Protoplasma (1998) 203: 144–152

PROTOPLASMA

The nuclear reticulum in placental cells of *Lilium regale* is a part of the endomembrane system

S. Singh, M. D. Lazzaro, and **B. Walles***

Department of Botany, Stockholm University, Stockholm

Received May 1, 1998
Accepted June 2, 1998

Summary. Placental cells line the ovarian transmitting tract in *Lilium regale* and produce exudates for secretion. Sections through the highly lobed nuclei of these cells reveal the presence of membrane profiles which form vesicles with varying dimensions in cross section. Computer reconstruction of the nucleus reveals that the vesicle profiles form a complex reticulum of tubular cisternae, which spans the whole nucleus, enclosing a maze of continuous lumen space. Connections between the vesicles and the inner nuclear envelope are visible at various points along the nuclear envelope. This complex network of tubules which constitutes the reticulum arises from the inner nuclear membrane. The nuclear reticulum dramatically increases the inner-envelope surface area, comprising 82% of the total membrane perimeter of inner nuclear envelope and nuclear reticulum. The inner nuclear envelope invaginates into the nucleus forming the nuclear reticulum and the outer nuclear envelope evaginates into the endoplasmic reticulum (ER), indicating that there is a continuity between the lumens of the nuclear reticulum and the ER. The nuclear reticulum is labelled with zinc iodide-osmium tetroxide, a staining pattern identical to that seen in the ER. Positive reaction to the zinc iodide-osmium tetroxide indicates that the nuclear reticulum is a site for Ca^{2+} deposition. The nuclear reticulum forms an extension of the endomembrane system which reaches deep into the nucleoplasm. The lumenal continuity of this system means that there is a channel for communication from the cytoplasm into the nucleoplasm, and that this channel sequesters calcium.

Keywords: Endomembrane system; *Lilium regale*; Nucleus; Placental cell; Transfer cell; Transmitting tissue.

Abbreviations: ER endoplasmic reticulum; TEM transmission electron microscope; ZIO zinc iodide-osmium tetroxide.

*Correspondence and reprints: Department of Botany, Stockholm University, S-106 91 Stockholm, Sweden.
E-mail: walles@botan.su.se

Introduction

The ovarian transmitting tissue in *Lilium regale* consists of a single layer of epidermal cells on the placenta. These epidermal cells, here referred to as placental cells, are papilla shaped with a domed top on the side facing the lumen (ovarian locus). The cell wall in this dome invaginates into the cell to produce branched convolutions (Singh and Walles 1992, 1995). These characteristics are typical of a transfer cell (Gunning and Pate 1969, Gunning 1977). The predominant physiological function of transfer cells is increased transport across the plasma membrane. In *Lilium* spp., transfer cells also line the stylar canal (Rosen and Thomas 1970, Vasil'ev 1970, Dashek et al. 1971, Yamada 1974, Dickinson et al. 1982, Hu et al. 1982, Gawlik 1984, Mike-Hirosige et al. 1987). The placental cells of *L. regale* are rich in organelles including mitochondria, chloroplasts, ribosomes, polysomes, lysosomes, multivesicular bodies, an extensive endomembrane system and a deeply lobed nucleus (Singh and Walles 1995). Cross sections through the nuclei often show the presence of numerous vesicular structures, some of which may connect to the inner nuclear envelope. Similar vesicles were found in stylar-canal cells (Walles unpubl.). A smaller number of nuclear vesicles was also found in the parenchyma cells underlying the placental cells. In a previous paper we suggested that these vesicular structures may be part of a continuous and extensive reticulum within the nucleus (Singh and Walles 1995). The purpose of the present study was to test

this hypothesis. We have therefore collected serial sections through the nucleus and generated digital reconstructions of the vesiculate structures. These serial sections were aligned with the public-domain computer program NIH Image, stacked on top of one another, and then projected as a reconstruction of the reticulum. This technique provides a more accurate interpretation of the structural arrangement of the components of the reticulum relative to one another inside the confined space of the nucleus.

Both the membrane perimeter and tubule diameter of this reticulate network inside the nucleus were measured, and comparative measurements of the endoplasmic reticulum (ER) in the cytoplasm adjacent to the nuclear envelope were obtained. In addition, the composition of the lumen of the reticulate network was analyzed histochemically with zinc iodide-osmium tetroxide (ZIO) staining.

Material and methods

Lilium regale bulbs were obtained from commercial suppliers. Potted bulbs were stored at 4 °C in the dark. The bulbs were brought into the greenhouse as needed and grown with average day and night temperatures of 22 °C and 17 °C, respectively.

For conventional transmission electron microscopy of placental cells, mature ovaries from one to six days after anthesis were cut into 1 mm^3 pieces and fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 4 h at 20 °C under constant rotation. The fixed tissue was rinsed in buffer and stored overnight at 4 °C. The tissue was then treated with 1% osmium tetroxide in 0.1 M buffer for 2 h, dehydrated through an acetone series and embedded in Spurr's epoxy resin. Serial sections (65 or 70 nm thick) were cut on an RMC MT 7000 ultramicrotome, collected on formvar-coated slot grids, and stained with uranyl acetate and lead citrate.

The ZIO reaction was completed with a method modified from Hall and Hawes (1991). Ovaries were dissected into 1 mm^3 pieces and fixed with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) for 4 h at 20 °C, rinsed in buffer and stored overnight at 4 °C. A stock solution of zinc iodide was freshly prepared by mixing 3 g of zinc powder in 20 ml of 0.1 M cacodylate buffer (pH 7.2) and adding 1 g of resublimed iodine. The tissue was then postfixed in either this mixture of zinc iodide and 1% OsO_4 in 0.05 M cacodylate buffer (pH 7.2) or in control solutions of 1% OsO_4 in 0.05 M cacodylate buffer (pH 7.2) or 1% OsO_4 in water, for 8 h at 20 °C. Both treated and control tissues were then dehydrated in acetone, embedded in Spurr's epoxy resin, sectioned, and post-stained with uranyl acetate and lead citrate. Sections were examined with a Zeiss EM 906.

