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The generative cell and its close association with the endoplasmic reticulum of the vegetative cell in pollen of Cyrtandra pendula (Gesneriaceae).

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PROTOPLASMA

ıe generative cell and its close association with the endoplasmic reticulum the vegetative cell in pollen of *Cyrtandra pendula* (Gesneriaceae)

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mmary. The development of the generative cell (GC) was examined the bicellular pollen of *Cyrtandra pendula*. The essential stages :: (1) GC attached to the intine, (2) GC detached and spheroidal shape, (3) GC mature and elongated. Cisternae of the endoplasmic iculum (ER) of the vegetative cell are in close contact with the C at all stages of development. In stages (1) and (2) the entire, ghtly undulated surface of the GC is surrounded by tightly apessed, single ER tubules or short stacks. In mature pollen grains age 3) the shape of the GC as well as the arrangement of the rrounding ER changes conspicuously. The GC is now spindleaped and its surface is wrinkled. An ER tubule is present in each vagination. These ER tubules form a cage-like framework around e GC. In the cytoplasm of the generative cell, 6 to 7 microtubular ındles with longitudinal orientation can be observed. They seem be responsible for maintaining the elongate shape of the GC. uring all stages of development vegetative ER cisternae are the ıly elements intimately associated with the GC wall. This feature dicates that the ER may contribute to the formation of the unılated outer shape of the GC. Also discussed is the possibility that ıergy-carrying substances are conveyed into the GC through the ıannels of ER.

eywords: *Cyrtandra pendula*; Generative cell; Endoplasmic reticuım; Pollen grain; Ultrastructure.

bbreviations: GC generative cell; GN generative nucleus; VC vegative cell; ER endoplasmic reticulum; rER rough endoplasmic reculum; MT microtubule; RF-FS rapid freeze fixation-freeze subitution.

ntroduction

The development of the generative cell (GC) has attracted the attention of a large number of cell biologists ince Strasburger (1878) first described mitotic cell division in pollen grains of angiosperms. Publications are focussing on the formation and nature of the GC wall (Angold 1968, Sanger and Jackson 1971 a, Heslop-Harrison 1968, Cresti et al. 1987, Brighigna et al. 1981, Schlag and Hesse 1992), the positional relationship between the vegetative nucleus and the GC (Heslop-Harrison et al. 1986, Mogensen 1986, Kaul et al. 1987, Palevitz 1993), and the process of GC division and sperm cell association (Cass and Karas 1975, Dumas et al. 1985, McConchie et al. 1985, Weber 1988 a, Charzynska and Lewandowska 1990, Mogensen 1992).

A striking feature during maturation of pollen is the alteration in shape of the GC from spheroidal to markedly elongate. It is generally agreed that microtubules are necessary to maintain this asymmetric configuration (Burgess 1970, Sanger and Jackson 1971 b, Cresti et al. 1984, Derksen et al. 1985, Lancelle et al. 1987, Zhou and Yang 1991). Some studies refer to the conspicuously undulated surface of the GC in mature pollen grains (Nakamura and Miki-Hirosige 1985, Cresti et al. 1990 a, Keijzer and Willemse 1988, Van Aelst and Van Went 1991). Van Aelst et al. (1989) obtained a three-dimensional image of the elongated GC and its longitudinally wrinkled surface by observing freeze-fractured frozen pollen of *Papaver dubium* with cryo-scanning electron microscopy. The involvement of the endoplasmic reticulum (ER) in the formation of the undulated outer shape of the GC in *Tradescantia reflexa* pollen is reported by Noguchi and Ueda (1990). The present paper describes three developmental stages of the GC in pollen of *Cyrtandra pendula*, a member

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of the highly evolved dicot plant family Gesneriaceae. Special attention is paid to its ultrastructure and the intimate association of the ER of the vegetative cell and the GC wall.

Material and methods

Cyrtandra pendula Bl., collected by A. Weber in the Malay Peninsula 870501-1/17, is cultivated in the greenhouses at the Botanical Garden of the University of Vienna (HBV). Anthers were fixed in 3% buffered glutaraldehyde (0.1 M phosphate buffer, pH 8) for 7 h at room temperature. After rinsing in buffer and distilled water, samples were postfixed in 2% OsO_4 plus 0.8% phosphate buffered $K_3Fe(CN)_6$ (in the ratio of 1 : 1) for 11 h at + 6 °C, dehydrated in an ethanol series and embedded in Spurr's low viscosity epoxy resin (Spurr 1969). Ultrathin sections, cut on a Reichert Ultracut with a diamond knife, were stained with uranyl acetate and lead citrate (UA-Pb staining) and examined in a Zeiss EM 109 transmission electron microscope at 50 or 80 kV. For detecting neutral polysaccharides ultrathin sections were collected on gold grids and treated with periodic acid (45 min), thiocarbohydrazide (12 h) and silver proteinate (30 min) (PATAg staining) according to Thiéry (1967).

