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MEMBRANE DIFFERENTIATION IN THE GOLGI COMPLEX OF *MICRASTERIAS DENTICULATA* BRÉB. VISUALIZED BY FREEZE-ETCHING

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SUMMARY

Freeze-etched dictyosomes of the alga *Micrasterias denticulata* (Bréb.) exhibit a gradual increase in the density of particles on the membranes of successive cisternae from the forming to the maturing face. At the maturing face a drop in particle numbers can be observed in the peripheral regions of the cisternae, where fenestration and the packaging of the secretory products takes place.

INTRODUCTION

In recent years the polarity of dictyosomes has been well established, both in functional and in morphological terms (Mollenhauer & Morré, 1966; Favard, 1969). Although many of the observed differentiations across stacks of dictyosome cisternae probably result from changes in the Golgi membranes and their associated enzymes, it has only recently been possible to obtain electron-microscope evidence for morphological changes in such membranes (Grove, Bracker & Morré, 1968). However, as these authors point out in their paper, 'the observed differences in membrane morphology represent differences in sites of stain deposition and do not necessarily reflect the morphological forms of the living membranes'.

In the present study we have attempted to correlate differences in the staining properties of Golgi membranes of the freshwater alga *Micrasterias denticulata* (Bréb.) (Kiermayer, 1970) with structural features of these membranes as revealed by the freeze-etching electron-microscope preparation technique (Moor & Mühlethaler, 1963), which allows the observation of membrane elements in the frozen state.

MATERIALS AND METHODS

Cells of *M. denticulata* (Bréb.) were cultivated under semisterile conditions in a Waris medium (Waris, 1950) at constant temperature (20° C) and with an illumination rhythm of 14 h of light and 10 h dark (Kiermayer, 1964). For normal sectioning the cells were fixed for

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15 min at room temperature in 1 % glutaraldehyde containing 0·05 M potassium phosphate (pH 6·8) and $3\cdot6 \times 10^{-5}$ M $CaCl_2$. After fixation, the cells were washed for 30 min in the buffer solution and post-fixed in 2 % OsO_4 for 4 h. They were then dehydrated in increasing concentrations of ethanol at 3–4 °C, and finally in propylene oxide at room temperature prior to embedment in Epon. Thin sections of embedded material were stained with uranyl acetate and lead citrate.

For the purpose of freeze-etching, the cells were first prefixed for 10 min at room temperature in 1 % glutaraldehyde prepared with 0·05 M potassium phosphate (pH 6·8) and then infiltrated for 2 h with 30 % glycerol in a 3 % glutaraldehyde/phosphate buffer solution. After centrifugation, drops of the cell suspension were rapidly frozen in Freon 12(−150 °C) and freeze-etched (Moor & Mühlethaler, 1963) on a Balzers BA 360 freeze-etch apparatus. The freeze-etch micrographs have been mounted so that the shadowing direction is from bottom to top as indicated by the encircled arrows.

RESULTS AND DISCUSSION

Conventionally cross-sectioned dictyosomes of the Golgi complex in *M. denticulata* (Bréb.) reveal a pronounced polar configuration (Fig. 1). Wide cisternae associated with elements of the rough endoplasmic reticulum (*er*) are found on the forming face (proximal pole), while narrow ones are limited to the maturing face (distal pole) of the stack. Similarly, peripheral blebs and vesicles with light and dark contents become prominent only in the distal pole region. Furthermore, the median splitting of these dictyosomes (single arrows, Figs. 1, 3), which probably represents a division stage (Kiermayer, 1970), seems to commence always at the proximal pole and to proceed towards the distal. The increased staining of the cisternal membranes on the maturing side of the stacks appears to be partly related to the narrowing of the cisternae and partly to changes in the staining properties of these membranes.

