars represent 5 μm (Fig. 3), 1 μm (Fig. 4).


V
N
Nu
A
3


LB
M
RER
ER
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4

3-to 6-Day-Imbibed Feeder Interface Cells

Ultrastructurally 2- and 3-day-imbibed feeder interface cells are very similar in appearance. The only significant differences are found in cell wall structure and in dictyosomal activity. The outer cell walls thicken. Whereas the inner surface of the cell wall becomes progressively thicker, the outer surface seems to disintegrate or at least becomes less compact (Fig. 5, double arrow). The beginning of this process is apparent at the 2-day-stage (Fig. 4). Dictyosome activity is prolific and the outer wall becomes very thick and mucilaginous. In the cytoplasm, minor ER dilations become more frequent (Fig. 6, arrows). Approaching the fourth day of germination small wall projections invested with plasmalemma form along the inner surface of the outer wall. Microfibrils are often evident at right angles to the plasmalemma (Fig. 7, arrow). Nuclei remain active in appearance. Apart from a few sporadic lipid bodies, reserve materials have largely disappeared. The cytoplasm contains many mitochondria which vary in size and shape, and dictyosomes which continue to actively proliferate vesicles. The dilations of smooth segments of ER become larger and more frequent and in many instances rough ER leads either into or out of the inflated areas (Fig. 7). Polysomes (Figs. 7, double arrow; 8, Ps) are extremely common.

Figure 8 is a fairly typical feeder interface cell after four days germination. Wall projections with associated plasmalemmal convolutions have increased and the outer wall has thickened considerably assuming a mucilaginous texture. At this stage the feeder adheres strongly to the gametophyte tissue by its ventral surface. The peripheral interface cells of the feeder's ventral surface begin to undergo some cell wall disintegration. They have an overlapping arrangement and degradation usually begins in the middle lamella region at the tips of overlapped cells (Fig. 8) in regions coinciding with areas where acid phosphatase is localised. The cytoplasmic features of interface cells on the fourth day of germination are similar to those described for Fig. 7 (3.5-day-stage). From the fourth day (Fig. 9) mitochondria increase dramatically in number while dictyosome activity diminishes. The entire inner wall surface now possesses wall projections and plasmalemmal convolutions (Figs. 8, 9).

By the sixth day of germination the plumule has usually emerged from the seed. Ventral interface cells near the tip of the feeder remain fairly active (Fig. 10) although many begin to show sings of senescence. Nuclei become increasingly irre-