Results

Generative cell (GC) attached to the intine

After the first pollen mitosis the GC is attached to the intine. Its cytoplasm contains numerous mitochondria,

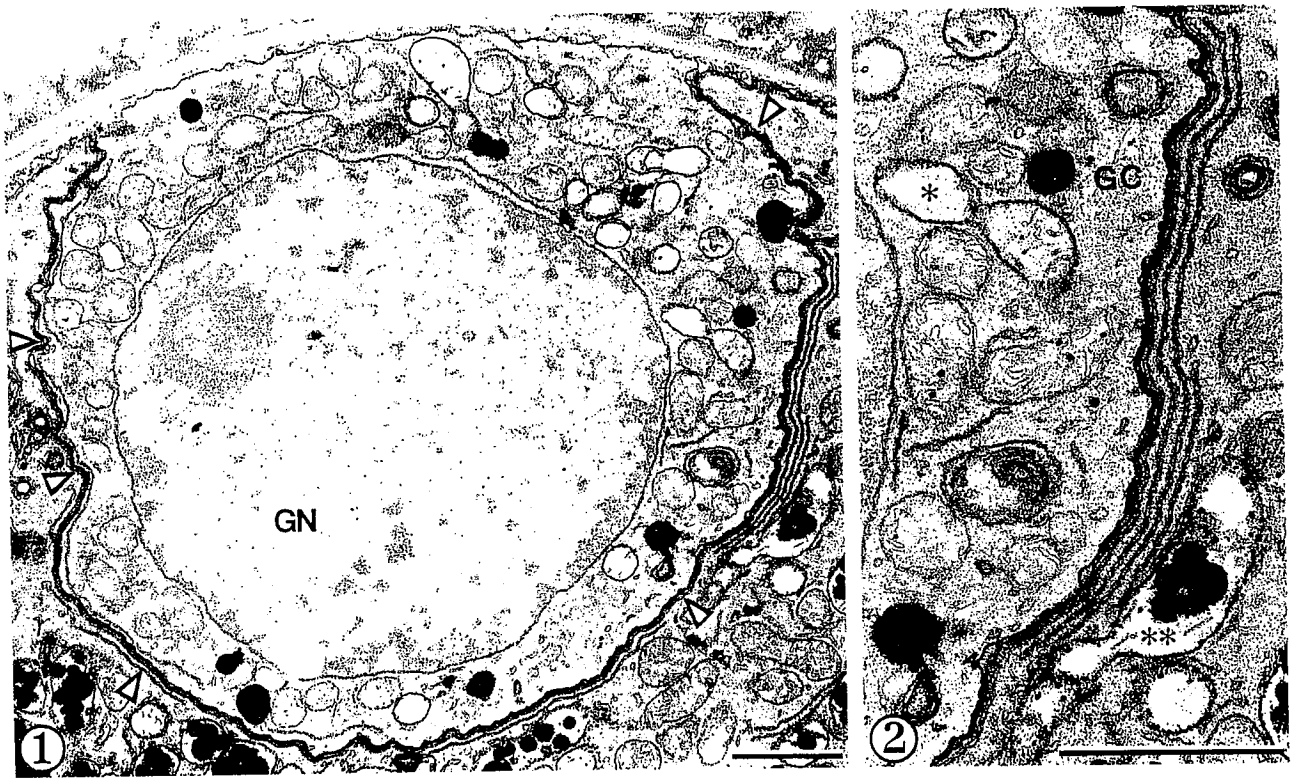


Fig. 1. Generative cell attached to the intine. Its cytoplasm contains a large nucleus (*GN*), numerous mitochondria, plastids, ER tubules, lipid bodies, small vacuoles, vesicles and a few single microtubules. The entire surface of the GC is surrounded by vegetative ER cisternae (arrowheads). PATAg stained: bar: 1 µm

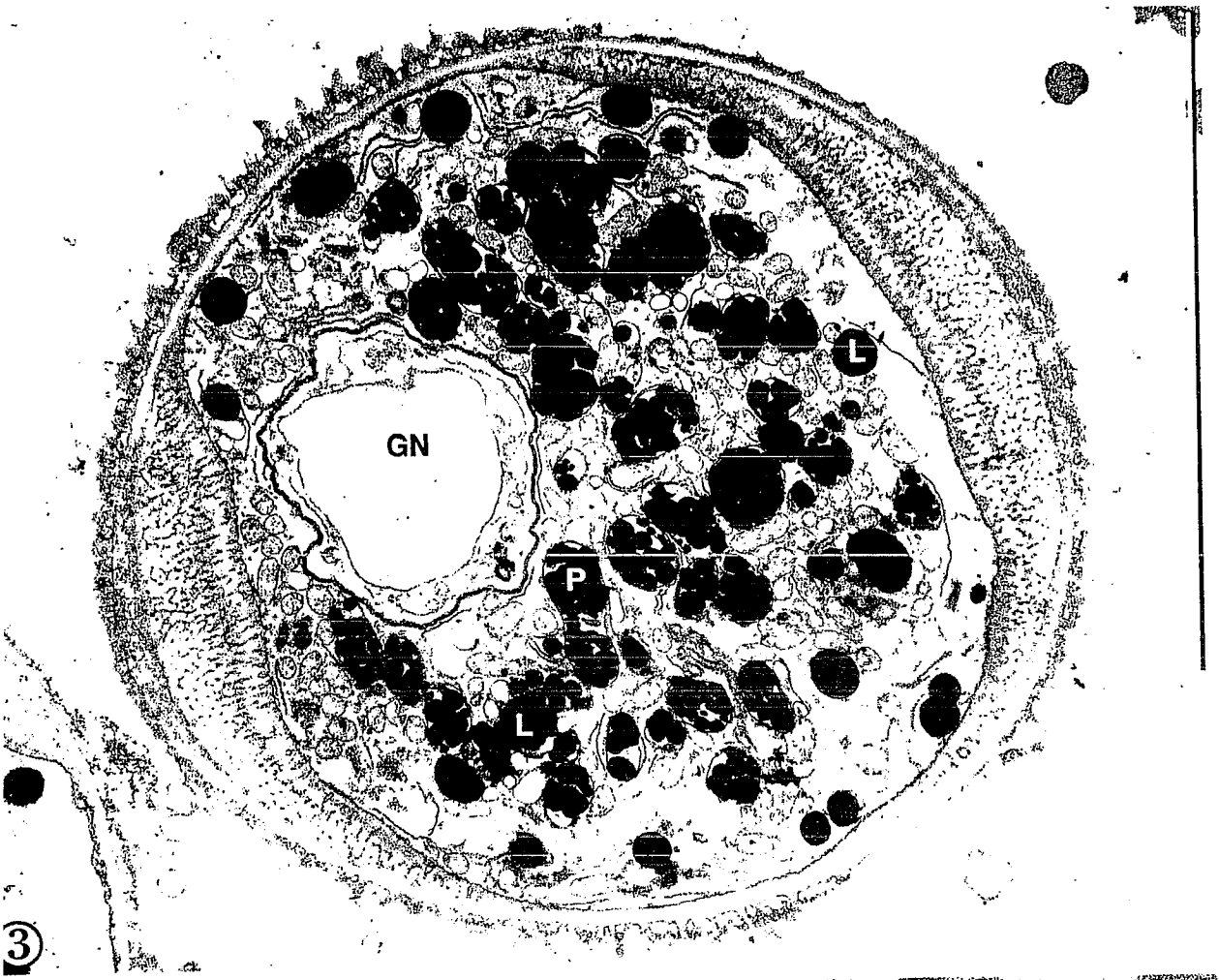
Fig. 2. Detail of Fig. 1, showing the border between generative (*GC*) and vegetative cell. The plastids of the GC (*) do not contain starch, while the plastids of the vegetative cell (**) are beginning to accumulate starch. Cisternae of vegetative ER are tightly appressed to the GC wall. PATAg stained: bar: 1 µm

