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The role of the intine and cytoplasm in the activation and germination processes of Poaceae pollen grains

JESUS MARQUEZ, JUAN A. SEOANE-CAMBA and MARIA SUAREZ-CERVERA

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Ultrastructural modifications of the intine and cytoplasm, during the maturation, activation and germination processes are described for several Poaceae pollen grains. Allergenic and antigenic proteins were found in the non apertural intine during the times of activation and germination, using TEM immunolabelling. This fact may be related to the function of the non apertural intine during the processes of pollen activation and pollen tube formation prior to fecundation. Changes in the granular particles of the cytoplasm are described and their role in pollen wall development is suggested. The pectic-cellulosic and callosic layers of the pollen tube were formed on the degraded intine, and a relationship between pollen tube wall development and the substances expelled from the fibrillar particles was observed. The immunolabelling of the starch granules may be in agreement with their role in the allergenic process.

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The free amino-acids, proteins and enzymes contained in pollen grains are found in the cytoplasm and in the inner (intine) and outer (exine) wall of the pollen grain. They are essential for pollen-stigma recognition, formation of the pollen tube and its passage through the style in order that fertilization may take place (Knox 1984). Immunocytochemical techniques using TEM have revealed the location and recognition of the antigens of some pollen grains, principally those of Poaceae (Knox et al. 1970, Knox & Heslop-Harrison 1971, Howlett & Clarke 1981, Vithanage et al. 1980, 1982, Abadie et al. 1988, Staff et al. 1990, Marquez et al. 1992, Grote et al. 1994, Fischer et al. 1996), Cupressaceae (Southworth et al. 1988, Miki-Hirosige et al. 1994), Betulaceae (Grote 1991, Grote et al. 1993, El-Ghazaly et al. 1996, Emilson et al. 1996) and Urticaceae (Casas et al. 1996).

Recently Marquez et al. (1997) used activated Poaceae pollen grains to show intine modifications and immunolabelling of proteins in the apertural region. The aim of this work is to study the non apertural intine and cytoplasm modifications in pollen grains of Poaceae, activated and germinated *in vitro*.

MATERIAL AND METHODS

Plant material

Young and mature anthers from *Paspalum dilatatum* Poir., *Digitaria sanguinalis* (L.) Scop. and *Lolium perenne* L. were collected on the campus of the Faculty of Pharmacy, University of Barcelona. Activation of mature anthers of *Lolium perenne* was induced *in vitro* in a wet chamber, for 5, 10 or 30 min at room temperature. After this time the process was interrupted and the anthers were removed from the wet chamber and fixed. Maize (*Zea mays* L.) inbred lines

Ky21 and B73 were grown under standard greenhouse conditions at Colorado State University. Mature pollen was collected from shedding tassels. For *in vitro* germination, pollen was collected from freshly exerted anthers and sprinkled onto a 0.7% agar medium with 0.35 M sucrose, 300 mg/l CaCl₂, and 100 mg/l boric acid (Walden 1993).

Fixation and embedding: Young, mature, activated anthers and germinated pollen grains were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer for 12 hours at room temperature. They were washed in 0.1 M cacodylate buffer for 30 min and postfixed with 1% osmium tetroxide in 0.8% phosphate buffered K₃Fe(CN)₆ for 7 hours at 4°C (Weber 1992a,b). This was followed by dehydration in acetone and embedding in Spurr's resin. Sections of the blocks containing the samples were obtained using a Reichert – Jung Ultracut E, and stained with uranyl acetate and lead citrate. For localization of neutral polysaccharides, sections were treated by the periodic acid thiocarbonyl silver protein (PA-TCH-SP) method (Thiery 1967). To detect total lipids we used the osmium-thiocarbonyl silver-osmium (OTO) technique (Seligman et al. 1966). The Thiery test was used without periodic acid oxidation for the detection of unsaturated lipids (Rowley & Dahl 1977).

Human sera

Sera from allergic patients with specific immunoglobulin E (IgE) were supplied by the Allergy Service of the Santiago Apostol Hospital in Vitoria. The specific IgE of the patients against a *Lolium* extract was 100, 97, 31 kU/l. Total IgE was 1327, 1049 and 94 KU/l (Pharmacia CAP system IgE FEIA: 10.39–40.05 kU/l is considered high). Serum from a non-allergic subject with negative specific IgE and the total IgE <100 kU/l was used as a control. The sera were stored in Eppendorf tubes at –40°C.

Immunogold labelling

For detection of allergens (IgE), ultrathin sections were incubated at room temperature on drops of 5% bovine serum albumin (BSA)

in 0.1 M phosphate buffered saline (PBS) for 15 min, followed by sensitized patient sera diluted 1/100 in 0.1 M PBS for 2 h. After three 10 min washes, sections were incubated for 1 h with a second antibody bridge, anti-human IgE developed in a goat (Sigma), diluted 1/40 in PBS. After three 10 min washes with drops of 0.1 M PBS, sections were incubated for 1 h with the antibody conjugated to 10 nm diameter colloidal gold particles, anti-goat IgG developed in a rabbit (Bio Cell), using a 1/60 dilution in PBS. This was followed by three washes with drops of 0.1 M PBS for 5 min, two washes with distilled water, and air drying. For the control of specific patient antibody the patient sera were replaced by non-allergenic subject serum. In the control for no specific binding of the antibody conjugated to colloidal gold, the patient sera and the anti-human IgE were omitted. For the control for non-specific binding of the bridge antibody, the patient sera were omitted.

For detection of antigens (IgG), ultrathin sections were incubated at room temperature on drops of 5% BSA in 0.1 M PBS for 15 min, followed by sensitized patient sera diluted 1/100 in 0.1 M PBS for 2 h. After three 10 min washes, sections were incubated for 1 h with the antibody conjugated to 10 nm diameter colloidal gold particles, anti-human-IgG developed in a goat (Amersham), using a 1/60 dilution in PBS. This was followed by three washes with drops of 0.1 M PBS for 5 min, two washes with distilled water, and air drying. For the control of specific patient antibody, the patient sera were replaced by non-allergenic subject serum. In the control for non-specific binding of the antibody conjugated to colloidal gold, the patient sera were omitted.

Finally, the sections were stained with uranyl acetate for 15 min and lead citrate for 5 min and observed in a Philips EM200 and 301 at 60 and 80 kV.

RESULTS

Young pollen grains

Paspalum dilatatum, *Digitaria sanguinalis* and *Lolium perenne*

Exine. – The ultrastructure of the ectexine was similar in the three species studied, with complete and spinulate tectum, short, non ramified columellae, and a foot layer. The presence of more microchannels in the tectum than in the foot layer was a general characteristic (Figs. 1–4). At this developmental stage abundant exchange of materials through these structures was observed; the microchannels were full of fibrillar material from the tapetum cells or the Ubisch body, and penetrated into the pollen wall (Figs. 1, 3, 4). Only traces of endexine lamellation were observed in the inner part of the exine.

Intine. – The first deposit corresponds to the outer layer (exintine or Z-layer), continues with the middle layer and finally with the inner layer (endintine). The exintine (Z-layer) developed into a thin and fibrillar layer (Fig. 1). The large, loose and channelled middle intine layer was deposited next (Fig. 2). Later the third intine layer (endintine) was formed by the undulated plasmalemma (Fig. 5).

Cytoplasm. – A high degree of cytoplasmic activity was observed with numerous mitochondria, ribosomes, endoplasmic reticulum and dictyosomes. The Golgi vesicles were clearly deposited near the plasmalemma and were expelled by exocytosis into the periplasmic space to form the exintine, which was found pressed against the exine (Fig. 1). The deposition of successive intine layers (middle intine and endintine) corresponds to a strong digitalization of the

plasmalemma (Figs. 2, 5). At this point the plastids were very active and a large number of starch grains were formed which tend to be located around the aperture. In addition, abundant vesicles containing polysaccharide material and with a reticulate appearance, were extruded in the endintine close to or in contact with the plasmalemma (Fig. 5).