Quantitative measurements and projections of the nuclear reticulum were completed with the public-domain program NIH Image on a Macintosh computer. TEM images were captured digitally with a Sony XC-77 CE videocamera connected to the side port on the column of the Zeiss 906. The video signal was displayed on a high-resolution video monitor and was also captured in digital form with a Scion VG-5 video card on a Power Macintosh 7200. Adjacent serial sections of the nuclear reticulum and nuclear envelope were examined. The first section in a series was displayed on the video monitor and captured to digital form. The details of the membrane profiles of the nuclear reticulum were carefully traced with NIH Image. To align adjacent sections the contours of the nuclear envelope were lightly traced on the video monitor and then the same cell area in the adjacent section was found with the TEM. The nuclear envelope in this section was aligned with the previous trace by rotating the TEM image (when needed) by the Zeiss 906 control for image rotation. When the nuclear envelope was aligned accurately with the envelope trace from the previous section, the TEM image was captured in digital form and the profiles of the nuclear reticulum traced by NIH Image. This process was then repeated with each successive section through the entire series, and proved to be the most accurate way to align adjacent serial sections.

The digital imaging system was calibrated by capturing a carbon replica of a line block shadowed with germanium on a 200-mesh copper grid. The spacing between each line on this block was 2.190 nm centre to centre. Images were captured through the entire magnification series of the Zeiss 906, and the line spacing on these images was measured with NIH Image, generating a set of calibration standards to convert pixels to nanometers. Aligned images from adjacent serial sections were stacked in image space and projected as a reconstruction.

Quantitative measurements were completed with NIH Image. The nuclear reticulum within 4.0 μm of the inner envelope and the endoplasmic reticulum within 4.5 μm of the outer envelope were measured. For the nuclear reticulum, serial sections (average of 8 per cell) were measured in 9 cells, while for the ER, serial sections (average of 7 per cell) for 10 cells were used. For membrane perimeter, the entire inner and outer nuclear envelope, nuclear reticulum, and ER reticulum in the captured images were measured. For the luminal diameter, on average 13 measurements for each section were taken at random across the membrane profiles.

Results

The nuclei of placental cells of *L. regale* are large and convoluted with deep cytoplasmic strands projecting into the interior, increasing the surface area of the nuclear envelope (Fig. 1). Sections through the nucleus reveal that membrane profiles are present inside the nucleus, throughout the nucleoplasm (Figs. 2 and 3). In cross section these profiles form vesicles with varying dimensions. The average diameter is 147 ± 2.5 nm (Table 1) but can vary from 20 nm to 500 nm. The vesicles are scattered throughout the nucleoplasm but are also closely associated with the heterochromatin. Sometimes the vesicles appear to protrude from or disappear into condensed masses of chromatin. Vesicles form long tubular extensions from the nuclear envelope into the nucleoplasm (Fig. 4). The vesicle lumen often contains a granulo-fibrillar material (Fig. 5) and the membranes have the characteristic lipid bilayer configuration (Fig. 6). No association with the nuclear-pore complex has been observed.

There are distinct connections between the inner nuclear envelope and the vesicles at several places along the nuclear envelope (Figs. 4, 7 and 9). At some places the inner membrane invaginates into the nucleoplasm forming a connection with the vesicles and across the perinuclear space, the outer membrane of the nuclear envelope projects into the cytoplasm forming a continuity with the ER (Fig. 7). The outer membrane of the nuclear envelope is continuous with the ER and the inner membrane forms the nuclear reticulum, resulting in a direct connection between their respective lumens through the perinuclear space.

Since the vesicular profiles within the nucleus were often tubular in shape, we collected serial sections to investigate the organization of this membrane system. Serial sections taken through the nucleus were photographed in the TEM (Fig. 8 a) and the TEM image was also captured on the computer. The membrane profiles were then traced with the image analysis software (Fig. 8 b). The lumen spaces were filled with white and the stacks of filled-in vesicles were then projected by the software in image space. These projections indicate that the vesicle profiles actually form a complex reticulum of tubular cisternae within the nucleus (Fig. 9). These tubular cisternae are extensively branched. The individual branches are usually short but can be quite elongated. The reticulum appears to originate from several places along the nuclear envelope. Each tubule branches repeatedly into the nucleoplasm, forming a labyrinth of tubular cisternae. These patches of reticulate growth can anastomose with each other, forming continuations between the isolated groups of protuberances. Even though they emerge from distinct positions on the inner nuclear envelope, the cisternae converge to form a single continuous network of interconnected tubular reticulum.

Since the nuclear reticulum is similar in three-dimensional organization to the ER, we measured the lumen diameter and membrane perimeter, and calculated proportions to the nuclear-envelope surface area for both the nuclear reticulum and ER. The lumen of the nuclear reticulum was 147 nm in diameter, ranging from 20 to 500 nm, while the ER lumen was smaller and less variable, only 28 nm (Table 1), ranging from 14 to 45 nm. Within 4 μm of the nuclear envelope, the ER perimeter was 37309 nm, compared to 16942 nm for the nuclear reticulum. The ER surface area was 93% of the membrane total of the ER and outer

Table 1. Quantitative comparison of various parameters of the ER and the nuclear reticulum