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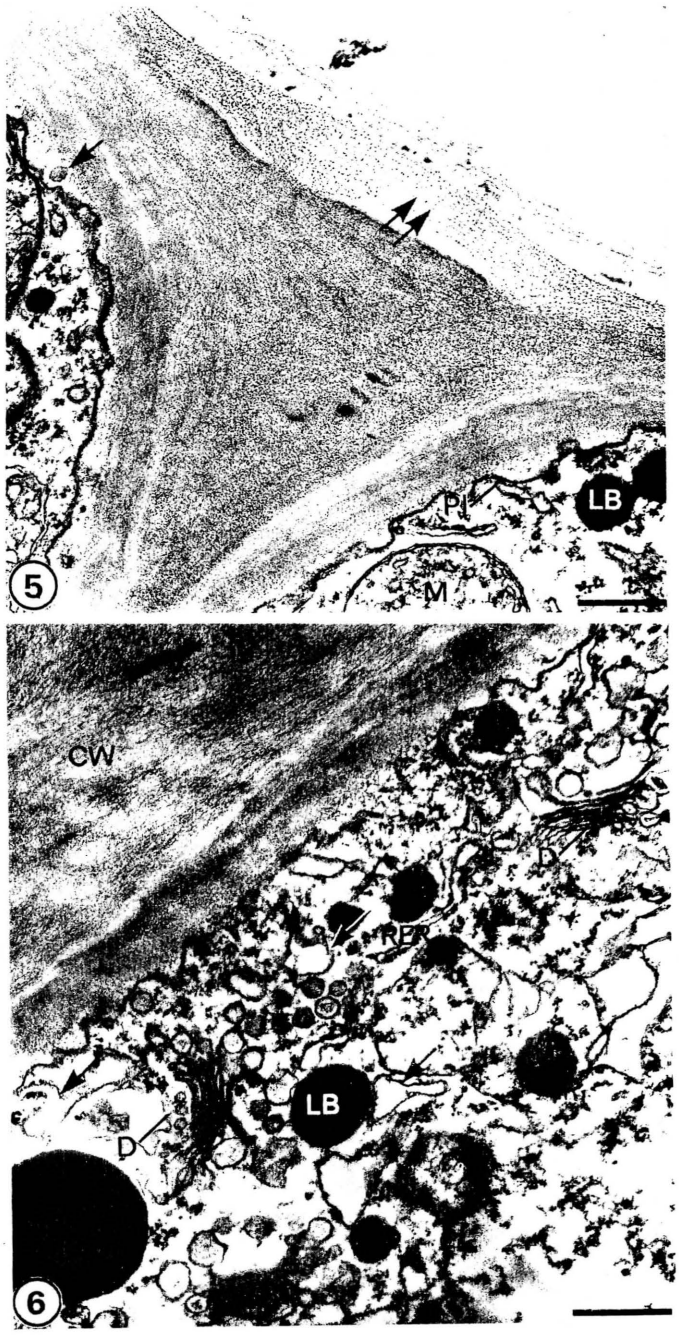
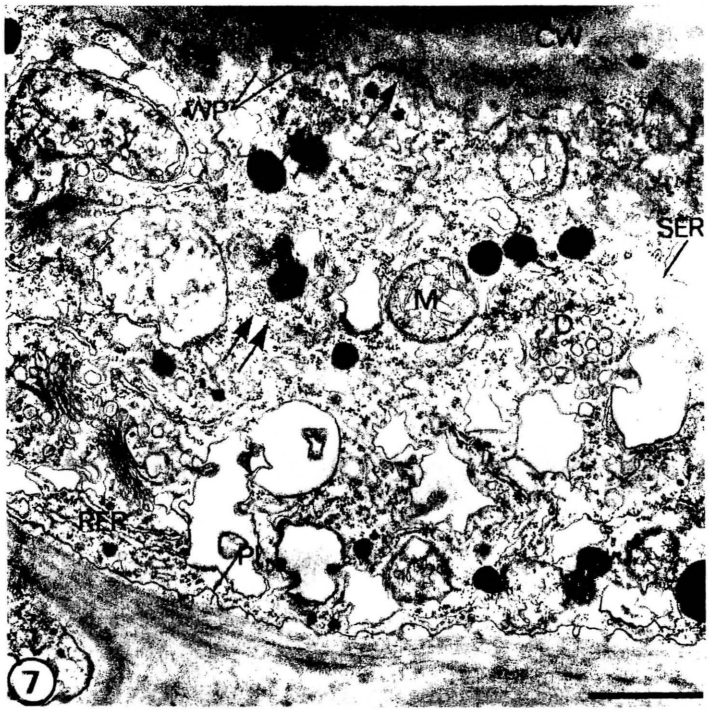
Figs. 5, 6: Feeder interface cells at 3-day-stage. – Fig. 5: Outer cell walls of feeder interface cells thicken. Dictyosome-derived vesicles continue contributing to inner surface of cell walls with vesicles (arrow) sometimes passing through the plasmalemma (Pl) prior to discharge of contents. At the same time the outer surface of the cell wall seems to disintegrate or become less compact (double arrow). LB = lipid body; M = mitochondrion. – Fig. 6: Prolific dictyosome (D) activity results in the outer cell wall (CW) becoming extremely thick and mucilagionous. Minor ER dilations (arrows) become more frequent. LB = lipid body; RER = rough ER. Bars represent 0.5 μm (Figs. 5, 6).