Ubisch bodies. – These were completely developed and connected by a sporopollenic orbicular-tapetal wall. These bodies have a central core with moderate electrondensity, and were surrounded by sporopollenin-like material with numerous microchannels similar to the exine. Abundant tapetum material was deposited in the tectum of the pollen grains, through the Ubisch bodies (Figs. 3, 4).

Ripe pollen

Paspalum dilatatum, *Digitaria sanguinalis* and *Lolium perenne*

Exine. – The structure was similar to that of the previous stage (Fig. 7). The passing of materials from the tapetum through the microchannels had finished (Fig. 6). The electron-lucent pollenkit was observed in the exine and between the columellae (Figs. 15, 16). The rudimentary endexine was difficult to observe.

Intine. – A well developed and three layered intine was observed (Fig. 9): the exintine or Z-layer, formed by a thin, fibrillar, granular and compacted layer; the wide middle intine formed by a loose, fibrillar and channelled layer; and the fibrillar endintine with thin fibrils. This intine contains a high concentration of polysaccharides (Fig. 9), only an isolated lipid reaction were observed (Fig. 8).

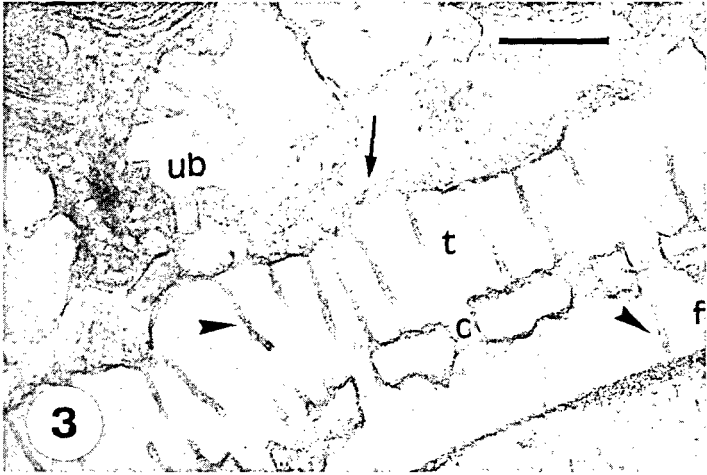
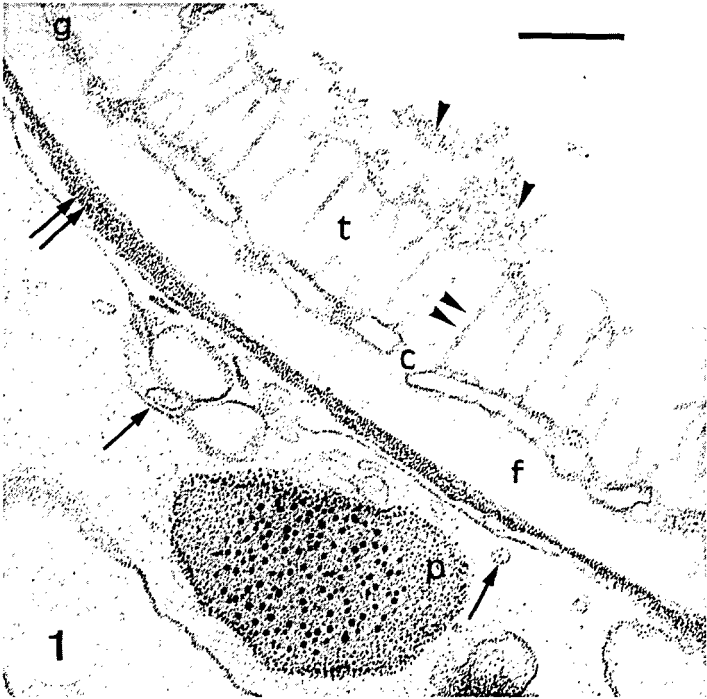
Cytoplasm. – At dehiscence the pollen grains were trinucleate (Fig. 11). Close to the aperture the starch granules were numerous and granular particles were abundant throughout the cytoplasm (Fig. 6). At this time these particles were spherical, granular and surrounded by a membrane (Figs. 6–9). Granules with a compacted electrondense appearance were also observed near the particle membrane (Fig. 9). These particles (referred to as granular particles) were faintly stained in the polysaccharide and lipid tests (Figs. 8, 9). Frequently these particles were located near the plasmalemma and appeared to be fused together (Fig. 9).

Ubisch bodies. – These remain intact and connected to each other with an orbicular-tapetal layer. The core of the Ubisch bodies was still present and was separated from the sporopollenin layer by a thin membrane (Fig. 10).

Labelling. – Scarce and no significant labelling was observed.

Activated pollen grains

***Lolium perenne* and *Zea mays*.** – Water absorption causes a significant increase in intine thickness (Fig. 11). The structure also undergoes a series of modifications: the exintine (Z-layer)



and endintine was diffuse; the middle intine was disorganized and had a loose appearance, with the electrondense granules moving to the outer part (Figs. 15–17). These granules were apparently expelled through the foot layer and the tectum microchannels (Fig. 17). In the cytoplasm the granular particles form evaginations with a fibrillar appearance. These evaginations (or gemes) increase in size, lose their membranes and fuse with the next ones forming new particles (fibrillar particles) not delimited by a membrane (Figs. 12, 13). At this point the granular particles are mixed with the fibrillar particles which have a variable form and size (Figs. 12, 13). All particles (granular and fibrillar) react faintly in the tests for polysaccharides and lipids (Fig. 13). Starch granules are also abundant (Figs. 12, 13, 20, 21).

Significant labelling was observed in the hydrated intine (Figs. 15–17). Labelling shown by the antigenic proteins (IgG) was more abundant than that shown by the allergenic proteins (IgE). The IgE labelling presented a tendency to form groups and was more abundant in the exudates material than the IgG (Figs. 15–17, 18, 19, 22). In the cytoplasm the starch granules were labelled by the IgE, but not by the IgG (Figs. 20, 21). The labelling in the mitochondria was significant (Fig. 18) but no specific labelling in the granular and fibrillar particles was observed (Figs. 17, 18, 20, 21). Significant labelling in the anther cell walls and pollenkitt was also observed (Figs. 15, 19). No significant labelling was observed in the Ubisch bodies (Fig. 19).

Germinated pollen grains

Zea mays

Early germination. – The intine was strongly disorganized (Figs. 23–25). Abundant exudates were expelled mainly through the exine microchannels. These channels may be full of electrondense material, similar to that on the outside of the pollen grain, which has been transported from the intine at the moment of maximum exudation (Fig. 24). These exudates were deposited outside in the tectum surrounding the spinules and were released later (Fig. 25).

At this stage all particles have a fibrillar appearance (Fig. 14). They are abundant near the plasmalemma which expels its fibrillar material into the disorganized intine (Figs. 23, 24). The plasmalemma was well structured and only disappears in the exocytosis region (Fig. 23). These materials will form the new pollen tube wall. The deposition of the pectic-cellulosic pollen tube layer was observed inside the intine. It appeared as a thin, undulated, fibrillar and granulated layer (Fig. 25).

At this time significant labelling was observed in the inner

part of this fibrillar and granulated pollen tube layer and scarce labelling in the exudates by IgG (Fig. 25). No significant labelling was observed by IgE.

Germination. – The pollen tube wall was formed in the place where the intine was being degraded (Fig. 27, 29). Close to the aperture the pollen tube wall, inside the pollen grain, was undulated and had two layers: the inner, callosic layer was faintly electrondense, and the outer, pectic-cellulosic layer was electrondense and fibrillar, similar to that of the pollen tube wall (Fig. 29). Disorganized cytoplasm was seen in the pollen grain at this time (Fig. 29). The same fibrillar particles observed in the activated and early germinated pollen grains were now in the pollen tube with their fibrillar contents condensed in the central part (Fig. 28).

The immunolabelling by IgG was abundant and localized in the callose pollen tube wall (Fig. 26). No significant labelling was observed by IgE.