Parameter	Value (mean ± SE per cell)	No. of cells	No. of measurements
Inner-nuclear-envelope perimeter (nm)	3617 ± 211	9	71
Nuclear-reticulum perimeter (nm)	16942 ± 590	9	71
Outer-nuclear-envelope perimeter (nm)	2890 ± 87	10	66
ER perimeter (nm)	37309 ± 1234	10	66
Nuclear-reticulum lumen diameter (nm)	147 ± 2.5	9	969
ER lumen diameter (nm)	28 ± 0.4	10	876
% Nuclear reticulum of nuclear-reticulum-and-inner-envelope surface area	82	9	71
% ER of ER-and-outer-envelope surface area	93	10	66

Fig. 1. Secretory placental cells line the transmitting tract of the ovary and contain large, deeply lobed nuclei in this light micrograph. Bar: 10 μm

Fig. 2. The secretory placental cells contain a dense cytoplasm with numerous mitochondria, plastids, and an elaborate endomembrane system. Immature cells two days after anthesis. Bar: 4 μm

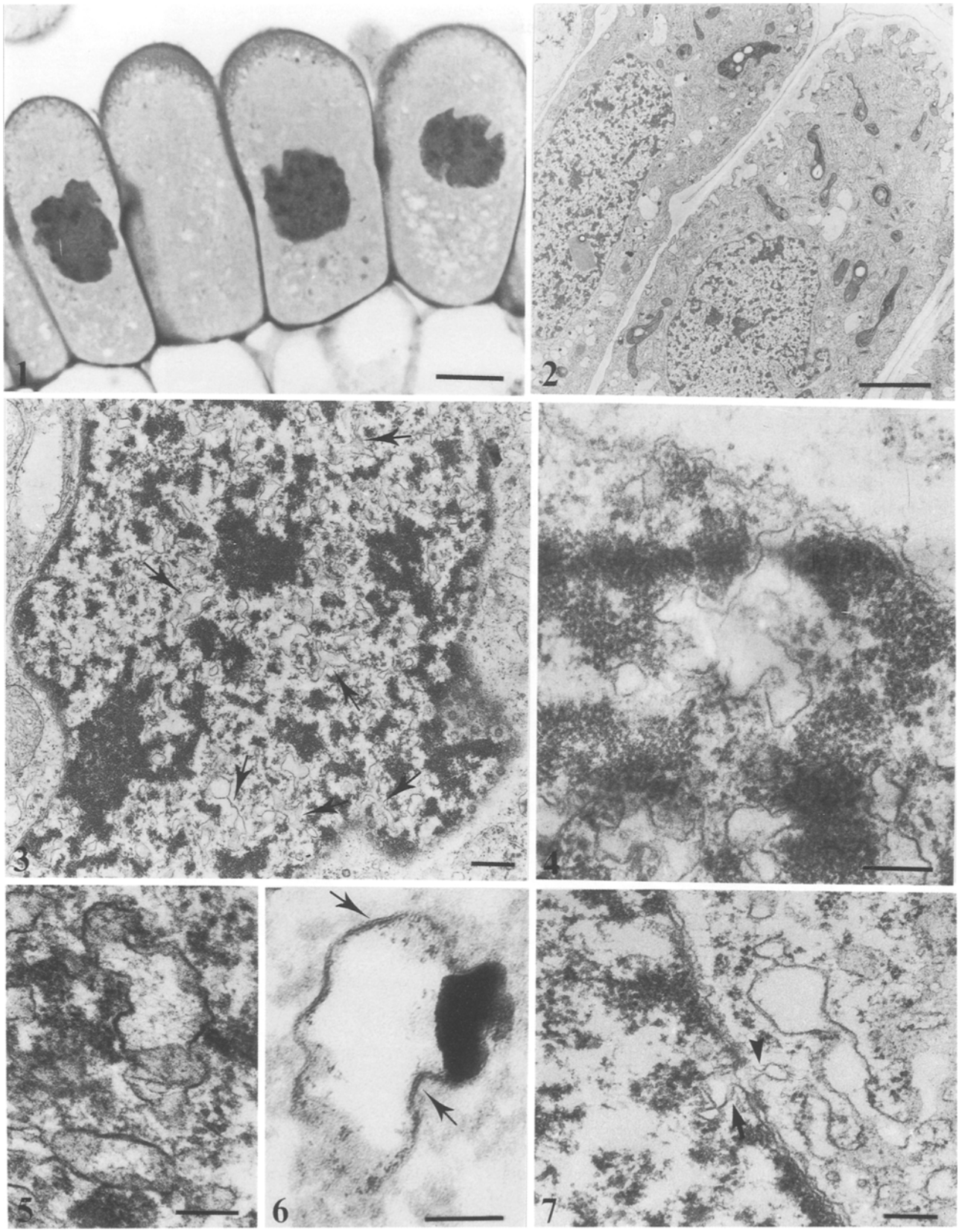
Fig. 3. The nuclear reticulum spans the entire nucleus in placental cells. Some profiles of the reticulum are elongated and branched (arrows). Bar: 500 nm

Fig. 4. Tubules of the nuclear reticulum branch as they emerge from the inner nuclear envelope. Several other branched tubules of the nuclear reticulum are also visible. Bar: 200 nm

Fig. 5. The nuclear-reticulum lumen contains a granulo-fibrillar matrix. Bar: 200 nm

Fig. 6. At high magnification, the triple-layer configuration (arrows) of the nuclear-reticulum membrane is distinct. The section is stained with ZIO. Zinc osmate precipitate is seen as electron scattering granules in the lumen. Bar: 100 nm

Fig. 7. The inner nuclear membrane invaginates into the nucleus forming the nuclear reticulum (arrowhead). Directly across the perinuclear space the outer nuclear membrane forms a connection with the ER in the cytoplasm (arrow). This indicates that the ER lumen and nuclear-reticulum lumen are contiguous. Bar: 200 nm