5
Pl
LB
M
6
CW
D
RER
LB
D


CW
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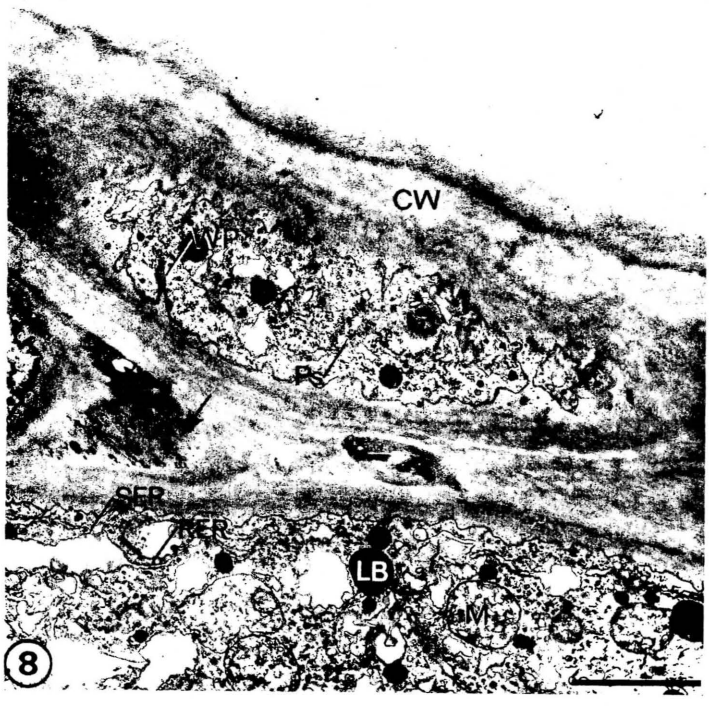
CW
P
SER
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8

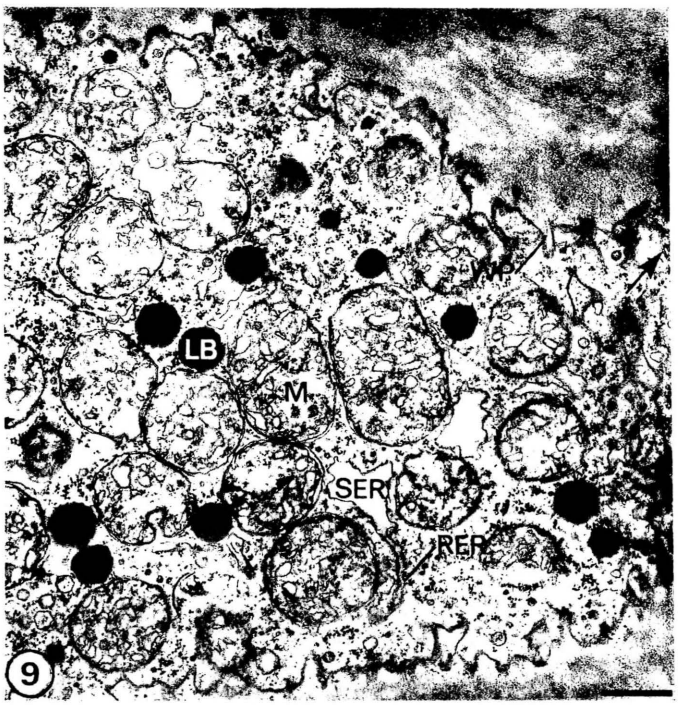
gular and ER continues to dilate. Polysomes are conspicuous (Fig. 10) but not profuse. Amyloplasts and microbodies are rare and dictyosome activity has apparently waned. Mitochondria are still numerous (Fig. 10) but a large number are beginning to swell and develop electron-transparent areas. From Fig. 10 it is apparent that many small vesicles (Ve) are discharged and accumulate between the plasmalemma and cell wall. They are unlike the previously described dictyosome-derived vesicles in that they do not contain fibrillar material. Figure 11 shows 6-day-stage interface cells situated near the base of the feeder. Comparison of Figs. 11 and 10 clearly illustrates that senescence accelerates from the tip (Fig. 10) to the base (Fig. 11) of the wedge-shaped feeder. Disintegration in the middle lamella region between interface and adjacent cells, first noticed in the 4-day-imbibed cells has progressed further in the 6-day-imbibed stage (Fig. 11). The condition of the cytoplasm of these interface cells (Fig. 11) reflects senescence: mitochondria are swollen and ER and dictyosomes are dilated; the protoplast of the lower interface cell in Fig. 11 has lost turgor and has shrunk away from the wall; the tonoplast has broken and the cytoplasm appears dilute; and at the extreme base of the feeder peripheral cells have senesced completely and become crushed against the gametophyte (Fig. 12, 6-day-imbibed, bracket). These cells, and cells in the process of degeneration, give a strong positive reaction for acid phosphatase (BUTLER et al., 1979 a).