Fig. 3. Pollen grain with detached, young generative cell. The GC is spheroidal and its surface is slightly convoluted. Its cytoplasm is filled mainly with the large nucleus (*GN*); plastids are no longer detectable in the GC. In the vegetative cell a large amount of reserve substances has accumulated (plastids, *P*, including starch granules and lipid bodies, *L*). PATAg stained; bar: 10 µm

Fig. 4. Detached young GC: dark-stained GC wall (arrows) and extremely closely associated short ER stacks. The innermost cistern does not bear ribosomes on the membrane adjacent to the GC wall. PATAg stained; bar: 1 µm

Fig. 5. Detached young GC: GC wall (arrows) and tightly appressed ER cisternae show similar contrast by staining with UA-Pb. Bar: 1 µm

Fig. 6. Tangential section of the nuclear envelope of the vegetative nucleus (*VN*) showing a large number of nuclear pores. PATAg stained; bar: 1 µm



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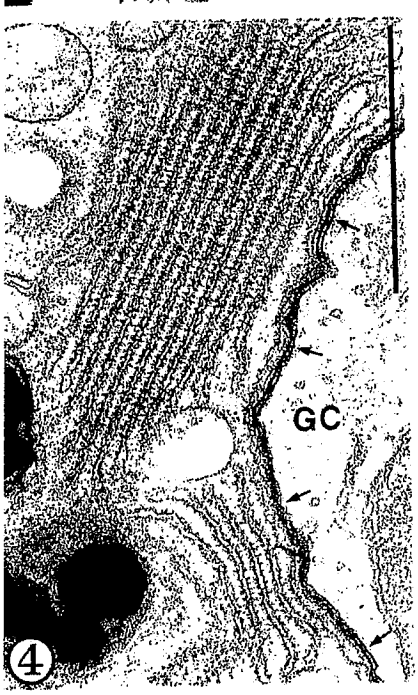

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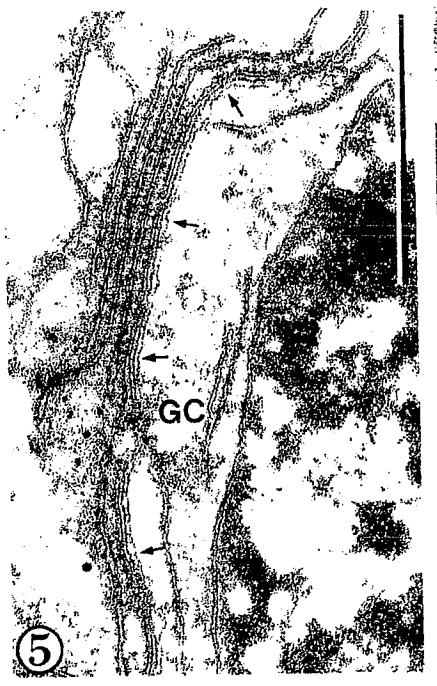

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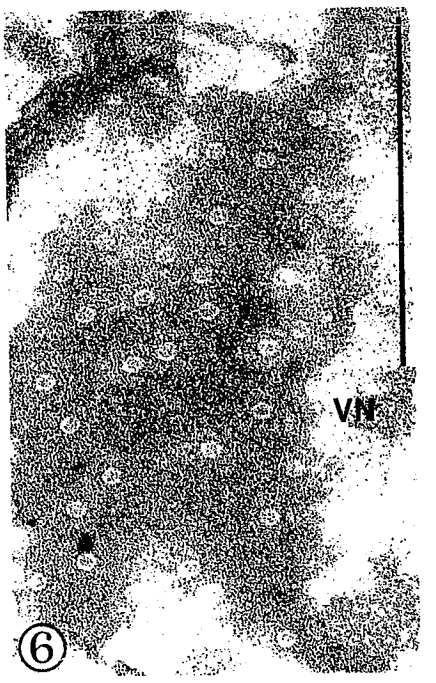

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ER tubules, lipid bodies, plastids without starch, few dictyosomes, small vacuoles and a few single MTs (Fig. 1).