1
2
3
4
5
6
7

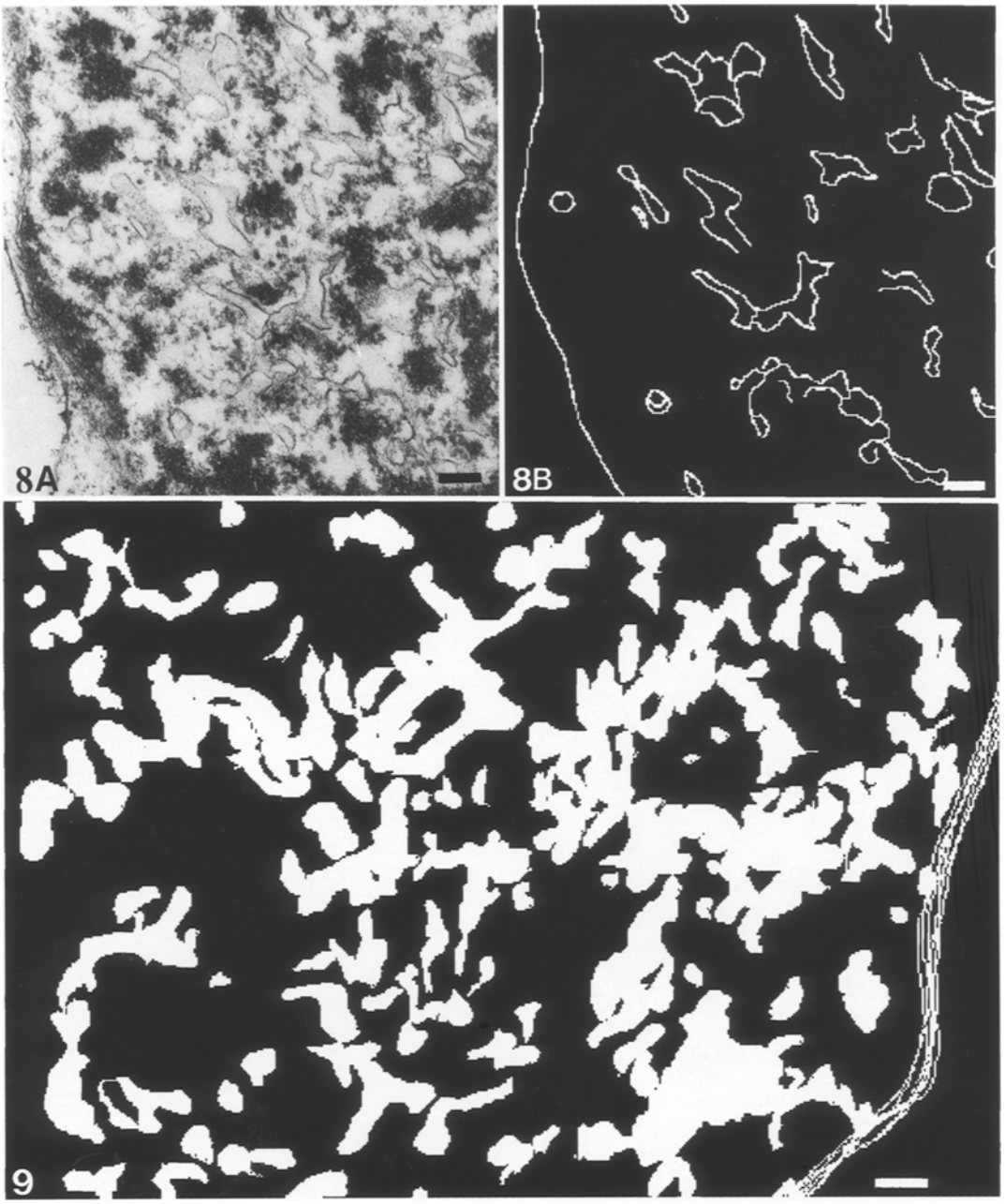


Fig. 8. The nuclear reticulum in the micrograph (**A**) was captured as a digital image and then traced as white lines on a black background (**B**). The nuclear envelope is also visible in the micrograph and trace. Bar: 200 nm

Fig. 9. A computer reconstruction of the nuclear reticulum. This is a single projection from a stack of 8 serial sections. The inner nuclear membrane emerges at various places along the nuclear envelope to produce invaginations into the nucleoplasm, forming a network of tubules. Bar: 200 nm

nuclear envelope, while the nuclear reticulum was 82% of the total of nuclear reticulum and inner nuclear envelope.

The nuclear reticulum frequently contained a granulo-fibrillar matrix (Fig. 5), so we investigated the histochemistry of the lumen. We first tried the osmium-ferricyanide technique for staining the reticulum, but this technique did not work on the placental cells. Even the ER remained unstained after many hours of incubation. We next tried ZIO, which worked well on these cells. ZIO is a histochemical stain for general impregnation of the endomembrane system, probably

