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Structural Aspects of Protein Secretion in Higher Plant Cells[1)]

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Summary

KRISTEN U., LOCKHAUSEN J. & KANDASAMY M. K. 1988. Structural aspects of protein secretion in higher plant cells. – Phyton (Austria) 28 (2): 183–191, with 4 figures (1 plate).–English with German summary.

Intracellular transport of secretory proteins in animal cells mainly occurs via the Golgi apparatus. Therefore, the animal dictyosomes are closely associated with the rough endoplasmic reticulum either by direct membrane connections or by transitory vesicles. In higher plant cells such associations are less frequent and less distinct. This fact is due to the relative small amounts of proteins which are usually mixed into the polysaccharide secretion.

We present some examples of higher plant cells which clearly exhibit associations between the endoplasmic reticulum and dictyosomes. By treatment of tobacco pollen tubes with pentachlorophenol (PCP) we demonstrate that certain ER regions, where transitory vesicles usually bud off, are compatible with portions of the dictyosomal cis face and with marginal regions of the Golgi cisternae. PCP causes energy depletion by interfering with the respiratory chain of the mitochondria.

A computer-generated three-dimensional reconstruction is used to show that both types of dictyosome-ER-associations, direct connections and transitory vesicles, are present in higher plant cells.

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Zusammenfassung

KRISTEN U., LOCKHAUSEN J. & KANDASAMY M. K. 1988. Strukturaspekte der Proteinsekretion in Zellen Höherer Pflanzen. – Phyton (Austria) 28 (2): 183–191, mit 4 Abbildungen (1 Tafel). – Englisch mit deutscher Zusammenfassung.

Der intrazelluläre Transport von Sekretionsproteinen erfolgt in tierischen Zellen hauptsächlich über den Golgi-Apparat. Daher sind die tierischen Dictyosomen entweder durch direkte Membrankontakte oder durch transitorische Vesikel mit dem rauhen Endoplasmatischen Retikulum verbunden. In Höheren Pflanzen kommen solche Verbindungen weniger häufig und weniger ausgeprägt vor. Diese Tatsache beruht vorwiegend auf der geringen Menge an Proteinen, die gewöhnlich den Polysaccharidsekreten beigemischt sind.

Wir behandeln einige Beispiele von Zellen Höherer Pflanzen, die deutliche Verbindungen zwischen dem ER und Dictyosomen aufweisen. Durch Inkubation von Tabakpollenschläuchen mit Pentachlorphenol (PCP) zeigen wir, daß bestimmte ER-Bereiche, an denen normalerweise transitorische Vesikel abgeschnürt werden, mit der cis-Seite der Dictyosomen und mit Randzonen der Golgi-Zisternen kompatibel sind. PCP verursacht Energieverarmung durch Eingriff in die Atmungskette der Mitochondrien.

Mit Hilfe der computergestützten dreidimensionalen Rekonstruktion zeigen wir, daß beide Typen von Dictyosomen-ER-Verbindungen, nämlich die direkte und die indirekte über transitorische Vesikel, in Höheren Pflanzen vorhanden sind.

Introduction

Secretion of polysaccharides in higher plants has been studied for a long time. It is now generally accepted that most types of non-cellulosic polysaccharides are synthesized in the Golgi apparatus (GA), transfered to the plasma membrane by Golgi vesicles and extruded by exocytosis. It has, however, been questioned for a long time whether proteins which derive from the rough ER follow the same secretional pathway in higher plant cells as in animal cells. The latter were shown to direct their secretory proteins from the rough ER to the plasma membrane via the Golgi apparatus. This classical route of intracellular transport and secretion of proteins suggested by PALADE 1975, involves transitory and secretory vesicles as protein-transfering derivatives of the ER or the dictyosomes, respectively.

In order to answer the question mentioned above it was necessary to search for cell types with protein secretion in higher plants. Some examples have been found during the past decade:

(1) The ovary gland cells of *Aptenia cordifolia* were shown to extrude substances rich in protein. However, their extrusion was concommitant with the degeneration of the cells (KRISTEN 1976, KRISTEN & al. 1980).

(2) It has been suggested that the stigma papillae of the same species secrete proteins directly by ER-derived vesicles bypassing the Golgi apparatus (KRISTEN 1977, KRISTEN & al. 1979). Support for this suggestion came from the studies of the stigma papillae in *Crocus* (HESLOP-HARRISON &

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HESLOP-HARRISON 1975), the digestive glands in *Dionaea muscipula* (ROBINS & JUNIPER 1980) and the marginal ligule cells of *Isoetes lacustris* (KRISTEN & al. 1982).

