Observation of Golgi Bodies in *Zea mays* Roots by Technovit DiOC₆-DAPI Double-Stain Microscopy

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Based on studies on intercellular secretion, the term Golgi apparatus has recently come to refer to the subcellular component that contains stacks of Golgi cisternae, called a Golgi stack or a Golgi body. In animal cells, these Golgi bodies are interconnected by tubular elements and located adjacent to the nucleus (Farquhar and Palade 1981). In plant cells, however, Golgi bodies appear to occur independently and in the cytoplasm and to be mobile, and plant Golgi bodies function in the formation of cell plates and the secretion of polysaccharides (Whaley and Mollenhauer 1963, Whaley *et al.* 1966, Hepler 1982, Morré *et al.* 1967). To function as these roles, Golgi bodies change their shape or number in a cell as it differentiates.

The plant root cap represents a fascinating model system for studying changes in Golgi bodies associated with the developmental progression of meristematic cells to polysaccharide-slime-secreting outer root-cap cells. The morphology of Golgi bodies in the root cap has been studied by electron microscopy (Mollenhauer *et al.* 1961, Steahelin *et al.* 1990), a useful tool for high resolution examination of intracellular components or structures. However, it is hard to distinguish individual cells within a tissue by electron microscopy, and thus it is not efficient for analysis of the distribution of Golgi bodies in a tissue. We therefore examined the dynamics of Golgi bodies in the maize root tip by a new method of fluorescence microscopy (Technovit DiOC₆-DAPI double-stain microscopy), which we developed to examine Golgi bodies in cultured tobacco cell line BY-2 (Kawazu *et al.* 1995). The specimen is fixed and embedded in Technovit resin. A section is cut 0.6 μm thick, double-stained with DiOC₆ (a dye specific for cellular membranes) and DAPI (a dye specific for DNA), and then examined with both an epifluorescence microscope and a color-image processor. Both cellular membranes and DNA can be observed with high resolution at the same time. The fact that Golgi bodies exhibit bright, rod-like DiOC₆ fluorescence, but lack DAPI fluorescence allows them to be distinguished from other organelles, which display both DiOC₆ and DAPI fluorescence. Bright, rod-like DiOC₆ fluorescence has been demonstrated to represent Golgi bodies by examining the same Technovit sections under both a fluorescence microscope and an electron microscope (Kawazu *et al.* 1995).

In the present study, we first investigated whether this new method could be applied to observing the behavior of Golgi bodies in plant tissues and then examined the dynamics of Golgi bodies compared to other organelles in the root of *Zea mays*.

Materials and methods

Culture of Zea mays, and fixation and embedding of samples

Zea mays L. seeds (Takii, Kyoto, Japan) were germinated on moist filter paper under

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continuous fluorescent light (Homolux FL15PG; National, Tokyo, Japan) at an intensity about $50 \mu\text{Em}^{-2} \text{s}^{-1}$. One week after sowing, maize roots were cut and fixed for 12 hr at 4°C in a solution containing 1.5% glutaraldehyde buffered by 20 mM sodium cacodylate at pH 7.2. The roots were dehydrated in a graded water-ethanol series, and then embedded in Technovit 7100 resin according to the manufacturer's instructions (Kulzer and Co., Wehrheim, Germany).

Observation of the maize root tip

Technovit sections were cut $0.6 \mu\text{m}$ thick using an MT7 rotary ultramicrotome (RMC-Eiko, Kawasaki, Japan) with a glass knife, then stretched in distilled water on a cover-glass, and air-dried. The sections were stained with $10 \mu\text{g/ml}$ 3,3'-dihexyloxacarbocyanine iodide (DiOC_6), a dye specific for cell membranes (Terasaki *et al.* 1984), washed with ethanol and then stained again with $0.1 \mu\text{g/ml}$ 4',6-diamidino-3-phenylindole (DAPI), a dye specific for DNA. The sections were examined under a BHS-RFC epifluorescence microscope (Olympus, Tokyo, Japan). Each DiOC_6 fluorescent image produced by blue-light excitation or DAPI fluorescent image produced by ultraviolet ray excitation was fed into a video-intensified microscope photon-counting system (VIMPCS; Hamamatsu Photonics, Hamamatsu, Japan) connected to the microscope, and then stored in a color image processor SPICCA-II (Nippon Avionics Co., Tokyo, Japan). Green was assigned to DiOC_6 fluorescence and blue to DAPI fluorescence for production of false-color images. The images were taken on RDP films (Fuji Photo Film Co., Tokyo, Japan) by using a film recorder, FR-1000 (Nippon Avionics Co.) connected to the color image processor.

Results and discussion

Maize root tips were examined by Technovit DiOC_6 -DAPI double-stain method (Fig. 1). Golgi bodies (g in Fig. 1) were observed in almost all regions of the root tip, as bright and rod-like DiOC_6 fluorescence, the same as in cultured tobacco cells BY-2 (Kawazu *et al.* 1995). Mitochondria were also observed as doughnut-like DiOC_6 fluorescence with intense DAPI fluorescence (m in Fig. 1). The Technovit DiOC_6 -DAPI double-stain method is useful for observing Golgi bodies within plant tissues as well as in cultured cells.