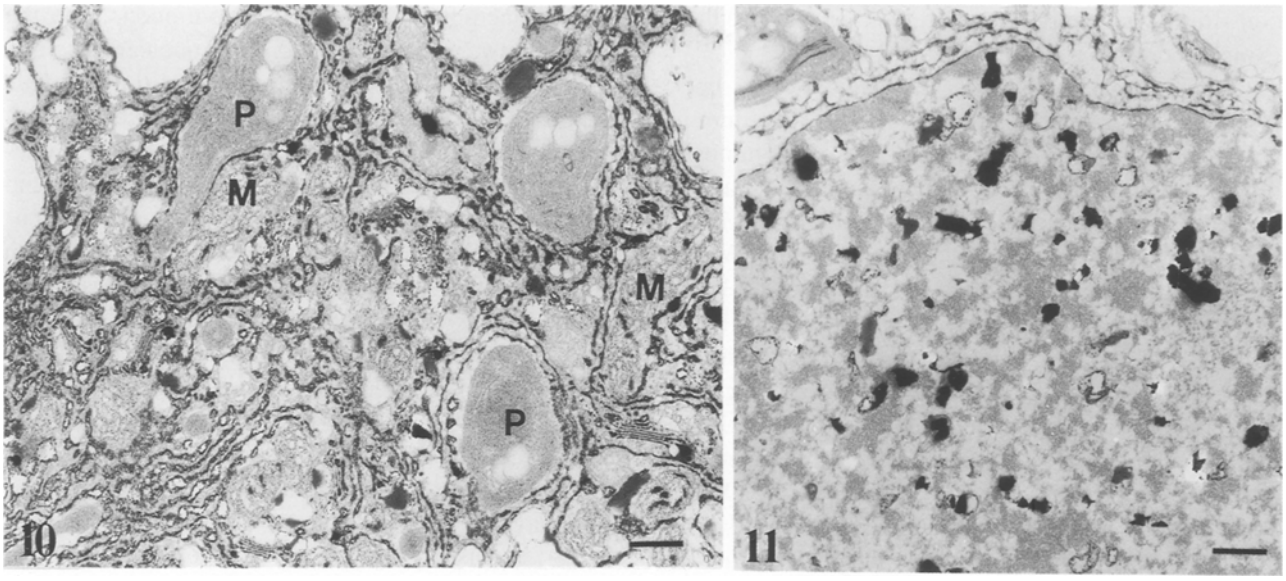


Fig. 10. The entire endomembrane system of the placental cell is stained by the ZIO technique. Note that the plasma membrane and the outer envelope of the plastid (*P*) and mitochondria (*M*) remain unstained. Bar: 500 nm

Fig. 11. The nuclear envelope as well as the profiles of the nuclear reticulum are stained by the ZIO technique. The staining pattern of the nuclear-reticulum tubules is similar to that of dilated profiles of ER in the cytoplasm; some lumens are completely filled while in others the zinc osmate is precipitated only at the periphery. Bar: 500 nm

precipitating in areas of high calcium concentration. The stock solution for the ZIO technique is recommended to be made in an aqueous medium, but in the case of the placental cells, the nuclear reticulum was lost with the conventional method. By substituting cacodylate buffer in the procedure we were able to preserve the reticulum. Tissues stained with the ZIO technique show a selective staining of the endomembrane system, labelling the ER, dictyosomes, vesicles, and the nuclear envelope (Fig. 10). It also occasionally stains the mitochondrial cristae and the plastid thylakoids. In addition to these components the tubular projections of the nuclear reticulum are also stained (Fig. 11). Narrow segments of the reticulum were completely filled with precipitate but in the more dilated regions the precipitate was restricted to the periphery (Fig. 11). The cisternae of ER follow the same pattern of staining whereas the cisternae of the dictyosomes stain very strongly (Fig. 10).

Discussion

In Lilium flowers, the transmitting tissue is predominantly secretory (Welk et al. 1965, Gawlik 1984, Van Roggen et al. 1988, Janson et al. 1994). The exudate is rich in carbohydrates, proteins, lipids, glycoproteins, and arabinogalactans (Labarca et al. 1970, Labarca and Loewus 1972, Aspinall and Rosell 1978, Campbell 1983, Campbell and Linskens 1984, Miki-Hirosige et al. 1987, Jauh and Lord 1996) and provides nourishment, chemotropic guidance, and a substrate for pollen tube growth (Welk et al. 1965; Crang 1969; Kroh et al. 1970; Labarca and Loewus 1972, 1973; Van Roggen et al. 1988; Janson 1993; Janson et al. 1993). The secretory cells also play an important role in the recognition and selection of compatible pollen tubes at different levels along the transmitting tract (Rosen and Gawlik 1966; Ascher and Peloquin 1970; Ascher 1975; Dickinson et al. 1982; Amaki et al. 1989; Amaki and Higuchi 1991; Ichimura and Yamamoto 1991, 1992a, b).

The ultrastructural organization of the placental cells in *L. regale* supports their secretory function (Singh and Walles 1995). A deeply lobed nucleus is a characteristic of metabolically active cells. The lobes provide an increase in the surface area of the nuclear envelope and in the number of nuclear pore complexes. The deep cytoplasmic strands between adjacent lobes would increase the interaction between the nucleus and the cytoplasm and the potential transport of material through the nuclear pores. In the placental cells the inner membrane of the nuclear envelope invaginates into the nucleus at several points forming deep tubules which repeatedly branch and anastomose with adjoining tubules to form a complex network of short, highly branched tubules with varying diameters. These tubules span the whole nucleus and

constitute what we have named the nuclear reticulum (Singh and Walles 1995).

Although various nuclear inclusions have been reported, the organization of the nuclear reticulum in *L. regale* placental cells is different. Commonly reported nuclear inclusion are the so-called nuclear vacuoles (Rasmussen 1976; Sheffield et al. 1979; Karasawa and Ueda 1983a, b; Rowley and Walles 1985; Sangwan 1986; Li and Dickinson 1987; Majewska-Sawka et al. 1990; Y. Wang and Hu 1993; Y. Wang et al. 1994), which occur mainly during meiosis. Nuclear vacuoles have also been associated with degenerating cells (Meek and Moses 1961, Williams and Jordan 1982) and were found in the polar nuclei of the embryo sac of *Vicia faba* (X. Wang and Wang 1992). However, nuclear vacuoles are quite distinct from the nuclear reticulum in their structure as well as in their presumed function.

Various nuclear inclusions can also result from pathological infections (Hong et al. 1997, Gilberet and Gosh 1993, Kanyari 1996, Pinner et al. 1993, Koning et al. 1993, Maeno et al. 1993). Viral infections in plants (Gao and Nassuth 1993, Pinner et al. 1993) can cause nuclear alterations and changes in some cytoplasmic organelles like mitochondria and chloroplasts. However, virus particles were never seen in the plant material used in the present study. Moreover, all organelles in the placental cells appeared healthy and showed no sign of viral influence. For about a decade the nuclear reticulum was routinely observed in the transmitting tract of healthy Lilium plants in our laboratory, and so we are confident that it is not a pathological phenomenon.