ER is abundant within the vegetative cell (VC). Some of the cisternae are in close association with its inner plasma membrane (Figs. 1 and 2). The entire surface of the GC is surrounded by one or more ER cisternae. Furthermore, the VC contains elongated plastids with starch granules, lipid bodies, numerous mitochondria and a few inactive dictyosomes.

GC detached and spheroidal in shape

After detachment from the intine the GC moves towards the centre of the pollen grain. Its content of organelles is comparable with the previous stage, but plastids are lacking. At this stage the shape of the GC is spheroidal to oblong; its surface is slightly undulated (Fig. 3). Some ER cisternae of the vegetative cell are closely appressed to the GC and their arrangement follows exactly its outline. Single sheets of ER, but also short ER stacks, surround the entire surface of the GC. The innermost cistern does not bear ribosomes on the membrane adjacent to the GC wall (Figs. 4 and 5). ER sheets and the GC wall can be clearly differentiated after PATAg staining because of the strong contrast of the plasma membranes around the GC (Fig. 4); UA-Pb does not allow sufficient discrimination (Fig. 5).

The vegetative cytoplasm is characterized by the accumulation of high amounts of storage substances (Fig. 3). Plastids are numerous and densely packed with starch. Mitochondria, small vacuoles and rER are frequent; the latter are mainly arranged in single layers, some cisternae are arranged in small stacks. Lipid bodies are evenly distributed throughout the cytoplasm and, at this stage, are not in close association with ER. Dictyosomes are in an inactive state.

GC mature and elongated

In mature pollen grains the generative cell appears roundish in cross section (Fig. 7), while in longitudinal section it is spindle-shaped (Fig. 10). It is mainly filled with the large, spindle-shaped generative nucleus. Most of its cytoplasm is located in the terminal parts of the cell. It contains small vacuoles, mitochondria, rER tubules, lipid droplets and dictyosomes, but no plastids. About 6–7 microtubular bundles, which could not be observed in previous stages, are now arranged peripherally and orientated along the longitudinal axis of the cell (Fig. 10). The two plasma membranes around the GC are conspicuously undulated and closely appressed to a layer of wall material (Fig. 8). Plasmodesmata-like structures link the cytoplasm of the GC and the vegetative cell (Fig. 9). Cross sections of the central region of the GC show that its undulated surface consists of roughly 30 lobes (Fig. 7). The microtubular strands of the GC are located usually near these lobes (Fig. 9).

In the vegetative cytoplasm the presence of stacked rER is a striking feature; some of the large stacks contain over 70 cisternae (Fig. 12). Other cisternae, however, are single and frequently associated with lipid bodies and plastids (Figs. 7 and 10). Moreover, the ER of the vegetative cell is in close contact with the GC. Tubules of the ER enter into all invaginations of the GC surface, which is wrinkled mainly longitudinally, but also transverse (Figs. 10 and 11). Cross sections of the GC show circular profiles of ER in each concavity (Fig. 7).

In the vegetative cell the irregularly shaped nucleus lies next to the GC. A large number of nuclear pores are visible in tangential sections of the nuclear envelope (Fig. 6). The plastids are often amoeboid in shape and no longer contain starch granules. Each lipid body is surrounded by at least one rER cistern. Mitochondria with many cristae are present, as are many small electron-translucent vacuoles and very active dictyosomes.

Discussion

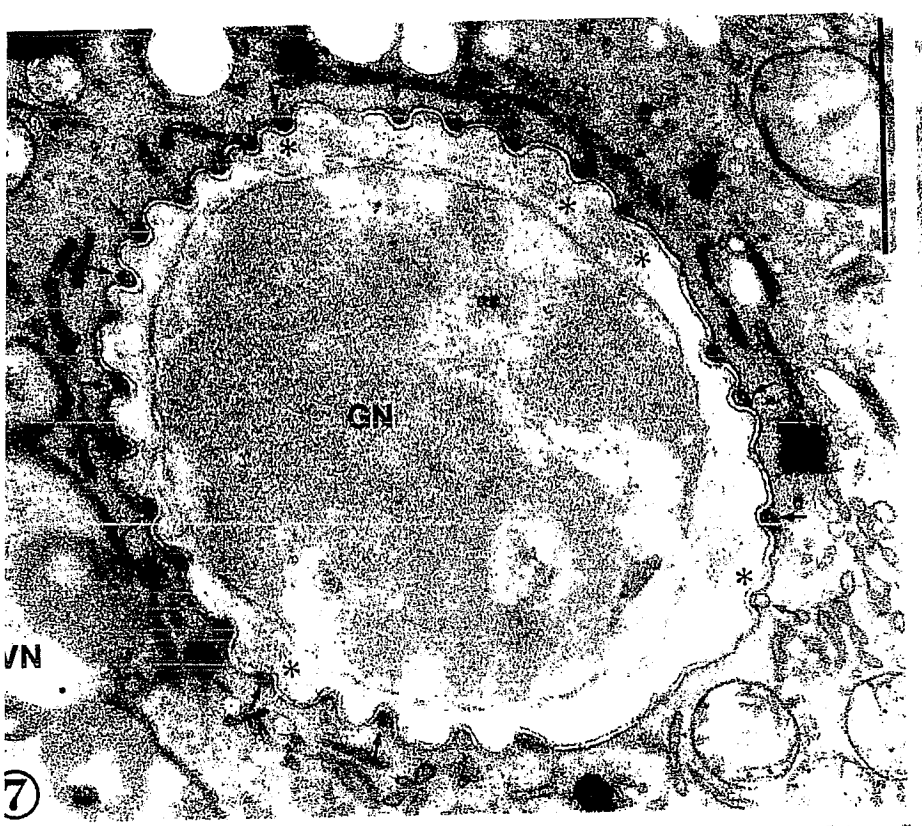
ER in pollen grains is mostly described in relation to its association with lipid droplets (Ciampolini et al. 1988, Noguchi 1990) and the outer plasma membrane