DISCUSSION

Exine

The Poaceae pollen grains are morphologically similar to each other, only *Pariana* pollen grains have a different structure (Salgado-Labouriau et al. 1993). In our material, the ultrastructure of the exine was similar in the four species studied. *Triticum* seems to have a greater number of microchannels in the tectum compared to other genera of the family (El Ghazaly & Jensen 1985, 1986) and similar to those observed in *Paspalum*. The presence of more microchannels in the tectum than in the foot layer was a general tendency that has also been observed in other Poaceae (*Poa*, Rowley 1962, 1963, 1964; *Zea*, Skvarla & Larson 1966; *Sorghum*, Christensen et al. 1972, Christensen & Horner 1974; *Secale*, Heslop-Harrison 1979a; *Triticum*, El Ghazaly & Jensen 1985, 1986, 1987; *Zea*, *Avena* and *Dactylis*, Peltre et al. 1987). The presence of microchannels in the exine of the pollen grains with a complete tectum has also been described for other taxa and for diverse families such as Onagraceae (Rowley 1973), Betulaceae (Dunbar & Rowley 1984, Rowley et al. 1987) and Chenopodiaceae (Rowley et al. 1987). These microchannels, which are distributed throughout the tectum, including the apertural sporoderm and the operculum, have been considered by other authors as adaptive mechanisms for the interchange of materials from the inside to the outside of the pollen grain (Rowley 1973, El Ghazaly & Jensen 1985). In our material, at the moment of intine formation, fibrillar material filled the exine and Ubisch body microchannels, and we were able to verify the possible exchange of materials through these structures. In the activated and early

Figs. 1–5. Young *Paspalum dilatatum* pollen grains. (1) Thiéry test. Deposit of exintine or Z-layer (double arrows). Golgi vesicles near the plasmalemma (arrows) and polysaccharide inclusions (P). Tectum (t), columellae (c), and foot layer (f). Note the microchannels (double arrowheads) filled with fibrillar material from the tapetum cells (arrowheads). Bar=0.25 µm. (2) Thiéry test. The exintine (Z-layer) was developed in a thin and fibrillar layer (arrow). The large, loose and channelled middle intine layer was deposited next (i₂). Note the dictyosome (g) and their vesicles (arrow) near the undulated plasmalemma. Bar=0.25 µm. (3) Abundant tapetum material (arrow), filled the microchannels (arrowheads). Ubisch body (ub), tectum (t), columellae (c), foot layer (f). Bar=0.25 µm. (4) Ubisch body (ub) in contact with the tectum (t). Note Ubisch body channels similar to those in the exine (arrows). Bar=0.25 µm. (5) Thiéry test. The third intine layer (endintine) was formed (arrow). The abundant fibrillar and polysaccharide vesicles (v) near the plasmalemma. Numerous starch granules (s). Ubisch body (ub) in contact with the exine (e). Bar=0.5 µm.

germinated pollen grains abundant exudates were expelled through these microchannels. Channels were observed which were full of electrondense material, similar to the material on the outside of the grain, which had been transported from the intine at the moment of maximum expulsion. According to our observations there is clear relationship between these exudate materials and the materials of the degraded intine. When the germination process begins, exudations end and the highest number of microchannels are empty or nearly empty.

Intine

The deposition of successive layers of intine corresponds to a strong digitalisation of the plasmalemma. This mechanism was very similar to that observed in *Triticum* (El-Ghazaly &

Jensen 1986, 1987). In *Lolium* the deposition of the three intine layers is consistent with the general intine stratification in grasses (Heslop-Harrison & Heslop-Harrison 1991, Marquez et al. 1997).

Grass pollen is released in a tricellular state. The gametophyte is completely developed at the moment of dehiscence. The precursor materials for the formation of the pollen tube have already been synthesized. Before dehiscence, rapid and dramatic dehydration occurs and dispersion of the pollen grains by the wind is facilitated (Stanley & Linskens 1974). After dispersion and pollination, the pollen grain undergoes rehydration on the surface of the stigma. Hydration is achieved by connection between the stigma and the pollen grain, through stigmatic secretion and the pollenkit. When the pollen grain grows in volume, a large amount of secretion is produced which passes through the pollen grain wall, and

Figs. 6–10. Ripe pollen. 6,7. *Digitaria sanguinalis*. 8–10. *Lolium perenne*. (6) Tri-nucleate pollen grain with starch granules (*s*) and numerous granular particles throughout the cytoplasm. Note the thin intine (*arrow*). Bar = 2 µm. (7) Detail of three-layered intine, exintine (*arrowhead*), middle intine (*i₂*) and endintine (*arrow*). Cytoplasm with mitochondria (*m*) and granular particles (*gp*). Bar = 0.25 µm. (8) Lipids test. The middle intine shows an isolated lipidic reaction (*double arrows*). Dictyosomes (*g*), granular particles (*gp*) faintly stained with the homogeneous granule close to their membrane (*arrow*). Bar = 0.25 µm. (9) Thiéry test. Polysaccharide intine, exintine (*short arrow*), middle intine (*i₂*) and endintine (*large arrow*). The faintly polysaccharide reaction of granular particles (*gp*) with homogeneous granules near their membrane (*thin arrow*). Note the granular particles located near the plasmalemma (*arrowhead*). Bar = 0.25 µm. (10) The Ubisch bodies remain intact and connected to each other by an orbicular-tapetal layer (*arrowhead*). The core of the Ubisch bodies (*c*) was still present and was separated from the sporopollenin layer by a thin membrane (*arrow*). Bar = 0.25 µm.

Figs. 11–14. 11–13. Activated pollen grains of *Lolium perenne*. (11) Significant increase in intine thickness (*arrow*). Starch granules (*s*), spermatophyte (*sn*) and vegetative (*vn*) nucleus. Bar = 2 µm. (12) Granular particles (*gp*) form evaginations (*arrow*) with a fibrillar appearance. Fibrillar particles (*fp*) not delimited by a membrane. Dictyosome (*g*), starch (*s*). Bar = 0.25 µm. (13) Thiéry test. The granular (*gp*) and fibrillar (*fp*) particles react faintly with the tests for polysaccharide. The granular particles with an homogeneous granule (*arrow*). Note the strong starch reaction (*s*). Bar = 0.25 µm. (14) Germinated pollen grains of *Zea mays*. All particles have a fibrillar appearance (*fp*). Bar = 0.25 µm.