As far as we know, the only other nuclear inclusion similar to the nuclear reticulum is a case reported from *L. longiflorum* (Janson 1992). Within the embryo sac of this species, it was found that the nucleus of the antipodal cell at the chalazal end contains membranous inclusions. These structures, which look similar to the nuclear reticulum, were not found in the antipodal cells towards the micropylar end, nor in any other cells of the embryo sac. The two kinds of antipodal cells are also distinct in that the antipodal cell with the nuclear inclusions has a much denser cytoplasm (Janson 1992). The placental cells in the present study are also characterized by a dense cytoplasm.

The nuclear reticulum forms a continuous assembly of membranes enclosing an interconnected internal space, separating it from the rest of the nucleoplasm. Since the nuclear reticulum originates from the inner nuclear membrane, its lumen is continuous with the lumen between the inner and outer nuclear envelope which is a continuation of the lumen of the ER. This means that the lumina of the nuclear reticulum and the ER are continuous. However, the average diameter of the ER lumen (28 nm) is much smaller and more uniform than the diameter of the nuclear reticulum lumen, which averages 147 nm but can vary from 20 to 500 nm. Structurally the labyrinth of branching tubules of the nuclear reticulum is similar to the tubular cisternae of the smooth ER. However, the nuclear reticulum lacks the sheet-like (lamellar) configuration typical of the rough ER (Hepler et al. 1990, McCauly and Hepler 1990).

The inner and outer membranes of the nuclear envelope have different molecular characteristics (Smith and Blobel 1993, Akao et al. 1994, Gerace and Foisner 1994, Malviya 1994, Humbert et al. 1996, Buendia and Courvalin 1997, Yang et al. 1997). Since the nuclear reticulum is an extension of the inner nuclear envelope, it is possible that their lipid and protein composition is similar. The outer membrane of the nuclear envelope is continuous with rough and smooth ER and is biochemically and functionally similar to the bulk ER membrane (Akao et al. 1994, Gerace and Foisner 1994, Yang et al. 1997), facilitating an exchange of soluble proteins between the ER lumen and the perinuclear space (Stinchcombe et al. 1995, Simos et al. 1996, Subramanian and Meyer 1997, Yang et al. 1997). The lumen of the nuclear reticulum is contiguous with the ER lumen and the perinuclear space, so soluble proteins would be able to move throughout this lumenal continuity. The nuclear reticulum does contain a granulo-fibrillar matrix, which is precipitated during sample preparation.

The ZIO technique impregnates the endomembrane system (Harris 1979; Hawes 1981, 1985; Juniper et al. 1982). It is postulated that the Zn^{2+} ions either bind to or displace Ca^{2+} ions from their binding sites on membranes and reduce osmium to form an electron-opaque deposit of zinc osmate (Gilloteaux and Naud 1979). The staining of the nuclear reticulum with the ZIO technique shows that this reticulum is a component of the endomembrane system. It also indicates that the nuclear reticulum is a site for Ca^{2+} deposition. Calcium channels have recently been identified in isolated patches of the inner nuclear envelope (Longin et al. 1997). Since calcium has been implicated in the control of secretion (Read et al. 1992), the impregnation with zinc osmate is an indication

that the nuclear reticulum might function in controlling secretion from these placental cells in *L. regale*. The lumen of the ER and the perinuclear space are sequestration sites for calcium (Hepler et al. 1990).

In conclusion, the secretory pathway in the placental cells of *L. regale* is a functionally interconnected endomembrane system. The nuclear reticulum forms an extension of this endomembrane system which penetrates deep into the nucleoplasm. This reticulum is apparently an additional storage site for calcium, and may provide a pathway for the transport of molecules in and out of the nucleus. It may play a role in controlling secretion.

References

Akao Y, Otsuki Y, Kataoka S, Ito Y, Tsujimoto Y (1994) Multiple subcellular localisation of bcl-2: detection in nuclear outer membrane, endoplasmic reticulum membrane, and mitochondrial membrane. Cancer Res 54: 2468–2471

Amaki W, Higuchi H (1991) Effects of stylar exudates collected from pollinated pistils on pollen tube growth in *Lilium longiflorum*. Sci Hort 46: 147–154

– Tezuka T, Yamamoto Y (1989) Effect of pistillary sap on the in vivo pollen tube growth and changes in components of stylar sap by pollination in *Lilium longiflorum* Thunb. J Jpn Soc Hort Sci 57: 648–654

Ascher PD (1975) Special stylar property required for compatible pollen tube growth in *Lilium longiflorum* Thunb. Bot Gaz 136: 317–321

– Peloquin SJ (1970) Temperature and the self-incompatibility reaction in *Lilium longiflorum* Thunb. J Am Soc Hort Sci 95: 586–588

Aspinall GO, Rosell K-G (1978) Polysaccharide component in the stigmatic exudate from *Lilium longiflorum*. Phytochemistry 17: 919–922

Buendia B, Courvalin J-C (1997) Domain-specific disassembly and reassembly of nuclear membranes during mitosis. Exp Cell Res 230: 133–144

Campbell RJ (1983) Release and nature of pistil fluids in Lilium. In: Proceedings of the VII International Cytoembryo Symposium, High Tatra, Czechoslovakia, 14–17 June, 1982, pp 231–234

– Linskens HF (1984) Successive flushing of stylar exudate of *Lilium longiflorum*. Acta Bot Neerl 33: 177–184

Crang R (1969) Pollination effects on style degradation in *Lilium philippinense*. Trans Am Microsc Soc 88: 294–299