Fig. 7. Cross section of the generative cell in a mature pollen grain. Its cell wall is conspicuously undulated and circular profiles of ER (arrows) are situated in each concavity. In the cytoplasm the large generative nucleus (*GN*) and some microtubular bundles (*) are visible. The vegetative nucleus (*VN*) lies close to the GC. PATAg stained; bar: 1 µm

Fig. 8. Undulated GC wall in a mature pollen grain. Note ER profiles (arrows) in each concavity. UA-Pb stained; bar: 0.1 µm

Fig. 9. Microtubules are located close to the undulated GC wall. Plasmodesmata-like structures (arrow) seem to link the cytoplasm of the generative (*GC*) and vegetative cell. UA-Pb stained; bar: 0.1 µm

Fig. 10. Longitudinal section of the GC in a mature pollen grain. Its cytoplasm is filled mainly with the large spindle-shaped nucleus (*GN*); a microtubular bundle (*) runs along the longitudinal axis of the GC. Each surface undulation is occupied by an ER tubule. PATAg stained; bar: 1 µm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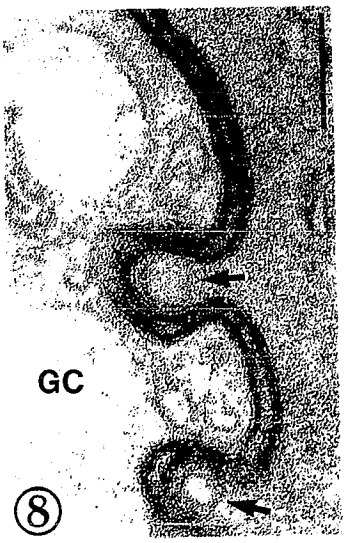
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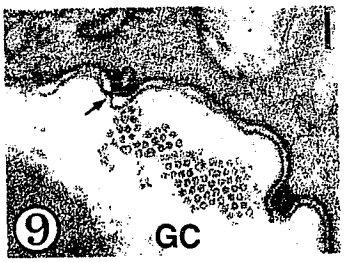
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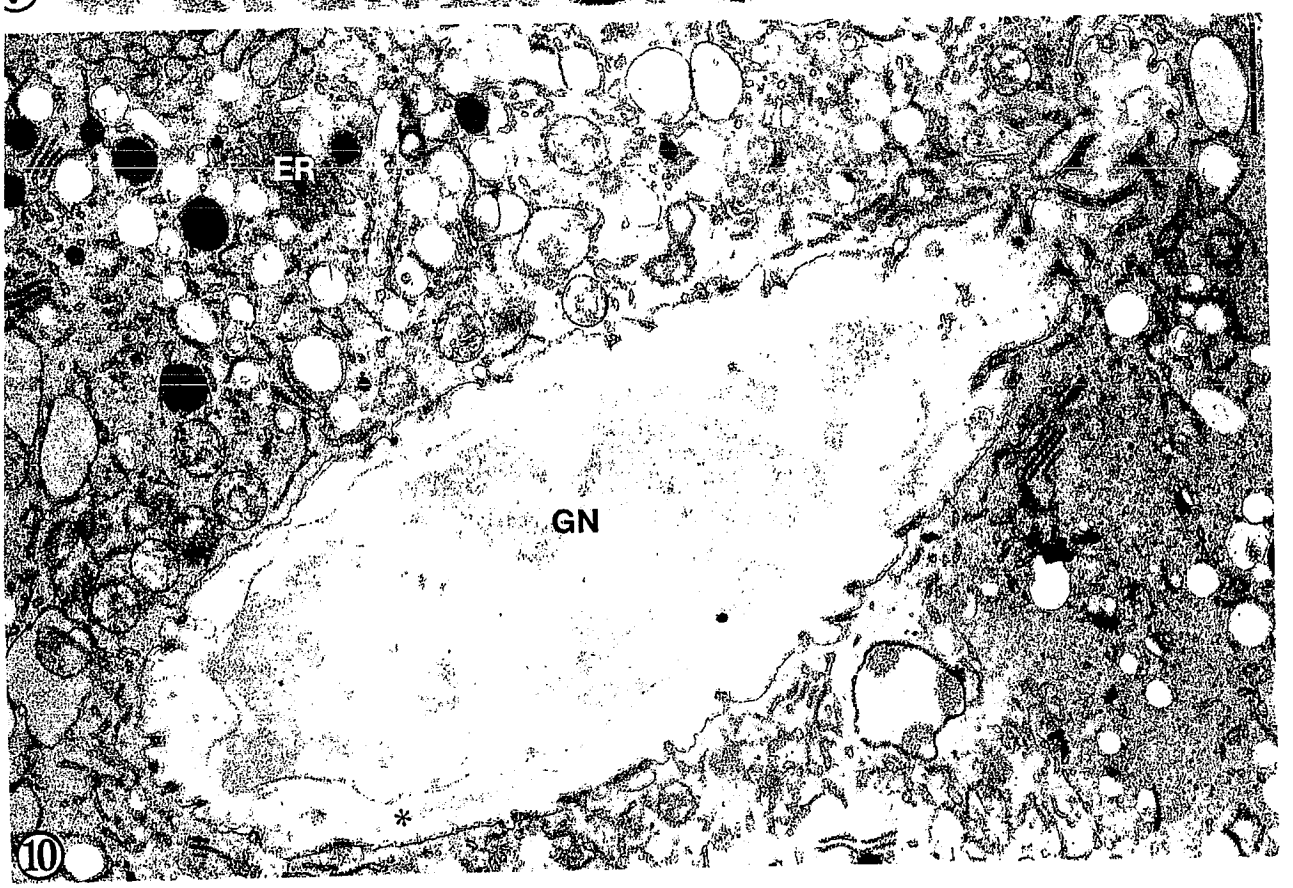
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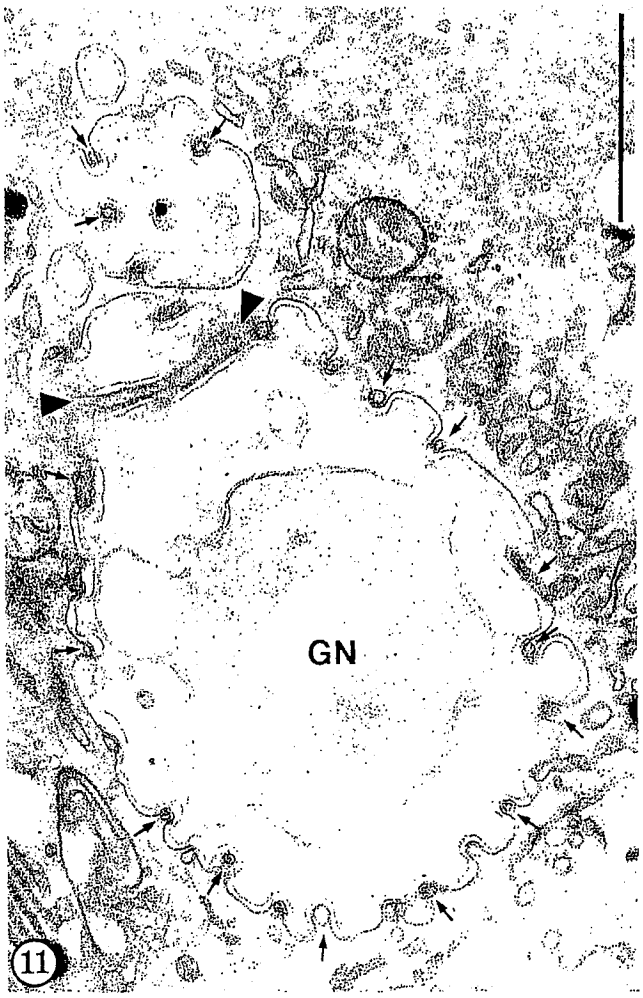