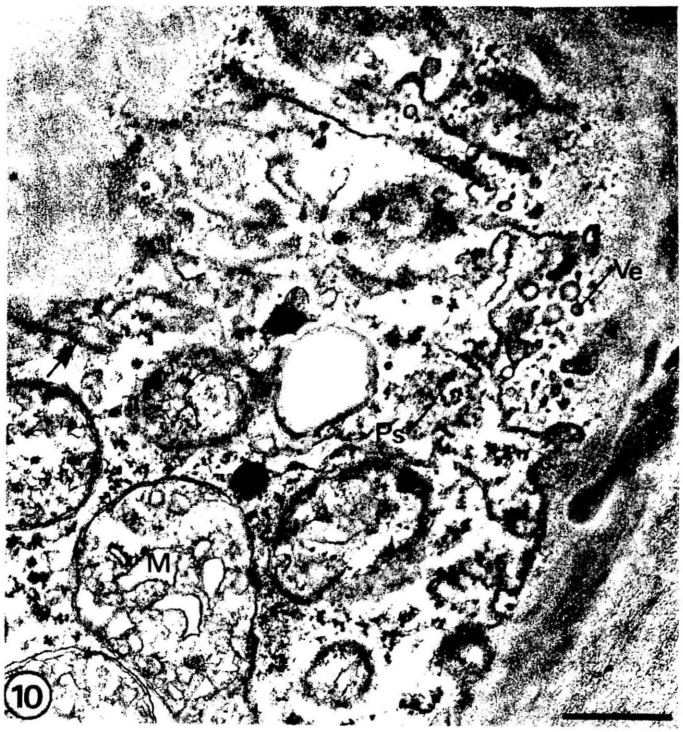
Discussion

Nucleus

Upon hydration collar nuclei are rapidly activated, with surface contours assuming slight irregularities as metabolic activity intensifies. While the irregularities are noticeable they are not as pronounced as the convolutions common to gametophyte nuclei. After approximately 4 to 5 days nuclei of feeder cells in contact with the gametophyte become increasingly irregular. While initial irregularity in nuclear contours is probably associated with heightened cellular activity, ultimate accentuation of these nuclear lobes seems to be connected with senescence.

Fig. 7: LS of feeder interface cell after 3.5 days germination. Small wall projections (WP) invested with plasmalemma (Pl) have begun forming along inner surface of outer cell wall (CW). Microfibrils (arrow) are evident at right angles to plasmalemma. Reserve materials have largely disappeared. The cytoplasm contains many mitochondria (M) and active dictyosomes (D). Dilation of smooth segments of ER (SER) become larger and more frequent. Note rough ER (RER) either leading into or out of inflated areas. Polysomes (double arrows) are common. – Fig. 8: LS of feeder interface cell after approximately 4 days germination. Wall projections (WP) with associated plasmalemma convolutions have increased. Outer cell wall (CW) is thick and mucilaginous. Peripheral interface cells have an overlapping arrangement. Some cell wall degradation begins in the middle lamella region at the tips of overlapped cells (arrow). LB = lipid body; M = mitochondrion; Ps = polysomes; RER = rough ER; SER = smooth ER. Bars represent 1 μm (Figs. 7, 8).


LB
M
SER
RER
9


Ve
Ps
M
10

Protein and Lipid Bodies

Protein body reserves are rapidly degraded leaving numerous small aqueous vacuoles which fuse to form a single large vacuole, characteristically located close to the outer interface wall. At the 3- to 4-day-stage interface cells near the base of the feeder begin to senesce. Breaks occur in vacuole membranes leading to the release of acid phosphatase and presumably other hydrolytic enzymes. Approximately 3 to 4 days after germination the protein and lipid stores have been greatly reduced. At this stage the plumule is still enclosed within the seed and is therefore incapable of photosynthesis. However, the feeder is developing rapidly and it is believed that breakdown products from the reserves of the gametophyte are absorbed by or via the feeder.

During the next 12 h much of the starch is replaced as collar lipid reserves are hydrolysed. As the feeder develops starch reserves fluctuate but are usually a constant cell feature. A large percentage of the starch present in the mature feeder is probably formed *de novo* as the result of mobilisation and transport of sugars from the gametophyte into the embryo.