Dashek MV, Thomas HR, Rosen WG (1971) Secretory cells of lilly pistil II: electron microscope cytochemistry of canal cells. Am J Bot 58: 909–920

Dickinson MV, Moriarty J, Lawson J (1982) Pollen pistil interaction in *Lilium longiflorum*: the role of pistil in controlling the pollen tube growth following cross and self pollination. Proc R Soc Lond B Biol Sci 215: 45–62

Gao J-G, Nassuth A (1993) Alteration of major cellular organelle in wheat leaf tissue infected with wheat streak mosaic virus (Bymovirus, Potyviridae). Phytopathology 83: 206–213

Gawlik SR (1984) An ultrastructural study of transmitting tissue development in the pistil of *Lilium leucanthum*. Am J Bot 71: 512–521

Gerace L, Foisner R (1994) Integral membrane proteins and dynamic organization of the nuclear envelope. Trends Cell Biol 4: 127–131

Gilberet R, Gosh HP (1993) Immunoelectron microscopic localization of herpes simplex virus glycoprotein gB in the nuclear envelope of infected cells. Virus Res 28: 217–231

Gilloteaux J, Naud J (1979) The zinc iodide-osmium tetroxide staining-fixative of Maillet. Histochemistry 63: 227–243

Gunning BES (1977) Transfer cells and their role in transport of solutes in plants. Sci Prog Oxford 64: 539–568

– Pate JS (1969) "Transfer cells": plant cells with wall ingrowths, specialization in relation to short distance transport of solutes – their occurrence, structure and development. Protoplasma 68: 107–133

Hall JL, Hawes C (1991) Electron microscopy of plant cells. Academic Press, London

Harris N (1979) Endoplasmic reticulum in developing seeds of *Vicia faba*: a high voltage electron microscope study. Planta 146: 63–69

Hawes CR (1981) Applications of high voltage electron microscopy to botanical ultrastructure. Micron 12: 227–257

– (1985) Stereo-transmission electron microscopy of thick sections and whole cells. In: Robards AW (ed) Botanical microscopy 1985. Oxford University Press, Oxford, pp 65–90

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

Hong T, Summers MD, Braunagel SC (1997) N-terminal sequences from Autographa californica nuclear polyhedrosis virus envelope proteins ODV-E66 and ODV-E25 are sufficient to direct reporter proteins to the nuclear envelope, intranuclear microvesicles and the envelope of occlusion derived virus. Proc Natl Acad Sci USA 94: 4050–4055

Hu Sy, Zhu C, Xu LY (1982) The structure of the canal cells in the style of *Lilium regale*. Acta Bot Sci 24: 395–407

Humbert J-P, Matter N, Artault J-C, Koppler P, Malviya AN (1996) Inositol 1,4,5-triphosphate receptor is located to the inner nuclear membrane vindicating regulation of nuclear calcium signaling by inositol 1,4,5-triphosphate: discrete distribution of inositol phosphate. J Biol Chem 271: 478–485

Ichimura K, Yamamoto Y (1991) Effects of the quantity and composition of stylar canal exudate on in vitro and in vivo growth of pollen tubes in self-incompatible *Lilium longiflorum*. J Jpn Soc Hort Sci 60: 659–668

– – (1992a) Correlation between the accumulation of canal exudate and the diminution of self-incompatibility in ageing flowers of *Lilium longiflorum*. J Jpn Soc Hort Sci 61: 159–165

– – (1992b) Changes in the amount and composition of stylar canal exudate after self- and cross-pollination in self-incompatible *Lilium longiflorum* Thunb. J Jpn Soc Hort Sci 61: 609–617

Janson J (1992) Pollen tube–pistil interaction and fertilization in *Lilium longiflorum*. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands

– (1993) Placental pollination in *Lilium longiflorum* Thunb. Plant Sci 90: 105–115

– Reinders MC, Van Tuly JM, Keijzer CJ (1993) Pollen tube growth in *Lilium longiflorum* following different pollination techniques and flower manipulation. Acta Bot Neerl 42: 461–472

– – Valkering AGM, Van Tuly JM, Keijzer CJ (1994) Pistil exu-

date production and pollen tube growth in *Lilium longiflorum* Thunb. Ann Bot 73: 437–446

Jauh GY, Lord EM (1996) Localization of pectins and arabinogalactan-proteins in lily (*Lilium longiflorum* L.) pollen tube and style, and their possible roles in pollination. Planta 199: 251–261

Juniper BE, Hawes CR, Horne JC (1982) The relationship between the dictyosomes and the forms of endoplasmic reticulum in plant cells with different export programs. Bot Gaz 143: 135–145

Kanyari PWN (1996) A comparison of the ultrastructural aspects of macrogametogenic stages of two *Eimeria* species of goats (*Capra hircus*). Disc Innov 8: 181–188

Karasawa R, Ueda K (1983a). Nuclear vacuoles and synthesis during meiotic prophase in *Haplopappus gracilis*. Cytologia 48: 119–126

– – (1983b). Occurrence of nuclear vacuoles in meiotic prophase nuclei in Compositae. Caryologia 36: 145–153

Koning AJ, Lum PY, Williums JM, Wright R (1993) DIOC-6 staining reveals organelle structure and dynamics in living yeast cells. Cell Motil Cytoskeleton 25: 111–128

Kroh M, Miki-Hirosige H, Rosen W, Loewus F (1970) Incorporation of label into pollen tube wall from myoinositol-labeled *Lilium longiflorum* pistils. Plant Physiol 45: 92–94

Labarca C, Loewus F (1972) The nutritional role of pistil exudate in pollen tube wall formation in *Lilium longiflorum* I: utilization of injected stigmatic exudate. Plant Physiol 50: 7–14

– – (1973) The nutritional role of pistil exudate on pollen tube wall formation in *Lilium longiflorum* II: production and utilization of exudate from stigma and stylar canal. Plant Physiol 52: 87–92