Fig. 11. Section through the terminal part of the generative cell. Profiles of ER are visible in each invagination (arrows). One invagination is orientated transverse to the longitudinal axis of the GC (arrowheads). *GN* Generative nucleus. PATAg stained; bar: 1 µm

Fig. 12. Huge stack of rough endoplasmic reticulum in the vegetative cytoplasm of a mature pollen grain. *V* Vacuole, *P* plastid. UA-Pb stained; bar: 1 µm

of the vegetative cell (Horner and Pearson 1978, Weber 1988 b, Van Aelst and Van Went 1991). A close association of ER and the GC is only briefly mentioned by Keijzer and Willemse (1988), Cresti et al. (1990 a), and Lancelle and Hepler (1992). The first detailed report on the latter phenomenon was published by Noguchi and Ueda (1990). These authors studied mature pollen grains of *Tradescantia reflexa* by rapid freeze fixation-freeze substitution (RF-FS). They found branches of the vegetative cell ER in all surface concavities of the GC. The authors discussed two possible roles of the ER: (1) Since the generative cytoplasm lacks reserve material, it is possible that energy-carrying substances are conveyed through the channels of ER into the GC. Spherical ER terminals at the bottom of the concavities are thought to facilitate transport of molecules. (2) Vegetative ER strands are always attached to the bottom of the concavities in the undulated surface of the GC, while microtubules and microfilaments are not found near these lobes. Because of this unique feature Noguchi and Ueda presumed that the ER depresses the plasma membranes inward to form concavities and thus is involved in the formation of the outer shape of the GC.

A similar role may be played by the ER in pollen of *Cyrtandra pendula*. Occasionally a lipid body is found in a mature GC; nevertheless a function of the vegetative ER as transport channels for mobilized and decomposed lipids seems to be likely. Some of the ER cisternae are arranged in huge stacks, containing up to

cisternae. These ER-aggregations, which are rded as storage sites (Jensen et al. 1974), are also orted in mature pollen of the closely related plant nily Scrophulariaceae (Jensen et al. 1974, Cresti et al. 88), in the Solanaceae (Kroh 1967) and Euphorbiae (Murgia et al. 1986). However, other ER cisterae are solitary and closely associated with lipid bodies, astids and the GC wall. Moreover, plasmodesmatae structures are observed in the wall of the GC. asmodesmata in the GC wall have also been described Lancelle and Hepler (1992) in RF-FS pollen tubes *Lilium longiflorum*, while Cresti et al. (1987) and oguchi and Ueda (1990) did not find structures that ould enable communication and transport of high olecular substances between vegetative and generae cell.