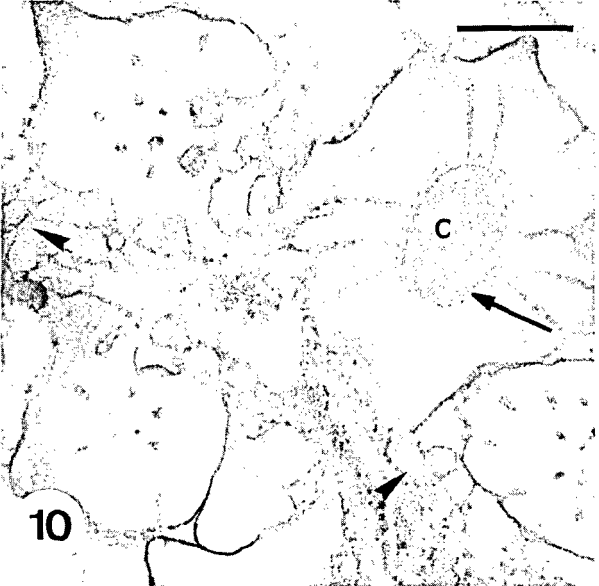
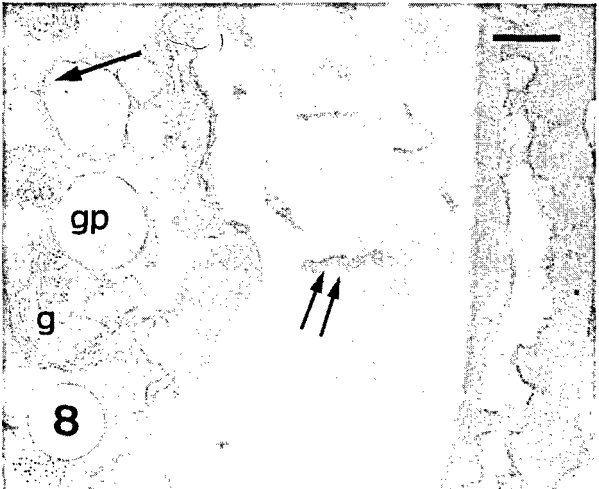
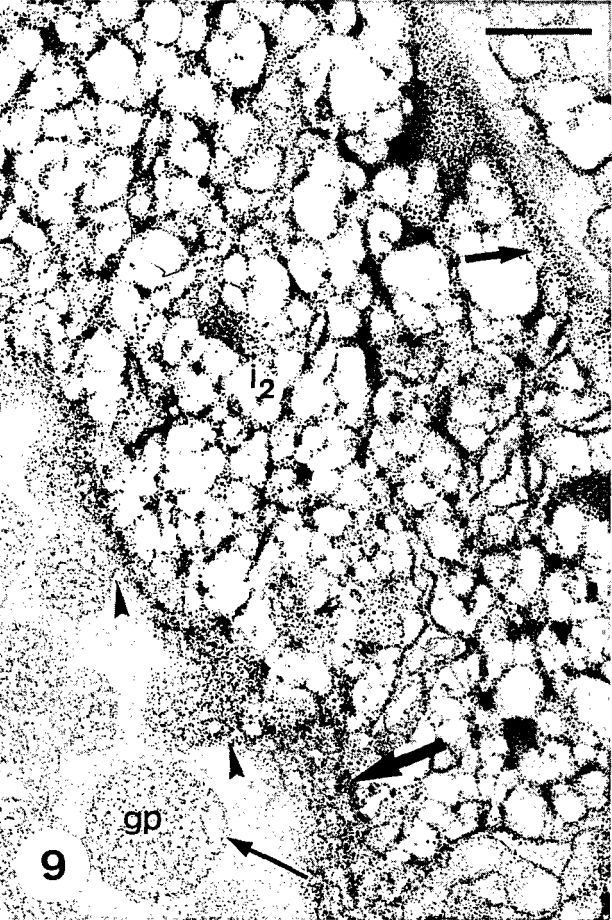
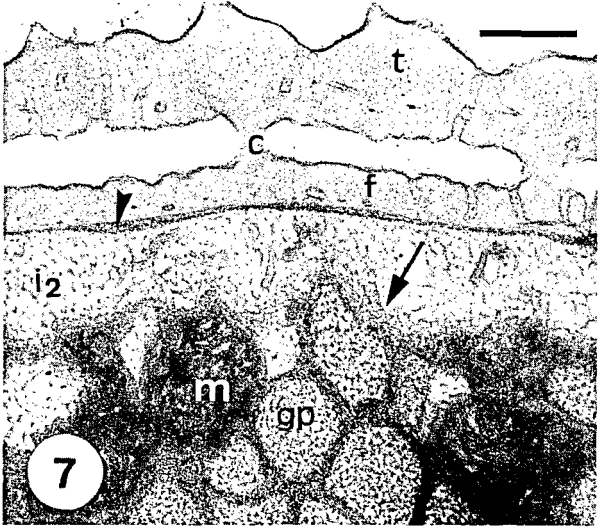
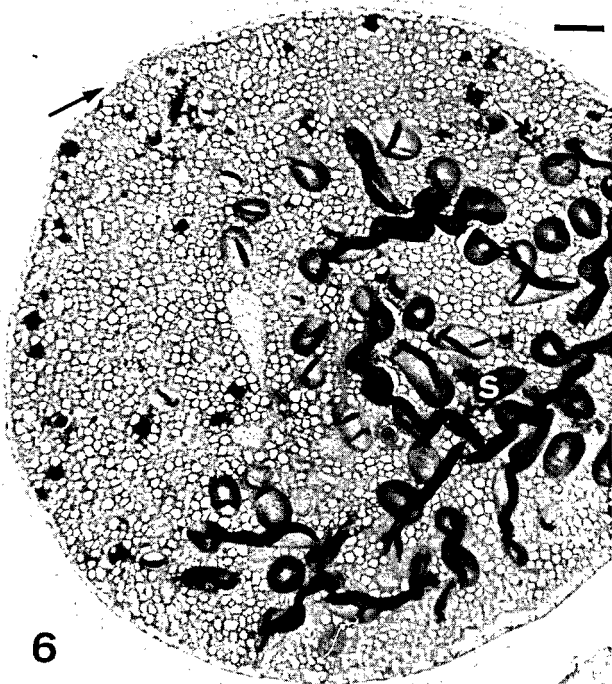
Figs. 15–17. Activated pollen grains of *Lolium perenne*. Immunogold labelling. (15) First stage of activation. Intine (*i*) starts to disorganize and shows scarce IgE labelling (*arrow head*). Note the labelling (*arrowhead*) in the pollenkit (*pk*). (16) Intine structure shows the disorganized middle intine (*i₂*) with a loose appearance, and the electrondense granules moving to the outer part (*arrows*); the exintine (*arrowhead*) and endintine (*double arrows*) were diffuse. Abundant IgG labelling in the hydrated intine, scarce labelling in the exine (*e*), pollenkit (*pk*) and Ubisch bodies (*ub*). (17) Significant IgE labelling in the intine (*i*) and exine (*e*). In the cytoplasm (*cy*) no significant labelling was observed in the granular and fibrillar particles. Arrows indicate grouped gold particles. Note the foot layer microchannels filled with material from the intine (*arrowhead*). Mitochondria (*m*), fibrillar particles (*fp*). Bars = 0.25 µm.

Figs. 18–22. Activated pollen grains. 18, 19. *Lolium perenne*. (18) IgE labelling in the cytoplasm. Granular particles (*gp*) with a homogeneous granule (*arrows*), fibrillar particles (*fp*), mitochondria (*m*). (19) IgG labelling. Abundant labelling in the anther walls (*w*) and insignificant labelling in the Ubisch bodies (*ub*). 20, 21. *Zea mays*. (20) IgE labelling in the cytoplasm. Note the significant labelling in the starch granules (*s*). The fibrillar particles (*fp*) were not labelled. (21) IgG labelling in the cytoplasm. Insignificant labelling in the starch granules (*s*) and fibrillar particles (*fp*). 22. *Lolium perenne*. IgE labelling. Note the group of labelling in the exine microchannels (*arrows*). Bars = 0.25 µm.