– Kroh M, Loewus F (1970) The composition of stigmatic exudate from *Lilium longiflorum*: labelling studies with myo-inositol, D-glucose and L-proline. Plant Physiol 46: 150–156

Li FL, Dickinson HG (1987) The structure and function of nuclear invaginations characteristic of microsporogenesis in *Pinus banksiana*. Ann Bot 60: 321–330

Longin A-S, Mezin P, Favier A, Verdetti J (1997) Presence of zinc and calcium channels in the inner membrane of the nuclear envelope. Biochem Biophy Res Commun 235: 236–241

Maeno Y, Toyoshima T, Fujioka H, Ito Y, Meshnick SR, Benakis A, Milhous WK, Aikawa M (1993) Morphologic effects of artemisinin in *Plasmodium falciparum*. Am J Trop Med Hyg 49: 485–491

Majewska-Sawka A, Bohdanowicz J, Jassen B, Rodriguez-Garcia MI (1990) Development of nuclear vacuoles in the sugar beet male meiocytes. Ann Bot 66: 139–146

Malviya AN (1994) The nuclear inositol 1,4,5-triphosphate and inositol 1,3,4,5-tetrakisphosphate receptors. Cell Calcium 16: 301–313

McCauly MM, Hepler PK (1990) Visualization of the endoplasmic reticulum in living buds and branches of the moss *Funaria hygrometrica* by confocal laser scanning microscopy. Development 109: 753–764

Meek GA, Moses MJ (1961) Microtubulation in the inner membrane of the nuclear envelope. J Biophys Biochem Cytol 10: 121–131

Miki-Hirosige H, Hoek IHS, Nakamura S (1987) Secretion from the pistil of *Lilium longiflorum*. Am J Bot 74: 1709–1715

Pinner MS, Medina V, Plaskitt KA, Markham PG (1993) Viral inclusions in monocotyledons infected by maize streak and related geminiviruses. Plant Pathol 42: 75–87

Rasmussen SW (1976) The meiotic prophase in *Bombyx mori* females analysed by 3-dimensional reconstructions of synaptonemal complex. Chromosoma 54: 245–293

Read ND, Allan WTG, Knight H, Knight MR, Malho R, Russel A, Shacklock PS, Trewavas AJ (1992) Imaging and measurement of cytosolic free calcium in plant and fungal cells. J Microsc 166: 57–86

Rosen WG, Gawlik SR (1966) Relation of lily pollen tube fine structure to pistil compatibility and mode of nutrition. In: Proceedings of the VI International Congress of Electron Microscopy. Maruzen, Tokyo, pp 313–314

– Thomas HR (1970) Secretory cells of lily pistils I: fine structure and function. Am J Bot 57: 1108–1114

Rowley JR, Walles B (1985) Cell differentiation in microsporangia of *Pinus sylvestris* III: late pachytene. Nord J Bot 5: 255–271

Sangwan RS (1986) Formation and cytochemistry of nuclear vacuoles during meiosis in *Datura*. Eur J Cell Biol 40: 210–218

Sheffield E, Cawood AH, Bell PR, Dickinson HG (1979) The development of nuclear vacuoles during meiosis in plants. Planta 146: 597–601

Simos G, Maison C, Georgatos SD (1996) Characterizatioin of p 18, a component of the lamin B receptor complex and a new integral membrane protein of the avian erythrocyte nuclear envelope. J Biol Chem 271: 12617–12625

Singh S, Walles B (1992) The ovarian transmitting tissue in *Lilium regale*. Int J Plant Sci 153: 205–211

– – (1995) Ultrastuctural differentiation of the ovarian transmitting tissue in *Lilium regale*. Ann Bot 75: 455–462

Smith S, Blobel G (1993) The first membrane spanning region of the lamin B receptor is sufficient for sorting to the inner nuclear membrane. J Cell Biol 120: 631–655

Stinchcombe JC, Nomoto H, Cutler DF, Hopkins CR (1995) Anterograde and retrograde traffic between the rough endoplasmic reticulum and the golgi complex. J Cell Biol 131: 1387–1401

Subramanian K, Meyer T (1997) Calcium-induced restructuring of nuclear envelope and endoplasmic reticulum calcium stores. Cell 89: 963–971

Van Roggen PM, Keijzer CJ, Wilms HJ, Van Tuly JM, Stals AWDT (1988) An SEM study of pollen tube growth in intra- and interspecific crosses between *Lilium* species. Bot Gaz 149: 365–369

Vasil'ev AE (1970) Ultrastructure of stigmatoid cells in Lilium. Fiziol Rast 17: 1240–1248

Wang XL, Wang YZ (1992) Studies of ultrastructure and ATPase localization of mature embryo sac in *Vicia faba* L. Acta Bot Sin 34: 862–867

Wang Y, Hu S-Y (1993) Development of nuclear vacuoles in prophasic nucleus of *Populus simonii*. Chin J Bot 5: 189–192

– Yang S-J, Li M-Y, Lou C-H (1994) Nuclear invagination and nuclear vacuole formation in several plants. Acta Bot Sin 36: 963–966

Welk M, Millington WF, Rosen WG (1965) Chemotropic activity and the pathway of the pollen in lily. Am J Bot 52: 774–781

Williams LM, Jordan EG (1982) Nuclear and nucleolar inclusions in root tip of *Brassica napus* L. Protoplasma 113: 189–192

Yamada Y (1974) An electron microscopic study of the secretory zone in the canal cell of *Lilium longiflorum*. Sci Rep Fac Educ Gunma Univ 23: 87–103

Yang L, Guan T, Gerace L (1997) Integral membrane proteins of the nuclear envelope are dispersed throughout the endoplasmic reticulum during mitosis. J Cell Biol 137: 1199–1210