addition to its role in transportation, there are sevral indications that ER also contributes directly to the rmation of the surface undulations of the GC. While rands of ER are in close contact to the GC at all ases of development, microtubular bundles are und only in mature, elongated GCs. Figures 2, 4, d 5 show extremely closely appressed ER tubules or ort stacks, which follow exactly the outline of the ung GC, maybe inducing the formation of surface vaginations. In a mature GC with its conspicuously dulated cell wall, tubules of vegetative ER are always tached to the bottom of the concavities but never to e top of a lobe; 6–7 microtubular bundles are oriated longitudinally in its cytoplasm. Cross sections f a mature GC reveal that its undulated surface consts of 30 lobes on average. Although microtubular undles are usually located near these lobes they are o few in number to have generated 30 lobes. In conast, ER tubules are found in each surface invagination. It is improbable that they would have occupied e invaginations after their formation. It is therefore kely that the vegetative ER has contributed to the rmation of the surface undulations of the GC. Nouchi and Ueda (1990) assumed an active role of the ER by depressing the plasma membranes of the GC ward to form concavities. However, it could also be terpreted as the ER being included passively in the avities as the GC and VC increase their water content uring pollen maturation.

This remarkable ER configuration around the GC was bserved not only in *C. pendula*, but also in other taxa f the Gesneriaceae (e.g., in *Cyrtandra sandwicensis*, *Aeschynanthus pulcher*, *Didymocarpus pyroliflorus*, *Monophyllaea horsfieldii*; Luegmayr, unpubl. data). Noguchi and Ueda (1990) were the first to report that the shape of cells or cellular organelles is determined by elements other than microtubules and microfilaments. Lancelle et al. (1987) noticed a striking MT-ER structural relationship in RF-SF pollen tubes of *Nicotiana alata*. They proposed that the ER, by being aligned along MT, may control cytoskeletal activity by regulating the concentration of calcium ions and must therefore be considered to elucidate cytoskeletal function. Hepler et al. (1990) considered that the cortical ER in plant cells may play a structural role in anchoring the cytoskeleton and organelles, and might also participate in the communication of signals.

Nevertheless, a possible involvement of microtubules in the formation of the undulated outer shape of the GC cannot be completely excluded. It is generally agreed that the main role of the microtubular bundles is to maintain the spindle shape of the GC (Burgess 1970, Heslop-Harrison et al. 1988), but they are probably also engaged in the movement of the GC during pollen tube growth (Derksen et al. 1985, Lancelle et al. 1987, Cresti et al. 1990 b). Zhou and Yang (1991) studied arrangement pattern of microtubules in isolated GCs. They observed MTs at all developmental stages and reported a great diversity in MT arrangement. The network pattern is reported as the standard type in spherical GCs while in spindle-shaped GCs all MTs are organized in long bundles parallel to the longitudinal axis. In *C. pendula* pollen, single MTs are occasionally detected in the young, spherical GCs, while conspicuous microtubular bundles are visible in mature, elongated GCs. Probably because of their disordered arrangement and the spatially restricted information provided by electron microscopy, MTs are not so easily detected in young GCs.

Another noteworthy feature in pollen of *C. pendula* is, that after the first mitosis young GCs contain a considerable number of plastids (Fig. 1). They have a simple structure, do not contain starch and are evenly distributed throughout the cytoplasm. During pollen maturation the plastids disappear from the GC; no plastids could be detected after its detachment from the intine. *Cyrtandra pendula*, therefore, has the Solanum type of plastid inheritance, which is strictly maternal (Hagemann 1981). This corroborates the findings of Corriveau and Coleman (1988) who reported a maternal inheritance of plastid DNA for two other taxa of Gesneriaceae.

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References

Angold RE (1968) The formation of the generative cell in the pollen grain of *Endymion non-scriptus* (L). J Cell Sci 3: 573–578

Brighigna L, Cecchi Fiordi A, Palandri MR (1981) Ultrastructural investigations on the two-nucleate pollen grain of *Tillandsia caput-medusae* Morr. (Bromeliaceae). Amer J Bot 68: 1033–1041

Burgess J (1970) Cell shape and mitotic spindle formation in the generative cell of *Endymion non-scriptus*. Planta 95: 72–85

Cass DD, Karas I (1975) Development of sperm cells in barley. Can J Bot 53: 1051–1062

Charzynska M, Lewandowska E (1990) Generative cell division and sperm cell association in the pollen grain of *Sambucus nigra*. Ann Bot 65: 685–689

Ciampolini F, Moscatelli A, Cresti M (1988) Ultrastructural features of *Aloe ciliaris* pollen. I. Mature grain and its activation in vitro. Sex Plant Reprod 1: 88–96

Corriveau JL, Coleman AW (1988) Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Amer J Bot 75: 1443–1458

Cresti M, Ciampolini F, Kapil RN (1984) Generative cells of some angiosperms with particular emphasis on their microtubules. J Submicrosc Cytol 16: 317–326

– Lancelle SA, Hepler PK (1987) Structure of the generative cell wall complex after freeze substitution in pollen tubes of *Nicotiana* and *Impatiens*. J Cell Sci 88: 373–378

– Milanesi C, Tiezzi A, Ciampolini F, Moscatelli A (1988) Ultrastructure of *Linaria vulgaris* pollen grains. Acta Bot Neerl 37: 379–386

– – Salvatici P, Van Aelst AC (1990 a) Ultrastructural observations of *Papaver rhoeas* mature pollen grains. Bot Acta 103: 349–354

– Murgia M, Theunis CH (1990 b) Microtubule organization in sperm cells in the pollen tubes of *Brassica oleracea* L. Protoplasma 154: 151–156