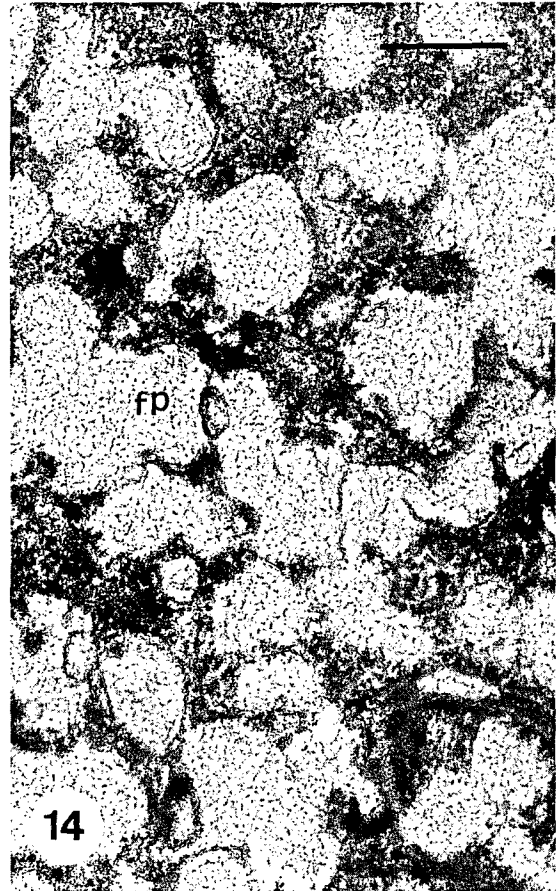
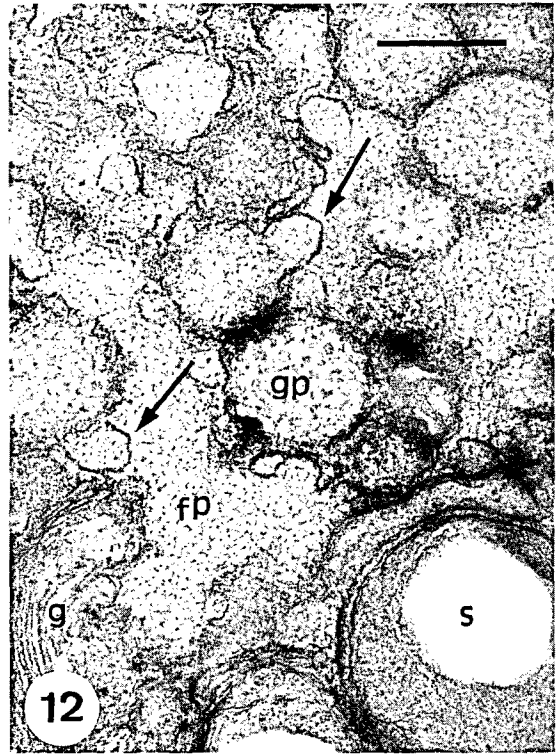
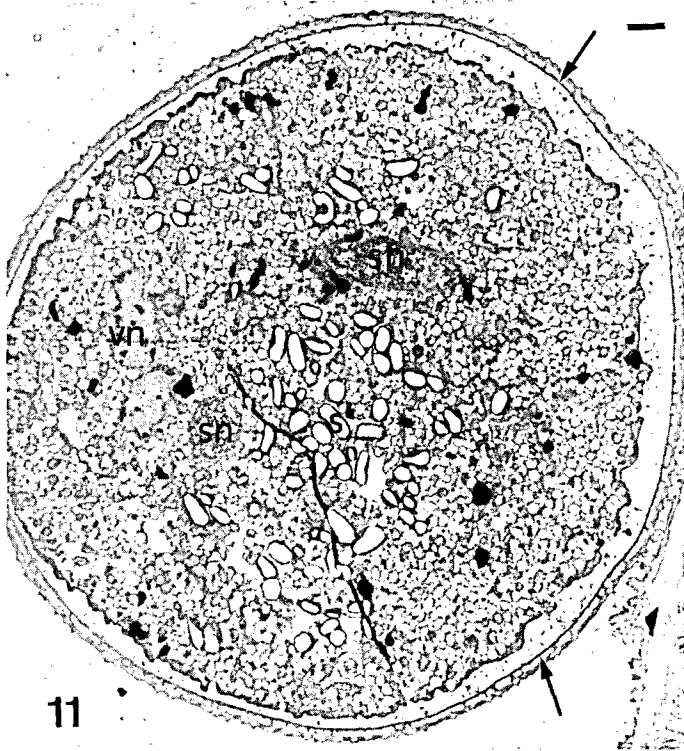
Figs. 23–25. Early germination of pollen grains of *Zea mays*. (23–24) Thick and disorganized intine (*di*). Exudates (*ex*) in the intercolumnellar region and in the exine microchannels (*long arrow*). Note the material coming from the intine (*arrowhead*). Abundant fibrillar particles (*fp*) near the plasmalemma which expels its fibrillar material (*short arrow*) into the disorganized intine (*di*). The pectic-cellulosic pollen tube layer was developed inside the intine (*double arrows*). (25) IgG labelling. Scarce labelling in the pectic-cellulosic pollen tube layer (*arrows*) and in the exudates (*arrowheads*). Bars = 0.25 µm.

Figs. 26–29. Germinated pollen grains of *Zea mays*. (26) IgG labelling of the callose layer (*cl*) of the pollen tube wall. (27) The exudates were deposited outside the tectum at the spinules (*arrow*). Electrontransparent pollenkit (*pk*). The tectum channels are full of electrondense material (*arrowhead*). Note the empty intercolumnellar region. Thin, undulated, fibrillar and granulated pectic-cellulosic layer (*long arrow*). (28). Pollen tube section. Faintly electrondense callosic layer (*cl*), electrondense pectic-cellulosic layer (*long arrow*) and exudates (*arrowhead*). Cytoplasm with fibrillar particles (*fp*) and starch granules (*s*). (29). Undulated and two layered pollen tube wall inside the pollen grain: faintly electrondense callosic layer (*cl*) and electrondense pectic-cellulosic layer (*long arrow*). Disorganized cytoplasm (*cy*). Bars = 0.25 µm.

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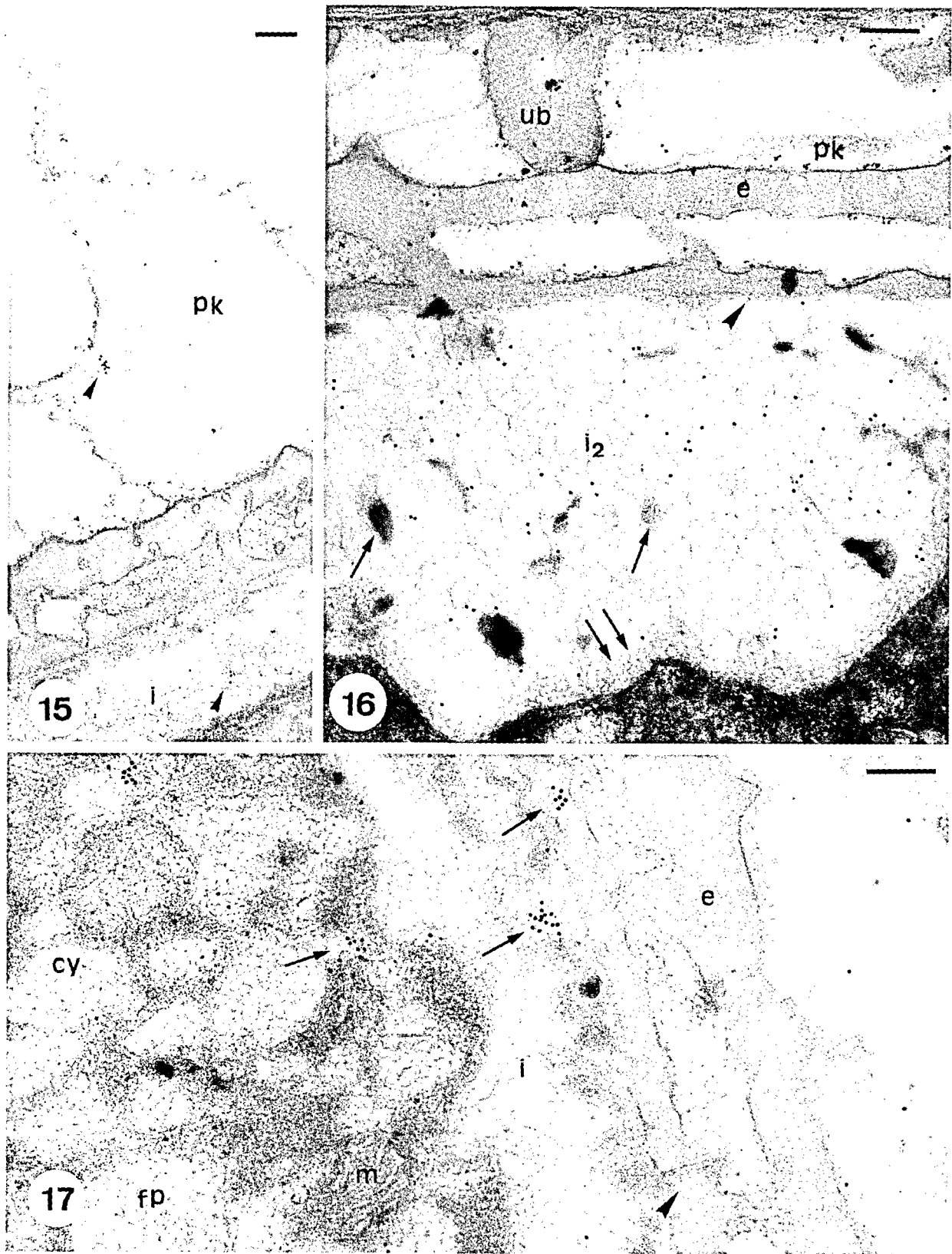


Figs. 6–10. (see caption p. 332).