Frequently the freeze-etch view of cross-fractured dictyosomes is similar to that of conventional sections (Fig. 3, upper half). Of more interest, however, are the surface views of membrane faces, which can be obtained with this method (Figs. 2, 3), because membranes have a far greater potential for differentiations within their plane than for changes within their depth (Staehelin, 1968*a*). Surface views of freeze-etched natural membranes appear in most cases as more or less smooth surfaces covered with varying numbers of small particles and occasional holes (Moor & Mühlethaler, 1963; Branton, 1966; Staehelin, 1968*b*; see also Figs 2, 3). It is generally accepted that these particles are not artifacts but represent real membrane structures, possibly membrane-bound globular enzymes or multienzyme-complexes (Mühlethaler, Moor & Szarkowski, 1966; Staehelin 1968*a*, *b*; Branton, 1969). This interpretation is supported not only by the fact that enzymically active membranes such as thylakoid membranes of chloroplasts carry large numbers of particles, while inactive ones such as nerve myelin carry few (Branton, 1969), but also by studies in which such particles have been isolated and subjected to biochemical tests (Matile, Moor & Mühlethaler, 1967; Arntzen, Dilley & Crane, 1969).

In the case of freeze-etched Golgi membranes, surface views usually reveal either of two faces, labelled *ga* and *gb* in Figs. 2 and 3. The former, which are characterized by large numbers of small particles (2·5–5·0 nm in diameter), are seen where the

cisternae have been opened by the fracturing process. The *gb* faces, on the other hand, carry only few particles and appear as external surfaces of cisternae. In the examples shown here some splitting of the membranes appears to have occurred. At present, however, it cannot be unequivocally determined whether *ga* and *gb* represent true membrane surfaces or split inner membrane faces. Despite this uncertainty with respect to the exact position of the fracture plane, it is clear that the tips of the particles seen on the *ga* faces project toward—and may protrude into—the lumen of the cisternae. Thus, if the particles were to represent globular enzymes, they would be located in a favourable position for synthesizing secretory products and for processing the contents of the cisternae.

Fig. 2 demonstrates that the density of the particles on the *ga* faces of successive cisternae is not constant but gradually increases from one side of the stack to the other. The lowest number of particles is found on cisternae of the forming face, the highest on those of the maturing face (in Fig. 2, e.g. the 3 large, successive *ga* faces have particle densities of approximately 2900, 3700 and 4300/μm^2). It is suggested that this increase in particle numbers might reflect an overall increase in the enzymic activity of Golgi membranes as the cisternae mature. In addition, the greater density of particles on membranes of the distal pole region could be partly responsible for the increase in stain deposition and in thickness of these membranes in conventionally sectioned material (Grove *et al.* 1968, and Fig. 1).

Besides the progressive change in membrane structure across stacks of dictyosome cisternae, we have also observed a radial differentiation of single cisternal membranes at the distal pole. Fig. 3 shows such a spatially differentiated membrane where the central region of the *ga* face is covered with densely packed particles (approximately 7000/μm^2), while the peripheral regions undergoing fragmentation show a sharp drop in particle numbers (approximately 4000/μm^2). This change indicates that the membranes of mature cisternae undergo further modifications before they are used for packaging secretory products. At present we cannot say if the change in particle distribution is due to a concentration of the particles (enzymes) in the central region of the cisternae, possibly to preserve the full set of enzymes as long as possible, or if it reflects a dilution of the particles in the marginal regions caused by the local incorporation of new, non-globular (e.g. phospholipid), membrane material.

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REFERENCES

ARNTZEN, C. J., DILLEY, R. A. & CRANE, F. L. (1969). A comparison of chloroplast membrane surfaces visualized by freeze-etch and negative staining techniques; and ultrastructural characterization of membrane fractions obtained from digitonin-treated spinach chloroplasts. *J. Cell Biol.* **43**, 16–31.

BRANTON, D. (1966). Fracture faces of frozen membranes. *Proc. natn. Acad. Sci. U.S.A.* **55**, 1048–1056.

BRANTON, D. (1969). Membrane structure. *A. Rev. Pl. Physiol.* **20**, 209–238.

FAVARD, P. (1969). The Golgi apparatus. In *Handbook of Molecular Cytology* (ed. A. Lima-De-Faria), pp. 1130–1155. Amsterdam: North-Holland Publishing Co.

GROVE, S. N., BRACKER, C. E. & MORRÉ, D. J. (1968). Cytomembrane differentiation in the endoplasmic reticulum-Golgi apparatus vesicle complex. *Science, N.Y.* **161**, 171–173.