(3) The classical route of protein transport and secretion, as postulated by PALADE (1975), appears to exist in the secretory trichomes of *Psychotria bacteriophila* (DEXHEIMER 1981), in the submarginal ligule cells of *Isoetes lacustris* (KRISTEN 1980, KRISTEN & al. 1982) and in the leaf glands of *Veronica beccabunga* (LOCKHAUSEN & KRISTEN 1986).

All these species were shown to secrete proteins as an admixture to polysaccharides. In their gland cells the Golgi apparatus is closely associated to the rough ER.

The general possibility of ER-GA associations in higher plant cells can be demonstrated by treatment of the cells with energy-depleting agents. MOLLENHAUER & al. 1975 inhibited the budding of transitory vesicles from the ER by cold treatment of root cap cells. As a result, the dictyosomes became laterally associated with tubular regions of the ER and formed membrane continuities with the ER.

We used the oxidative uncoupler pentachlorophenol (PCP) to inhibit vesicle budding in order to connect ER and dictyosomes in growing pollen tubes of tobacco. Moreover, we demonstrated the close structural relationship between ER and the Golgi apparatus using a computer-generated three-dimensional reconstruction of higher plant dictyosomes. Some results of both investigations, PCP treatment and reconstruction, are presented in this paper.

Materials and Methods

1. Semivivo culture and PCP treatment of pollen tubes

Flowers of *Nicotiana sylvestris* (SPEG. & COMES) var. SCR were emasculated and then pollinated in situ. 24 hr after pollination the pistils were cut just in front of the pollen tube front. The cut stylar ends were incubated in a culture medium (10% sucrose, 0.01% boric acid, 3 mM $Ca(NO_3)_2$ in distilled water; pH 6) for 4 hr at 25°C to obtain short, compact tube bundles protruding from the cut end of the pistil. The semivivo culture technique (according to KROH & KNUIMAN 1982 and to MULCAHY & MULCAHY 1985) and the determination of the tube growth in vivo is described in a previous paper (KANDASAMY & KRISTEN 1987).

1 mg of 99% pure PCP (Riedel de Haen, Hannover, F.R.G.) was dissolved in 100 ml of distilled water. This stock solution was added to the culture medium to obtain a PCP concentration of 2.0 mg L^{-1}. For tube growth inhibition the cut ends of the pollinated pistils were incubated in the culture medium containing PCP, as described above.

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The semivivo grown pollen tube bundles were prepared for ultrastructural analysis as described in a previous paper (KANDASAMY & KRISTEN 1987).

2. Three-dimensional reconstruction of dictyosomes

Transmission electron micrographs taken from a series of 30 successive ultrathin sections across a dictyosome of an ovary gland cell in *Aptenia cordifolia* (L.f.) SCHWANTES were used as basic material for the computer-generated reconstruction. The cells were conventionally fixed using glutaraldehyde/osmium tetroxide and were embedded in Spurr's medium (SPURR 1969) after ethanol dehydration. The ultrathin sections, contrasted with lead citrate, were photographed in a Philips EM 420 electron microscope. The negatives were scanned after being fitted into the drum of a microdensitometer (Optronics P-1000). For storage and further processing, the picture size used was 512 by 512 points. The profile contours of the Golgi and ER cisternae and of the adjacent vesicles were enhanced by a contrast enhancement program. In addition, the computer display process interactively encompassed (1) contour editing and manipulation, (2) connection editing, (3) automatic polygon tiling, (4) surface smoothing and (5) a shaded perspective display of the object at the monitor. The image processing system was centred on a VAX/11-8600 computer. The other devices used were a video display unit, a track-ball and a high-resolution colour-monitor hard-copy camera (Honeywell D-3000). All facilities were kindly provided by the Philips Research Laboratory (Hamburg, F.R.G.). A detailed description of the hardware and of all steps of the image processing is given in a previous paper (MENHARDT & al. 1986).

Results and Discussion

Treatment of the semivivo growing pollen tubes with 2.0 mg L^{-1} PCP inhibited pollen tube growth and caused the formation of Golgi apparatus-ER hybrids (KANDASAMY & KRISTEN 1987). These hybrids consist of two distinctly different portions of stacked cisternae (Fig. 3). One portion has membrane-attached ribosomes and can therefore be attributed to the rough ER; the other has smooth membranes and shows a tighter stacking of the cisternae similar to that of dictyosomes. In addition, the dictyosomal portion sometimes has more cisternae (3–6) than the rough ER portion (2–3) and appears to bud vesicles from the free ends of its cisternae (Fig. 3). In some cases, intercisternal elements can be detected in the dictyosomal portion. The cisternae of both portions show lateral membrane continuity with each other. The Golgi apparatus-ER hybrids are usually cup-shaped. The PCP-treated pollen tubes also contain enlarged dictyosomes which

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consist of more than 5 up to 12 cisternae with a plate diameter about two times larger than that of the control dictyosomes (cf. Fig. 1 and 2).