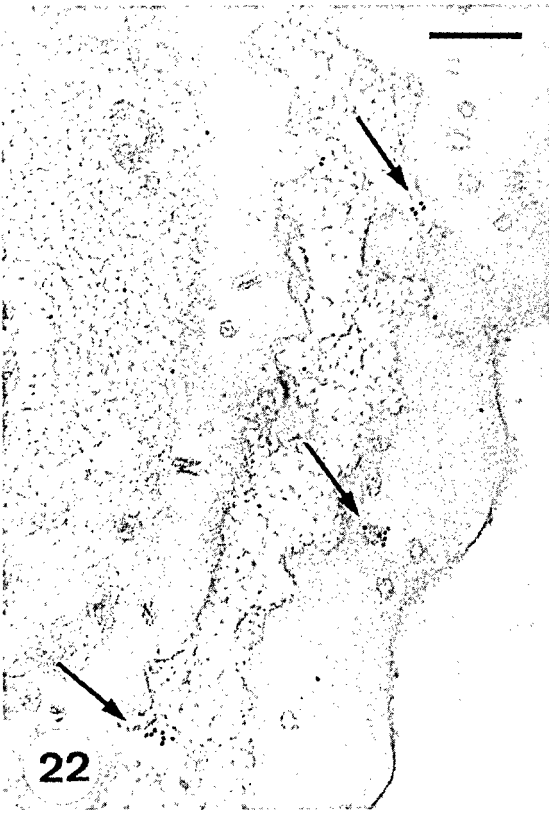
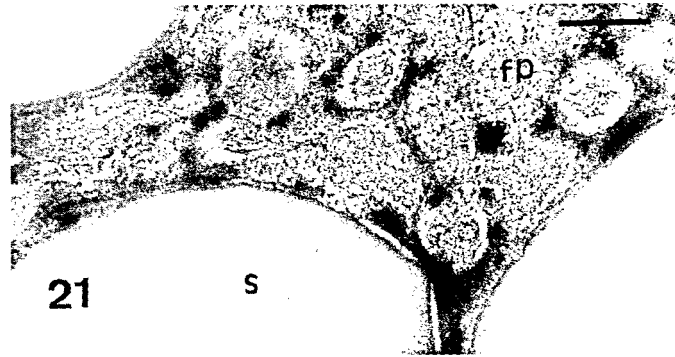
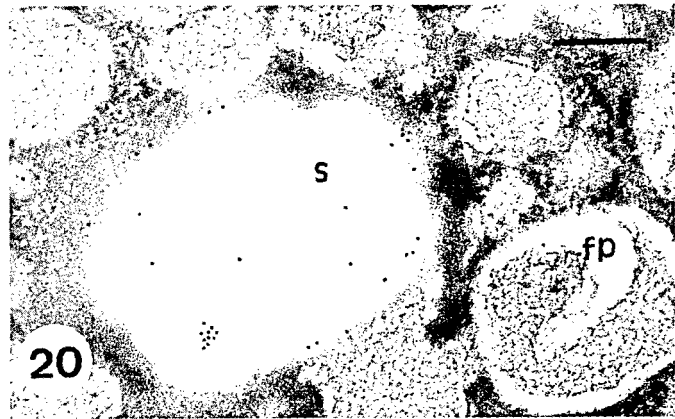
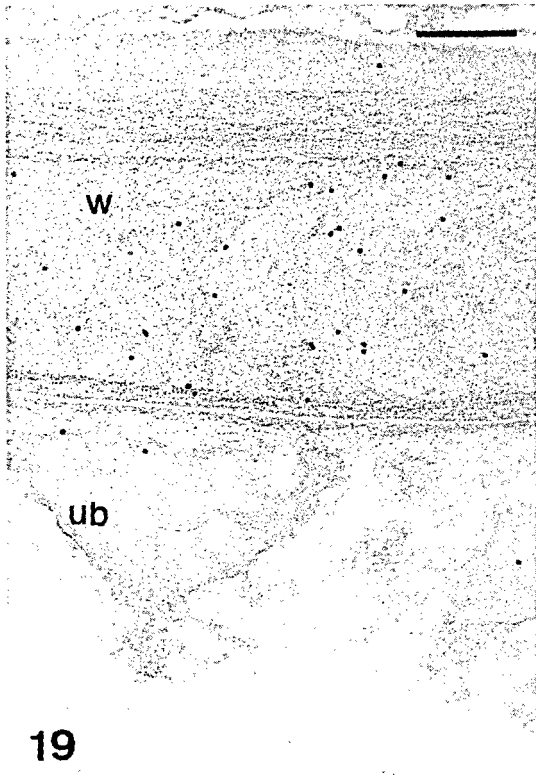
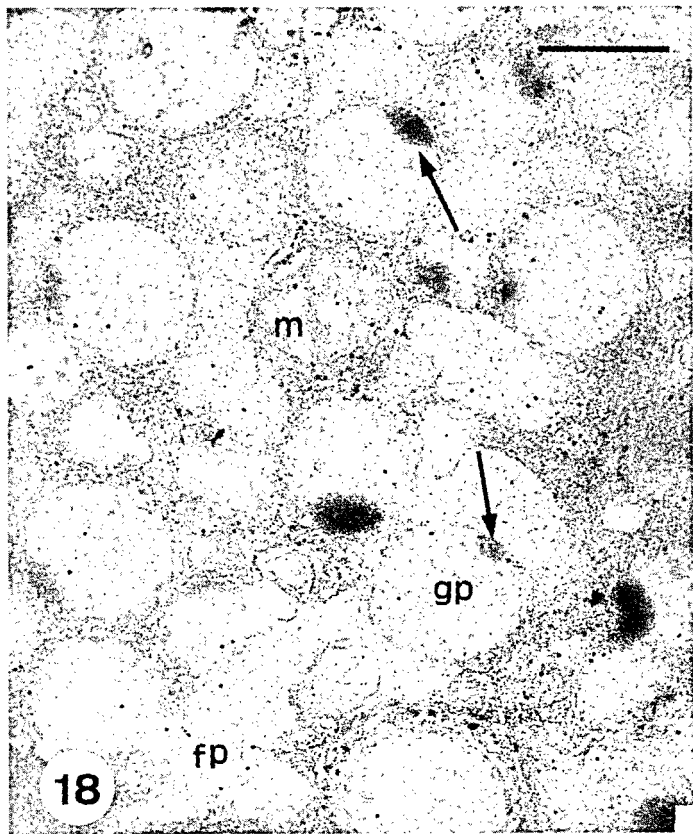


Figs. 11–14. (see caption p. 332).