KIERMAYER, O. (1964). Untersuchungen über die Morphogenese und Zellwandbildung bei *Micrasterias denticulata* Bréb. *Protoplasma* **59**, 76–132.

KIERMAYER, O. (1970). Elektronenmikroskopische Untersuchungen zum Problem der Cytomorphogenese von *Micrasterias denticulata* Bréb. *Protoplasma* **69**, 97–132.

MATILE, PH., MOOR, H. & MÜHLETHALER, K. (1966). Isolation and properties of the plasmalemma in yeast. *Arch. Mikrobiol.* **58**, 201–211.

MOLLENHAUER, H. & MORRÉ, D. (1966). Golgi apparatus and plant secretion. *A. Rev. Pl. Physiol.* **17**, 27–46.

MOOR, H. & MÜHLETHALER, K. (1963). Fine structure of frozen yeast cells. *J. Cell Biol.* **17**, 609–628.

MÜHLETHALER, K., MOOR, H. & SZARKOWSKI, J. (1966). The ultrastructure of the chloroplast lamellae. *Planta* **67**, 305–323.

STAEHELIN, L. A. (1968*a*). Ultrastructural changes of the plasmalemma and the cell wall during the life cycle of *Cyanidium caldarium*. *Proc. R. Soc.* B **171**, 249–259.

STAEHELIN, L. A. (1968*b*). The interpretation of freeze-etched artificial and biological membranes. *J. Ultrastruct. Res.* **23**, 326–347.

WARIS, H. (1950). Cytophysiological studies on *Micrasterias*. I. Nuclear and cell division. *Physiologia Pl.* **3**, 1–16.

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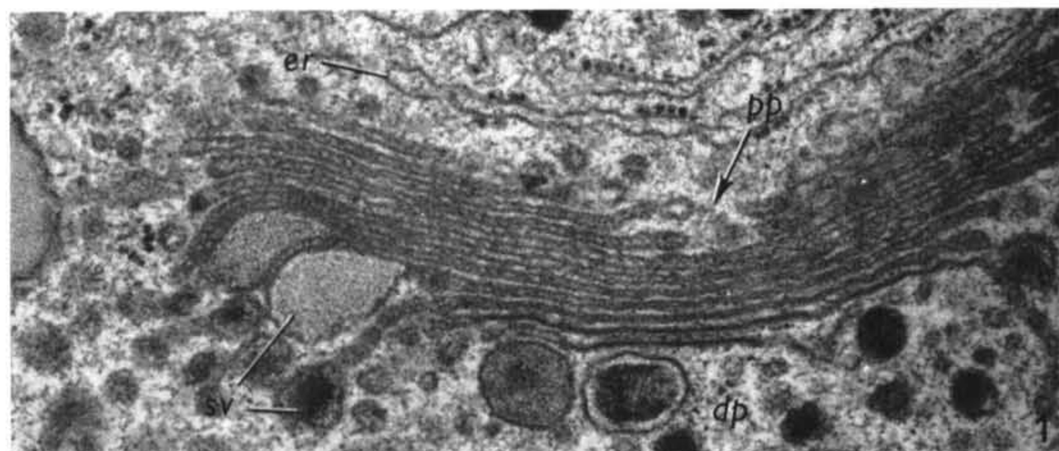
Fig. 1. Conventionally sectioned dictyosome showing at the proximal pole (*pp*) association with the endoplasmic reticulum (*er*), initiated dividing cleft (arrow), and well-separated cisternae; towards the distal pole (*dp*) the membranes of each cisterna become closely apposed and stain more intensely in their central region, while secretory vesicles (*sv*) are formed at their margins. × 68000.