Derksen J, Pierson ES, Traas JA (1985) Microtubules in vegetative and generative cells of pollen tubes. Eur J Cell Biol 38: 142–148

Dumas C, Knox RB, Gaude T (1985) The spatial association of the sperm cells and vegetative nucleus in the pollen grain of *Brassica*. Protoplasma 124: 168–174

Hagemann R (1981) Unequal plastid distribution during the development of the male gametophyte of angiosperms. Acta Bot Soc Pol 50: 321–327

Hepler PK, Palevitz BA, Lancelle SA, McCauley MM, Lichtscheidl IK (1990) Cortical endoplasmic reticulum in plants. J Cell Sci 96: 355–373

Heslop-Harrison J (1968) Synchronous pollen mitosis and the formation of the generative cell in massulate orchids. J Cell Sci 3: 457–466

– Heslop-Harrison JS, Heslop-Harrison Y (1986) The compartment of the vegetative nucleus and generative cell in the pollen and pollen tubes of *Helleborus foetidus* L. Ann Bot 58: 1–12

– Heslop-Harrison Y, Cresti M, Tiezzi A, Moscatelli A (1988) Cytoskeletal elements, cell shaping and movement in the angiosperm pollen tube. J Cell Sci 91: 49–60

Horner HT, Pearson CB (1978) Pollen wall and aperture development in *Helianthus annuus* (Compositae: Heliantheae). Amer J Bot 65: 293–309

Jensen WA, Ashton M, Heckard LR (1974) Ultrastructural studies of the pollen of subtribe Castilleiinae, family Scrophulariaceae. Bot Gaz 135: 210–218

Kaul V, Theunis CH, Palser BF, Knox RB, Williams EG (1987) Association of the generative cell and vegetative nucleus in pollen tubes of *Rhododendron*. Ann Bot 59: 227–235

Keijzer CJ, Willemse MTM (1988) Tissue interactions in the developing locule of *Gasteria verrucosa* during microgametogenesis. Acta Bot Neerl 37: 475–492

Kroh M (1967) Fine structure of *Petunia* pollen germinated "in vitro". Rev Paleobot Palynol 3: 197–203

Lancelle SA, Hepler PK (1992) Ultrastructure of freeze-substituted pollen tubes of *Lilium longiflorum*. Protoplasma 167: 215–230

– Cresti M, Hepler PK (1987) Ultrastructure of the cytoskeleton in freeze-substituted pollen tubes of *Nicotiana alata*. Protoplasma 140: 141–150

McConchie CA, Jobson S, Knox RB (1985) Computer-assisted reconstruction of the male germ unit in pollen of *Brassica campestris*. Protoplasma 127: 57–63

Mogensen HL (1986) Juxtaposition of the generative cell and vegetative nucleus in the mature pollen grain of Amaryllis (*Hippeastrum vitatum*). Protoplasma 134: 67–72

– (1992) The male germ unit: concept, composition, and significance. Int Rev Cytol 140: 129–147

Murgia M, Wilms HJ, Cresti M, Cesca G (1986) Ultrastructure of pollen development in *Euphorbia dulcis* L. I. Diploid plants. Acta Bot Neerl 35: 405–424

Nakamura S, Miki-Hirosige H (1985) Fine-structural study on the formation of the generative cell wall and intine-3 layer in a growing pollen grain of *Lilium longiflorum*. Amer J Bot 72: 365–375

Noguchi T (1990) Consumption of lipid granules and formation of vacuoles in the pollen tube of *Tradescantia reflexa*. Protoplasma 156: 19–28

– Ueda K (1990) Structure of pollen grains of *Tradescantia reflexa* with special reference to the generative cell and the ER around it. Cell Struct Funct 15: 379–384

Palevitz BA (1993) Relationship between the generative cell and vegetative nucleus in pollen tubes of *Nicotiana tabacum*. Sex Plant Reprod 6: 1–10

Sanger JM, Jackson WT (1971 a) Fine structure study of pollen development in *Haemanthus katherinae* Baker. I. Formation of vegetative and generative cells. J Cell Sci 8: 289–301

– – (1971 b) Fine structure study of pollen development in *Haemanthus katherinae* Baker. II. Microtubules and elongation of the generative cells. J Cell Sci 8: 303–315

Schlag M, Hesse M (1992) The formation of the generative cell in *Polystachya pubescens* (Orchidaceae). Sex Plant Reprod 5: 131–137

Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26: 31–43

Strasburger E (1878) Über Befruchtung und Zellteilung. Hermann Dabis, Jena

Thiéry JP (1967) Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. J Microsc 6: 987–1018

Van Aelst AC, Van Went JL (1991) The ultrastructure of mature *Papaver dubium* L. pollen grains. Acta Bot Neerl 40: 319–328

– Mueller T, Dueggelin M, Guggenheim R (1989) Three-dimensional observations on freeze-fractured frozen hydrated *Papaver dubium* pollen with cryo-scanning electron microscopy. Acta Bot Neerl 38: 25–30

r M (1988 a) Formation of sperm cells in *Galium mollugo* (Rubiaceae), *Trichodiadema setuliferum* (Aizoaceae) and *Avena sativa* 'oaceae). Plant Syst Evol 161: 53–64

988 b) Metabolism of P-particles (polysaccharide particles) in mature pollen grains of *Eryngium campestre* L. (Apiaceae). Protoplasma 146: 65–71

Zhou C, Yang HY (1991) Microtubule changes during the development of generative cells in *Hippeastrum vittatum* pollen. Sex Plant Reprod 4: 293–297

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