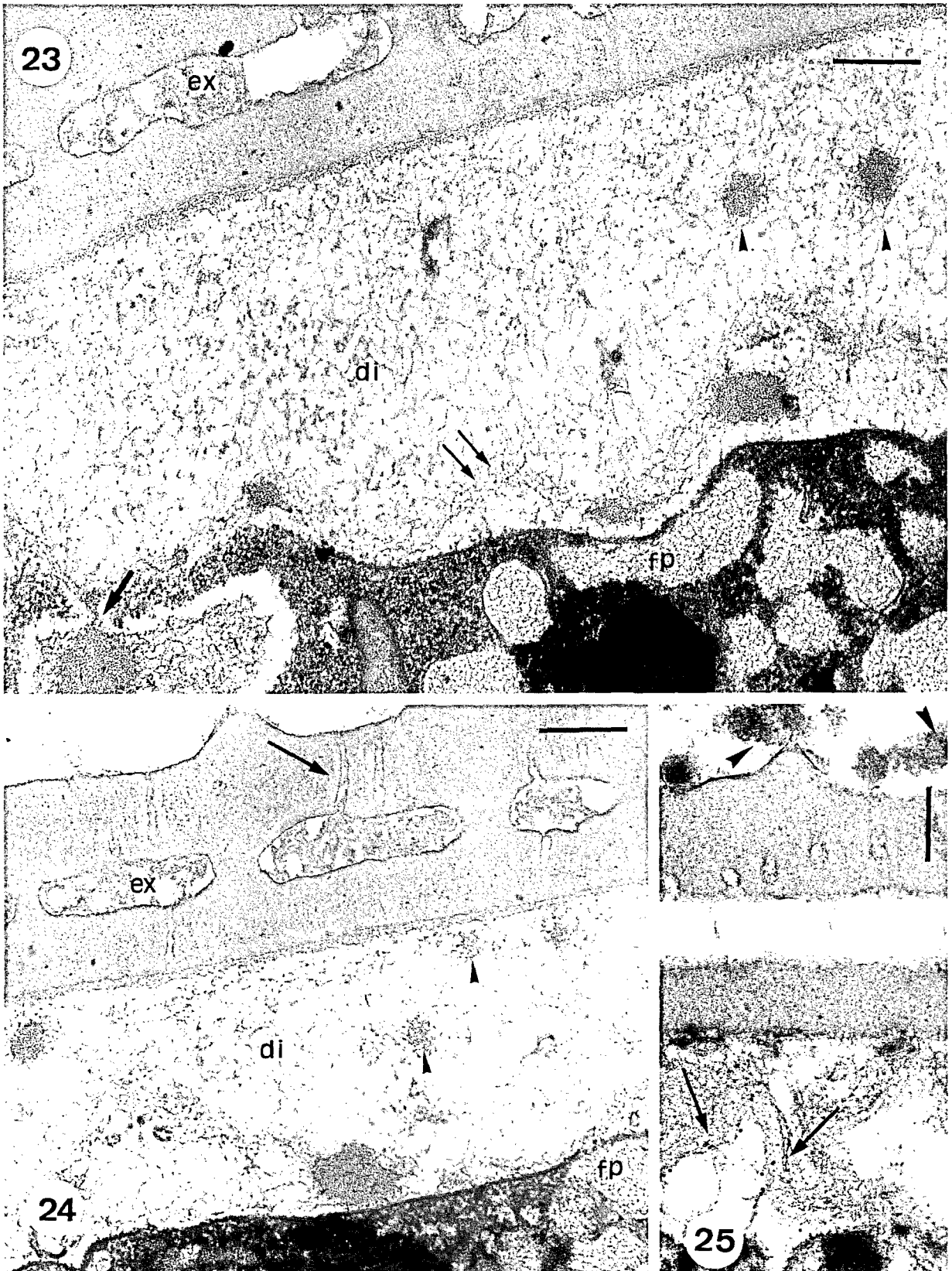
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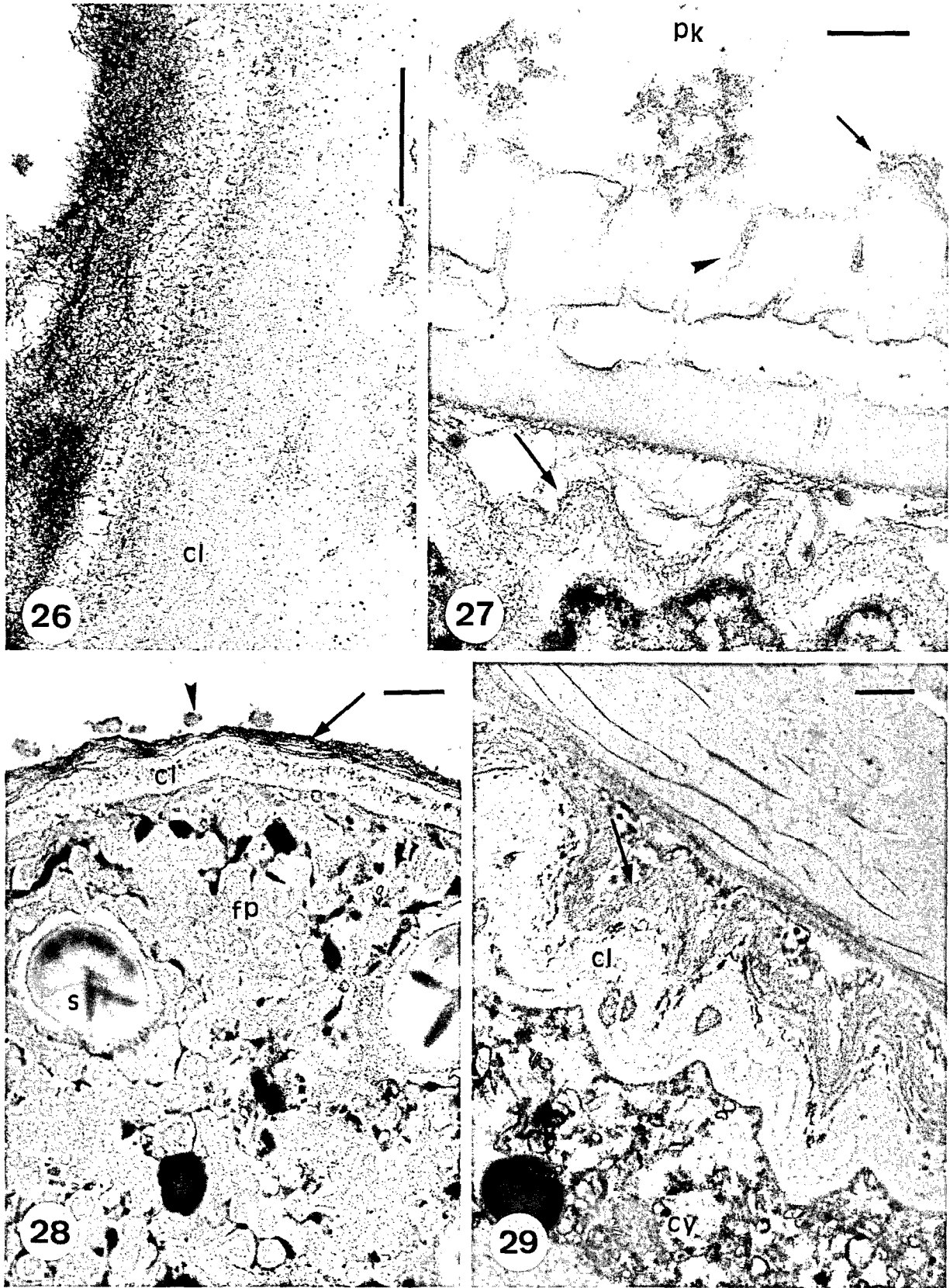
Figs. 15–17. (see caption p. 332).



Figs. 18–22. (see caption p. 332).



Figs. 23–25. (see caption p. 332).



Figs. 26–29. (see caption p. 332).

reaches the outside through the microchannels of the exine. If a compatible stigma is found, the pollen grain emits a tube that corresponds to the first morphological indication of germination (Heslop-Harrison 1979b, Dumas et al. 1984). In *Lolium*, after moisture activation, we were able to corroborate by TEM the steps in the process prior to germination. During hydration, the non apertural intine increased in volume, it was disorganized and the secretions were expelled through the exine channels. At this time immunolabelling of allergenic and antigenic proteins (IgE and IgG) was observed. The process of IgG protein diffusion through non apertural intine seems to be slower than that of IgE proteins. According to our findings, in *Lolium* the interapertural intine proteins are activated at the moment of hydration of the pollen grains. These proteins remained significantly in the intine and the labelling of exudates was scarce. Specific labelling in the activated Zwischenkörper of *Lolium* was also observed (Marquez et al. 1997), just as allergens surrounding the pollen grains of *Betula* were observed by Belin & Rowley (1971). Recently Casas et al. (1996) detected allergenic proteins in the intine and in material extruded from the activated pollen grains of *Parietaria judaica*, these proteins seem to be related to pollen-stigma recognition and they are activated after 10 min of hydration, prior to pollen tube formation. In Poaceae it seems that proteins from apertural intine could have the function attributed to the intine in the processes of pollination and fertilization (Heslop-Harrison & Heslop-Harrison 1991, Marquez et al. 1997), as well as in the possible activation mechanism of the pollen grains when they react with sensitive subjects (Seoane-Camba & Suarez-Cervera 1986, Casas et al. 1996). However the role of the intine proteins may be more related to pollen tube formation.