The apical meristem consists of small cells (Fig. 1B), and fewer Golgi bodies were observed than mitochondria. The Golgi bodies were uniformly small and approximately $0.8 \mu\text{m}$ in size. In the root cap initials, the source of the root cap cells, the Golgi bodies were as small as in the apical meristem (Fig. 1C). A larger number of Golgi bodies were observed within these cells than in apical meristemic cells. In the outer root cap, the Golgi bodies were clearly larger (Fig. 1D), and enlarged Golgi bodies appeared to be fenestrated (arrowheads in Fig. 1D). The cells in the upper end of the outer root cap were filled with these large Golgi bodies (Fig. 1E). Mitochondria and plastids were not so numerous as the Golgi bodies in this

Fig. 1. Fluorescence images of cross-sections of a maize root obtained by the Technovit DiOC_6 -DAPI double-stain method. Maize roots were embedded in Technovit 7100 resin and thin sections were double-stained with DiOC_6 (green fluorescence), which is specific for membranes, and DAPI (blue fluorescence), which is specific for DNA, and then examined under an epifluorescence microscope. Membranous organelles (Golgi bodies, mitochondria, and plastids) were stained with DiOC_6 . Cell nuclei, mitochondria nuclei, and plastid nuclei were stained with DAPI. The two images were superimposed by the image processor to create a double-fluorescent image. Golgi bodies (g, arrowheads), mitochondria (m, arrows), plastids (p, small arrows) and the nucleus (n) can be seen in each section, but plastids are very rare. A dividing Golgi body is seen in E (double arrowhead). A is a schematic diagram indicating the positions of region B, C, E, and F. B: apical meristem; C: root cap initials; D: outer root cap; E: upper end of the outer root cap. Bar indicates $10 \mu\text{m}$.

Fig. 1.

Fig. 2. Distribution map of Golgi bodies in a maize root tip. The plots indicate where Golgi bodies were observed in a thin Technovit section ($0.6\mu\text{m}$ thick). Golgi body density was high in two regions: the root cap initials (open circle) and the upper end of the outer root cap (arrows).

region. They were observed to have increased as a result of their dividing (double arrowhead in Fig. 1F).

To study the behavior of Golgi bodies, their distribution was examined in a whole maize root tip (Fig. 2). The density of Golgi bodies in the root cap was higher than in the root meristem, and they were particularly dense in two regions, the root cap initials and the upper end of the outer root cap. The number of Golgi bodies within one thin Technovit section of a cell and the density of the bodies were quantitatively examined (Fig. 3). Approximately 7.2 Golgi body units were present in a cell section in the root cap initials, and about 19 Golgi body units were observed in a cell section of the upper end of the outer root cap. In both two regions, the density of Golgi bodies was estimated to be more than $30,000\text{ unit/mm}^2$, more than twice as high as in the apical meristem. The sizes of the Golgi bodies was then measured (Fig. 4). The length of the Golgi bodies in the apical meristem was approximately $0.83\mu\text{m}$, versus $0.92\mu\text{m}$ in the root cap initials, where they were slightly longer than in the apical meristem. The Golgi bodies in the outer root cap were quite large. They were $1.42\mu\text{m}$ long, which is about 1.5 times as longer as in the apical meristem and the root cap. The standard deviation of the length was 0.01–0.02 in three regions. This shows that the Golgi bodies in each cell population are uniform.

Golgi bodies are thought to increase in number in the root cap initials. Their density decreases as the cells of the root cap initials divide and grow toward the outer region. There seem to be two reasons why the Golgi bodies increase in number in the root cap initials. First, the cells in this region divide frequently and cell division requires Golgi bodies, which are involved in the formation of the cell plate and wall. Secondly, the Golgi bodies divide in the root cap initial cells and they prevent the number of the Golgi bodies in the root cap from decreasing rapidly during cell division and differentiation.

In the outer root cap, Golgi bodies became large and produce polysaccharide-slime (Morré *et al.* 1967). The cells are filled with the Golgi bodies in the upper end of the outer root cap. The shape of the Golgi bodies in the outer root cap was the same as in other parts of the

Fig. 3. The number (A) and density (B) of Golgi bodies observed in the cells in different regions of a maize root: upper apical meristem (A), lower apical meristem (B), root cap initials (C), and upper end of the outer root cap (D). The Golgi bodies were analysed by Technovit DiOC₆-DAPI double-stain microscopy. The number of Golgi bodies observed in a thin Technovit section was calculated.

Fig. 4. The size of the Golgi bodies observed in the cells in different regions of a maize root: apical meristem (A), root cap initials (B), and outer root cap (C). The length of Golgi bodies was measured by Technovit DiOC₆-DAPI double-stain microscopy.

outer root cap, and thus their main function in the upper end of the outer root cap seems to be production of polysaccharide-slime. The upper end of the outer root cap is close to the epidermis, where cells frequently divide. If the epidermis is injured by soil, the root may not form normally. Accordingly, a large quantity of the polysaccharide-slime is thought to be necessary in the upper end of the outer root cap to protect the epidermis, and thus Golgi bodies,

one of factories producing the polysaccharide-slime, may be present there in great numbers.

Summary

The behavior of Golgi bodies in a maize root tip was examined by the Technovit DiOC₆-DAPI double-stain method. In almost all regions of the root tip, Golgi bodies were observed as bright and rod-like DiOC₆ fluorescence. The number or shape of Golgi bodies varied in different tissues. Their density was quite high in two regions, the root cap initials and the upper end of the outer root cap were especially full of Golgi bodies. The Golgi bodies were uniformly small in the meristem and the root cap initials, but were much larger in the outer root cap, and this seems to be because they produce polysaccharide-slime.

Key words: Golgi body, *Zea mays*, Root tip, Root cap

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