Enlargement and curvature of dictyosomes frequently occured when cells were treated with respiratory inhibitors (SCHNEPF 1963, see also ROBINSON & KRISTEN 1982, for review). Cold treatment also caused dictyosome enlargement and even the formation of Golgi apparatus-ER associations with lateral membrane continuity between both these compartments (MOLLENHAUER & al. 1975). It has been suggested that this continuity results from a decrease in the production of vesicles due to energy depletion. The tubular vesicle-budding transition region of the ER is thought to be changed into a cisternal structure which then interassociates with the Golgi cisternae of adjacent dictyosomes (MORRÈ & al. 1979).

Both experiments, cold treatment and PCP treatment, clearly indicate that membrane regions of the ER where transitory vesicles usually bud off are compatible with the margin or with the cis face of dictyosomes. This implies that the endomembrane system of higher plant cells has the structural and molecular precondition for membrane association between ER and Golgi apparatus, which again is a prerequisite for the transfer of secretory or storage proteins via the Golgi apparatus.

The same object, the tobacco pollen tube, was recently used to demonstrate the transfer of tritiated leucine from the ER to the Golgi apparatus. The experiment was performed using the techniques of pulse chase and preparative free-flow electrophoresis in combination. The results revealed membrane flow and/or protein transfer from the ER to the Golgi apparatus (KAPPLER & al. 1986). In barley aleurone cells, the ER-Golgi apparatus transfer route was demonstrated to be used by α-amylase (GUBLER & al. 1986). However, one type of this enzyme may be transfered from the rough ER to the plasma membrane (PM) bypassing the Golgi apparatus (R. L. JONES, personal communication), as suggested for secretory proteins in the stigma papillae of *Aptenia* (KRISTEN & al. 1979) and *Crocus* (HESLOP-HARRISON & HESLOP-HARRISON 1975) and also in the trap glands of *Dionaea muscipula* (ROBINS & JUNIPER 1980).

The attempts to find structural preconditions for the protein transfer via the Golgi apparatus (GA) in higher plants were predominantly disappointing. For example, serial ultrathin sections across dictyosomes were used for the search of connections between the ER and the Golgi apparatus (ROBINSON 1980). Such connections could not be unequivocally detected in the electron micrograph series of various higher plant cells. However, three-dimensional reconstructions based upon superimposition of these micrographs revealed close associations between ER tubules and the central region of dictyosomes (KRISTEN & al. 1984).

Better results were obtained by computer-generated three-dimensional reconstruction based on electron micrographs taken from serial sections

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across dictyosomes of *Aptenia* ovary glands. These reconstructions revealed rather spherical bodies which partially consist of a cluster of vesicles differing in size and shape (Fig. 4). The central plate-like and fenestrated region of the dictyosome is hidden by these vesicles, but it can be visualized by incomplete reconstruction, as shown by a previous paper (LOCKHAUSEN & al. 1987). The cloud of vesicles and vesicle-like cisternal buds is partially penetrated by ER tubules protruding towards the dictyosomal centre. In addition, small transitory vesicles are visible between one pole of the model and an adjacent ER cisterna indicating the cis face of the dictyosome. Moreover, the reconstruction indicates that both types of ER-Golgi apparatus associations may occur in a single dictyosome: (1) direct connections between the ER and the central region of the Golgi stack and (2) transitory vesicles between the ER and the Golgi cis face.

Conclusion

Three different possibilities of vesicle-mediated transport of hydrophilic macromolecules appear to exist in higher plant cells: (1) GA → PM for various non-cellulosic polysaccharides, (2) ER → PM for proteins, (3) ER → GA → PM for proteins which must be processed in the Golgi apparatus (e.g. glycoproteins).

The Golgi apparatus of higher plant cells is closely woven into the network of the endomembrane system. We suggest that even in higher plants, each dictyosome is temporarily connected with the ER, at least with one cisterna. In addition, transitory vesicles serve for the transport of secretory, storage or lysosomal proteins from the ER to the dictyosomes. The intensity of this traffic and of the ER-GA association depends on the

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Fig. 1–3. Electron micrographs of dictyosomes taken from semivivo grown *Nicotiana sylvestris* pollen tubes.