The tip of the pollen tube wall is covered by a pectic layer; on both sides of the tip the wall contains an external pectic-cellulosic layer under which is found the internal callose layer described by Heslop-Harrison (1987) in *Secale*. The most internal layer of the intine (endintine) is possibly the precursor of the pectic-cellulosic layer of the tube (Heslop-Harrison 1979a, 1987). The stratification that we observed in the non-tip regions in *Zea*, coincides with the descriptions of this author. In *Zea* pollen grains we also saw very clearly the deposition of substances expelled by the fibrillar particles into the degraded endintine; these substances begin to form the pectic-cellulosic and callosic layers of the pollen tube. Throughout the whole germination time, no labelling was observed by IgE. However labelling by IgG was observed in the pollen tube wall. On the other hand, specifically PEX glycoproteins were located in the internal callose layer of the *Zea* pollen tube wall by immunogoldlabelling (Rubinstein et al. 1995). In our opinion the IgG labelling could indicate that the non apertural intine and later the callose pollen tube wall, are two regions where important metabolic reactions related to the germination process could take place.

Ubisch bodies

The Ubisch bodies in grasses have an external sporopollenic part and a core or central part which is non-sporopollenic, and which is not destroyed at maturity. These bodies adhere to the wall of the anther, each one joined to another by an

orbicular-tapetal wall, containing sporopollenin. The presence of the orbicular wall that joins the bodies to each other even after the degeneration of the tapetal cells has been described in other Poaceae (Rowley 1962, 1963, Banerjee 1967, Christensen et al. 1972, El-Ghazaly & Jensen 1986). Miki-Hirosige et al. (1994) localized the allergenic proteins in the tapetum and Ubisch bodies of *Cryptomeria japonica*. Recently El-Ghazaly et al. (1995) showed the moderate staining of proteins of orbicules in *Betula pendula* and postulate their possible allergenicity. These Ubisch bodies could be released into the environment (Miki-Hirosige et al. 1994, El-Ghazaly et al. 1995). In this study the Ubisch bodies remained adhered to the wall of the anther, and its release was not detected. No significant labelling was observed which is in agreement with Taylor et al. (1994).

Pollenkitt

According to Hesse (1978, 1979), the pollenkitt in pollen grains from diverse entomophilous taxa has a homogeneous appearance, is electrondense and is located between the columellae and the tectum. The anemophilous pollenkitt is located mainly between the columellae, is not homogeneous, and is electrontransparent. Hesse (1980) described the pollenkitt in grasses, *Seslera* and *Alopecurus* as being transparent to electrons. In *Triticum* (El Ghazaly & Jensen, fig.10B, 1986) an abundant pollenkitt was observed with medium electrondensity on the exterior of the pollen grain. In *Lolium* we were able to observe the electronlucent pollenkitt located above the tectum and between the columellae. The test for polysaccharides and lipids was negative, but we also found glycoproteins in the pollenkitt by means of significant labelling. The observed labelling in the pollenkitt, orbicular-tapetal and anther walls may be an indication that some of the allergenic proteins are produced in the sporophytic cell.

Cytoplasm

In the processes of maturation, activation, and germination of pollen grains, the cytoplasm is extremely active and undergoes a series of modifications that play a decisive role in each process. In grasses, during the period of maturation (intine deposit) we were able to observe a high degree of cytoplasmic activity with numerous mitochondria, ribosomes, endoplasmic reticulum and dictyosomes. The Golgi vesicles are clearly deposited in the plasmalemma and are expelled by exocytosis into the periplasmic space to form the exintine, which is found pressed against the exine. The plastids are also highly activated and a large quantity of starch granules are formed, which tend to be located around the aperture. This mechanism is similar to the mechanisms observed in *Triticum* (El-Ghazaly & Jensen 1986, 1987) and in *Lolium* (Pacini et al. 1992).

The cytoplasm of the vegetative cells is characterized by numerous starch granules and a greater number of particles (P-particles in Heslop-Harrison 1979a, Heslop-Harrison & Heslop-Harrison 1982).

It was observed significant labelling by IgE in the starch granules of the activated and early germinated pollen grains, but scarce or no significant labelling was observed by IgG.

Taylor et al. (1993) show significant labelling in the starch granules of *Lolium* ripe pollen grains and El-Ghazaly et al. (1996) also found the major allergen of *Betula pendula* pollen grains predominantly in the starch granules. Suphioglu et al. (1992), Knox (1993), Vrtala et al. 1993, El-Ghazaly et al. (1996) and Knox et al. 1997 attributed a important role in the asthma process to the starch granules. This is in agreement with our results, the patient sera against *Lolium* appear to be sensitized to specific starch glycoproteins and hence the IgE response could be more specific than the IgG one.

The P-particles are responsible for the formation of the pollen tube and seem to represent 30% of the reserves of a vegetative cell (Heslop-Harrison 1979a, Heslop-Harrison & Heslop-Harrison 1982). These authors conducted an exhaustive study of the morphology and the chemical composition of these particles in *Secale* and *Pennisetum*. In other families similar structures are formed during pollen tube formation (Dickinson & Larson 1975, Cresti et al. 1977, 1985, Cresti & Tiezzi 1990, Geitmann et al. 1995). They are surrounded by a membrane and initially have a granular content. At the moment of activation of the pollen grain the contents become fibrillar and a loss of membranes occurs, as was observed in *Secale* and *Pennisetum* (Heslop-Harrison & Heslop-Harrison 1982). In *Eryngium* the granular particles fuse to each other, forming non-membrane-bound polysaccharide aggregates, which become amorphous (Weber 1988). In our study we were able to detect these particles in the cytoplasm of the vegetative cells of Poaceae pollen grains with a finely granular content, accompanied by one or two homogeneous but electrondense grains. During the pollen activation process, we observed that the transformation of granular particles into fibrillar particles occurred by gemma formation. They react faintly to the lipid and polysaccharide test. When germination begins all the cytoplasmic particles of pollen grains are fibrillar and have a similar appearance to the pollen tube particles. These fibrillar pollen tube particles have their fibrillar contents condensed in the centre and surrounded by an electronlucent halo. This is in agreement with the particles described by Geitmann et al. (1995) in the *Brugmansia* pollen tube. During formation and growth of the pollen tube, the large aggregates of fibrillar particles are found near the plasmalemma of the pollen grains, and release their contents into the periplasmic space, between the plasmalemma and the pollen tube wall, as observed in *Zea*. Also (Rubinstein et al. 1995) show the relationship between the fibrillar pollen tube particles and callose pollen tube wall.

Heslop-Harrison & Heslop-Harrison (1982) and Heslop-Harrison (1987) studied the chemical composition of the P-particles in the pollen tubes and in the cytoplasm of the vegetative cells in various species of monocotyledon, and found that the largest component of these particles was pectin. With immunogold pectin localization Geitmann et al. (1995) found that in Solanaceae, these particles contain pectins in a highly methyl esterified form, but no callose. The implication of these particles in the formation of the pectic-cellulosic layer of the pollen tube has been shown in *Zea* in this paper. We were also able to see the removal of the fibrillar component of these particles into the pectic-cellulosic pollen tube layer, in the pollen grain. No specific labelling (IgG and IgE) was detected in *Lolium* and *Zea*

particles, however. The Pex proteins were localized on the callosic sheath of the pollen tube wall of *Zea* but not in the fibrillar pollen tube particles (Rubinstein et al. 1995). So, it would seem possible that precursors to the allergenic proteins were activated in the extracellular matrix.

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