Fig. 2. Obliquely fractured stack of freeze-etched cisternae of a dictyosome showing 2 different types of membrane faces (*ga* and *gb*) belonging to successive cisternae. The density of particles on *ga* faces increases from the forming face or proximal pole (*pp*) to the maturing face or distal pole (*dp*, see also Fig. 3). The top *gb* face shows peripheral fenestration of a cisterna at the distal pole. × 76000.


er
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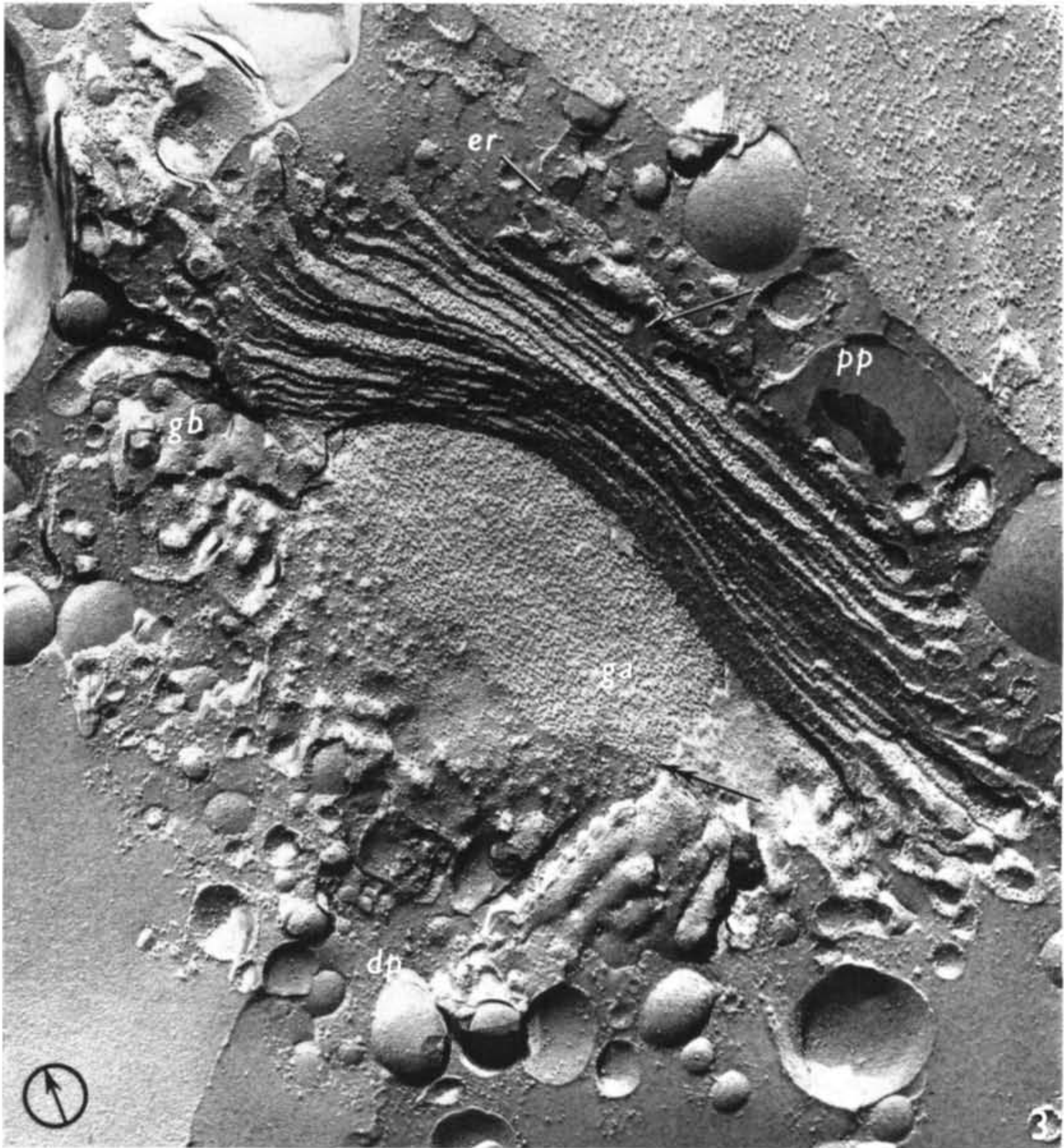

Fig. 3. Freeze-etched dictyosome with cisternae seen in cross-sectional (upper half) as well as in surface views (lower half). At the proximal pole (*pp*) note association with membranes of the *er* and initiated dividing cleft (single arrow). Membrane faces *ga* and *gb* belong to cisterna at the distal pole *dp*. Particle density on *ga* face declines sharply (double arrow) where fenestration begins. × 60000.