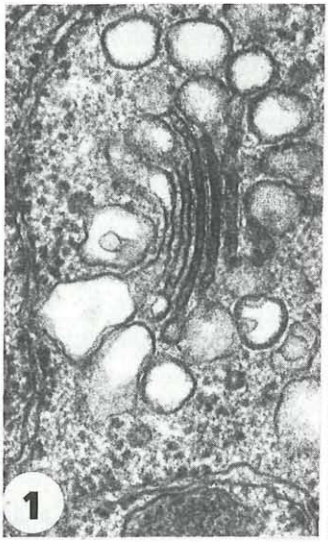
Fig. 1. Control. Normal feature of an active dictyosome consisting of 5 cisternae and producing large secretory vesicles. Connections between RER and Golgi cisternae are absent. 55,000×.

Fig. 2. Treatment with 2.0 mg L^{-1} PCP. Enlarged dictyosome consisting of 8 cisternae some of which being twice as long as in the control (Fig. 1). The number of vesicles produced is much lower than in the control. 55,000×.

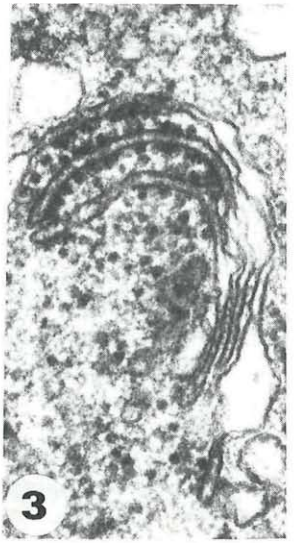
Fig. 3. Treatment with 2.0 mg L^{-1} PCP. Cup-shaped Golgi apparatus-ER hybrid structure consisting of RER and a dictyosome-like portion. 60,000×.

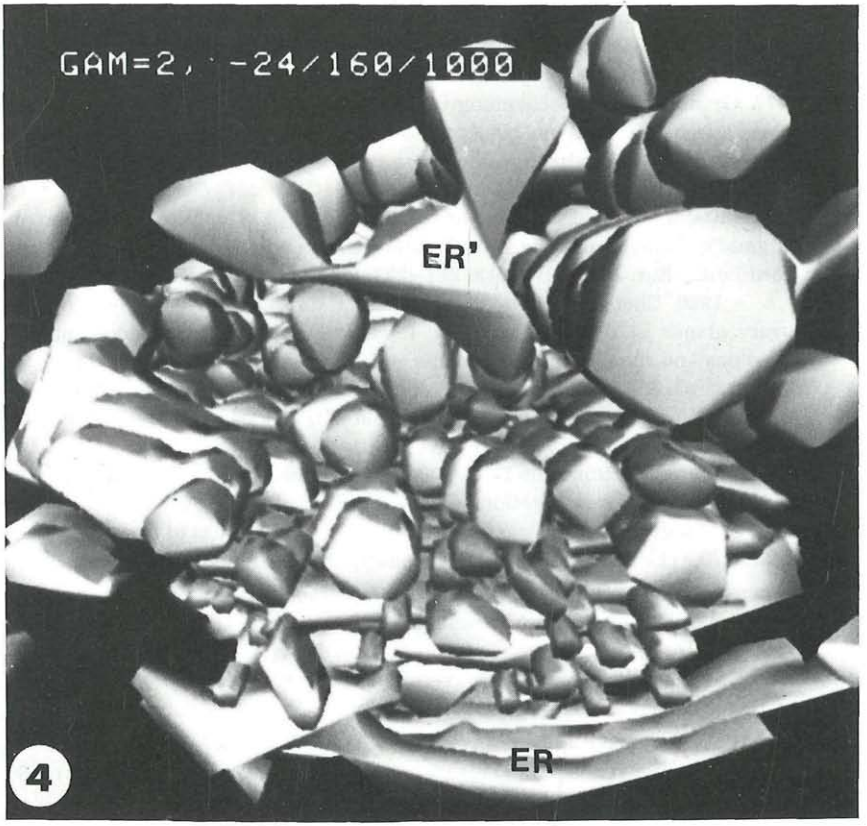
Fig. 4. Computer-generated three-dimensional reconstruction of a dictyosome of an ovary gland cell in *Aptenia cordifolia*. The central region is totally hidden by a cloud of vesicles. The large, secretory vesicles indicate the trans face of the dictyosome. Transitory vesicles (small) are visible between an ER cisterna (ER) and the cis face. An ER tubulus (ER') protruded the peripheral vesicular mantle towards the centre of the dictyosome.

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requirement of proteins to be processed in the Golgi apparatus for their different functions.

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