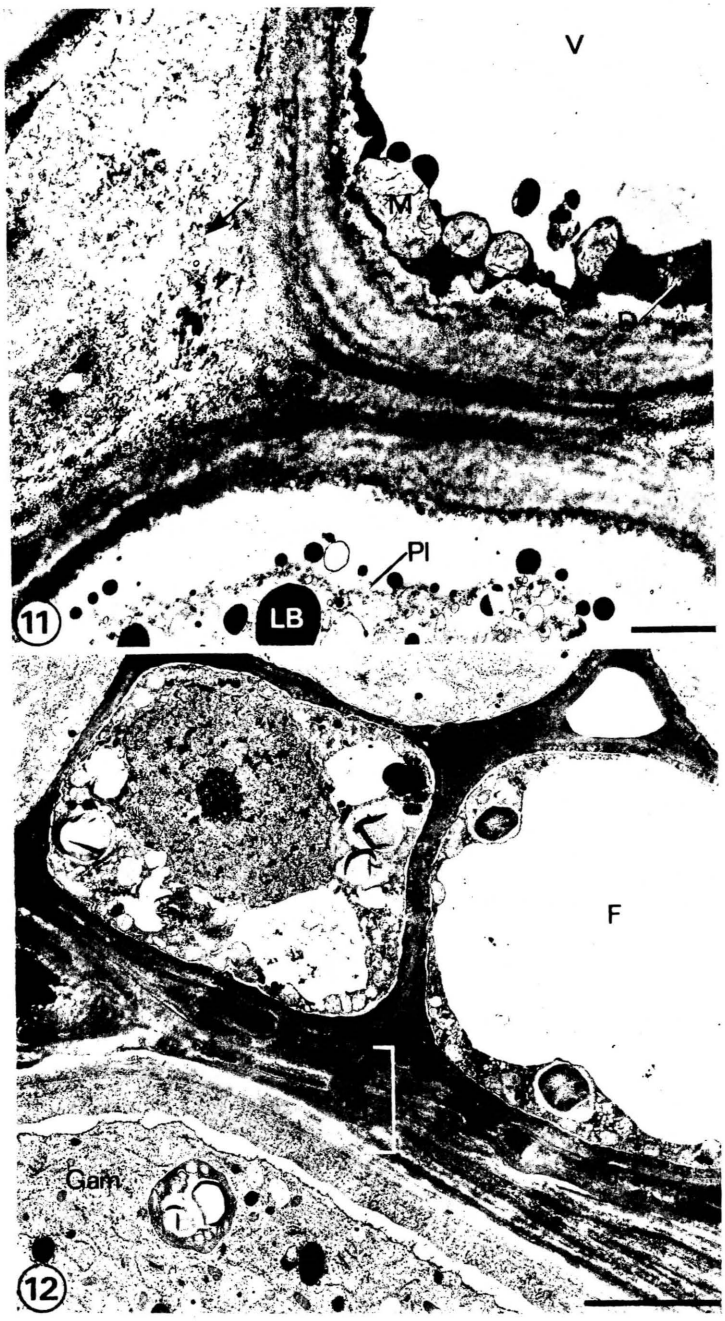
Microbodies

Microbodies are rare in dry collar tissue. Upon hydration they multiply during the first 24 h, but decline in number between 24 and 48 h. The build-up of the microbody population parallels the rapid decline of lipid stores observed during the early stages of cellular activity following imbibition. Forty-eight hours after germination lipid stores are greatly depleted and the microbody population has dropped. The close association of microbodies with lipid bodies and their corresponding increase as lipid stores decline suggests that they might belong to the class of microbodies known as glyoxysomes (BREIDENBACH et al., 1968).

Mitochondria

Randomly distributed mitochondria, containing an electron-transparent matrix and very few cristae are present in the cytoplasm. Between the second and fourth day of germination the mitochondria increase greatly in number especially in the feeder interface cells in contact with the gametophyte (Figs. 3, 9). In these interface cells the mitochondria are primarily concentrated along the cell walls which, at the 4-day-stage, have usually developed many small wall projections.

Fig. 9: LS of portion of feeder interface cell after 4 days germination. The entire inner wall surface possesses wall projections (WP) and plasmalemma convolutions. Note microfibrils (arrow). Mitochondria (M) have increased in number. LB = lipid body; RER = rough ER; SER = smooth ER. – Fig. 10: LS of portion of interface cell near tip of feeder after 6 days germination. Mitochondria (M) are still numerous and polysomes (Ps) are conspicuous. Many small vesicles (Ve) have accumulated between plasmalemma and cell wall. Unlike previously described dictyosome-derived vesicles (Figs. 4, 6) they do not contain fibrillar material. Note microfibrils (arrow). Bars represent 1 μm (Fig. 9), 0.5 μm (Fig. 10).


V
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Pl
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11
F
Gam
12

Dictyosomes

In common with many previous studies (VARNER and SCHIDLOVSKY, 1963; PALEG and HYDE, 1964; YATSU, 1965; PAULSON and SRIVASTAVA, 1968; ABDUL-BAKI and BAKER, 1973) dictyosomes are unrecognisable in the dry embryo. They appear initially on the first day of imbibition, increasing greatly in number between the 2- and 3-day-stage. In interface cells dictyosomal activity is prolific between the 2- and 4-day-stages. Dictyosome-derived vesicles containing fibrillar material migrate toward and fuse with the plasmalemma. The released fibrillar contents are contributed particularly to the outer cell walls.

ER

Except for short segments, ER is first observed in profile approximately 36 h after initial imbibition. Its prompt proliferation and formation of polysomes supposedly indicate the synthesis of enzymes and structural proteins. Rough and smooth ER are evident in cells of the interface zone at the 2-day-stage. They may occur as separate profiles or may interlead. From the second day onward smooth ER inflates and rough ER blebs. Approaching the fourth day of germination inflation of smooth ER becomes more frequent with rough ER invariably leading into and out of the localised dilations. Unidentified membranous fragments are frequently found within the inflations which apparently are autophagically active. These dilations may have a lysosomal function (BERJAK and VILLIERS, 1970). As outer interface cells senesce, the ER becomes increasingly dilated.

Cell Walls

In the dry state collar cells are thin-walled (0.2 μm). From imbibition until the 4-day-stage feeder interface cell walls thicken, particularly the outermost walls (0.9 μm) in contact with gametophyte tissue. After four days germination wall projections with associated plasmalemma convolutions cover the entire inner wall surface of the cell. These projections, together with the previously mentioned overlapping nature of the outer cells, result in a much greater absorptive surface area. Although the wall projections are not as pronounced as the transfer-type wall configurations described by PATE and GUNNING (1972), these cells probably function as transfer cells with the wall projections facilitating absorption and/or secretion of substances. The large numbers of mitochondria occurring in these cells argues for an active uptake of nutrients via the feeder.

Fig. 11: 6-day-stage interface cells near base of feeder. The condition of the cytoplasm reflects senescence. Organelles are swollen, the protoplast has lost turgor and, in the lower cell, has shrunk away from the wall. The tonoplast has also broken and the cytoplasm appears dilute. Note disintegration in middle lamella region (arrow) between interface and adjacent cells. D = dictyosome; LB = lipid body; M = mitochondrion; Pl = plasmalemma; V = vacuole. – Fig. 12: Interface between gametophyte (Gam) and extreme base of feeder (F) at 6-day-stage. Peripheral feeder cells have senesced and become crushed against gametophyte (bracket). Bars represent 1 μm (Fig. 11), 5 μm (Fig. 12).

Interface cell walls thicken as a result of dictyosomal activity, but while fibrillar material is contributed to the inner surface of the cell walls, the outer part seems to disintegrate or become less compact. At the 4-day-stage the thick outer walls become mucilaginous and it is perhaps due to this that the feeder and gametophyte adhere so firmly. With the gametophyte and feeder adhering firmly together, the transfer of nutrients could be facilitated. The adherance might also have a secondary function: the anchoring of the feeder in the gametophyte tissue, providing the plumule with a firm base as it emerges from the seed. The overlapping of outer interface cells coupled with their relatively short life span and development of thick mucilaginous walls led us to suspect that these cells might be of root cap origin. Light microscopical investigation of a 7 h-imbibed embryo (BUTLER, 1970) revealed a root tip protected by a massive root cap that telescopes backwards until approximately to the midpoint of the collar. If these cells are in fact root cap cells, this could explain the positive geotropism of the feeder.

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Prof. Dr. CHRIS H. BORNMAN, Department of Plant Physiology, University of Lund, Fack, S-220 07 Lund, Sweden.