Three-dimensional visualization of the Golgi apparatus: Observation of Brunner's gland cells by a confocal laser scanning microscope

Running head: Three-dimensional visualization of the Golgi apparatus

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Abstract

The three-dimensional structure of the Golgi apparatus was visualized in the mouse Brunner's gland cells by using a confocal laser scanning microscope. Two lectins, FITC-labeled soybean agglutinin and Texas red-labeled *Griffonia simplicifolia* agglutinin II, were used to visualize the whole Golgi apparatus. The staining with the former lectin, which has been known to label the *cis*-stacks, showed a lacy dome-like structure situated in the supranuclear region. The latter known to label the intermediate to *trans*-stacks and the secretory granules showed a dome-like structure consisting of network and cobblestone-like patterns in the similar region, and also granular stainings near the surface of the cobblestone-like patterns, inside the dome, and the apical region of a cell. The double staining demonstrated that the soybean agglutinin-labeled network always surrounded the *Griffonia simplicifolia* agglutinin II-stained structure in the supranuclear region. Based on these observations, we propose a new three-dimensional model of the Golgi apparatus: it forms a dome-like structure over a nucleus, the network of the *cis*-stacks forms its outer boundary, which is lined and paved with intermediate and *trans*-stacks, successively. Secretory granules are suggested to be released toward its internal space and transported to the apical region through the holes of the network.

Introduction

The three-dimensional structure of the Golgi apparatus has been studied by using an electron microscope. Based on conventional electron microscopic observations, it has been reported that several sets of the Golgi stacks are distributed in the supranuclear region, generally with their convex side facing the nucleus and the concave side facing a group of secretory granules (Beans, 1968; Yamashina, 1994). Precise appearances of Golgi stacks and their three-dimensional arrangements have been partially shown by high-voltage electron microscopic studies in several types of cells, such as pancreatic exocrine cells, ganglion cells, atrial muscle cells and Brunner's gland cells (Noda, 1984; Rambourg, 1986; Rambourg, 1984, 1987). However, the spatial arrangement of the Golgi apparatus is too extensive to study electron-microscopically, and the whole architecture has still remained controversial.

We have studied the lectin-binding sites in the Golgi apparatus of the mouse Brunner's gland cells electron-microscopically, and have demonstrated that soybean agglutinin (SBA) strongly labels the *cis*-stacks of the Golgi apparatus and *Griffonia simplicifolia* agglutinin II (GSA II) the intermediate to *trans*-stacks and the secretory granules (Suzaki, 1992). Therefore, these two lectins will be convenient tools to distinguish the stacks of the Golgi apparatus.

A confocal laser scanning microscope (CLSM) lightens a new sight of stereoscopic examination, which offers depth-discriminated images with reasonably high resolution and contrast. Thus, a CLSM will make it possible to examine three-dimensional architecture of the Golgi apparatus in thick specimens. Moreover, it allows us to examine the individual Golgi stacks stained in different colors. The purpose of the present study is to visualize the whole architecture of the Golgi apparatus in the mouse Brunner's gland cells by the combination with confocal laser scanning microscopy and the lectin cytochemistry using SBA and GSA II.

Materials and Methods

Chemicals

Texas red-labeled GSA II (GSA II-Texas red) and FITC-labeled SBA (SBA-FITC) were purchased from EY Laboratories (San Mateo, CA, USA).

Animals and Preparation of Tissues

Adult ICR male mice (2 months old), fed ad libitum, were used. Under sodium pentobarbital anesthesia, they were perfused with 4% formaldehyde in 0.1 M phosphate buffer with 2.5% sucrose (pH 7.2) via the left ventricle of the heart, and the duodenum was dissected into small pieces. They were fixed by immersion in the same fixative for 6 hours at 4°C and soaked in 0.1 M phosphate buffer (pH 7.2) overnight. The fixed tissue blocks were dehydrated in a graded series of ethanol and embedded in paraffin as following a routine procedure. Thin sections (10 μm-thick) were cut and mounted on a gelatin-coated glass slide. The paraffin-embedded sections were deparaffinized and processed for lectin cytochemistry.

Labeling of the Golgi apparatus

For lectin cytochemistry, all incubations were performed at room tempetature. The sections were treated with 10 mM phosphate-buffered saline containing 0.5 mM $CaCl_2$ (pH 7.2) (PBS) for 5 minutes and then with 1% bovine serum albumin in PBS for 10 minutes, followed by a brief wash with PBS. The sections were subsequently incubated with lectins as described below. After PBS washes, the specimens were mounted and examined.

Single Staining: The sections were incubated with either SBA-FITC (0.5 mg/ml) for 50 minutes or GSA II-Texas red (0.5 mg/ml) for 30 minutes.

Double Staining: The sections were incubated with GSA II-Texas red (0.5 mg/ml) for 30 minutes, washed with PBS, and subsequently incubated with SBA-FITC (0.5 mg/ml) for 50 minutes.

CLSM observation

The stained specimens were examined with a CLSM (LSM-410, Carl Zeiss, Jena, Germany) with the fluorescence mode. Optically sectioned serial images (0.5 μm-step) were collected and were reconstructed to a three-dimensional architecture.

Results

The Golgi apparatuses stained with SBA-FITC were examined from the apical, apicolateral, lateral and basal sides in Brunner's gland cells (Fig. 1). The staining revealed a dome- or beret -like structure of a network over a dark nucleus (Fig. 1a). The holes of the network tended to be larger at the apical surface (Fig. 1b and c, arrows) than those at the lateral. Another example stained with SBA-FITC is shown in Fig. 2, which was seen from the apical cytoplasm toward the cell base through the Golgi apparatus. Fig. 2a shows a three-dimensionally reconstructed image from 15 optical sections shown in Fig. 2b-p. The Golgi apparatuses in Fig. 2b-d consisted of a network at the ceiling of the dome-like structure, then wavy loops with many small gaps appeared in consecutive sections (Fig. 2g-j). The loop images continued toward the base of the Golgi apparatus where the nucleus appeared as a dark area. These results also showed that SBA-stained stacks formed a dome-like structure over a nucleus, the wall of which was composed of a fine network. In Fig. 3a, GSA II-Texas red stains revealed a dome-like structure over a nucleus and accumulated secretory granules at the cell apex. The staining appeared to be paved with cobblestone-like structures (Fig. 3b, arrows).

The sections doubly stained with SBA-FITC and GSA II-Texas red clearly demonstrated spatial relationship between the two differently stained structures (Fig. 4). A Golgi apparatus was seen from the base of the cell, i. e., from the nuclear side. Fig. 4a shows a stereopair of a Golgi apparatus. This example is three-dimensionally reconstructed from 15 optical sections, four of which are shown in Fig. 4b-e. Although the appearance of the two stains changed in each optical section, the SBA-labeled structure always surrounded GSA-II labeled one. The network stained with SBA-FITC was mainly found at the level of 1.0 μm in depth (Fig. 4b), which was followed by that stained with GSA II-Texas red at the level of 1.5 μm (Fig. 4c). The image at the level of 3.0 μm in depth, which corresponded to about the middle of this Golgi apparatus, demonstrated a SBA-stained wavy loop surrounding a parallel GSA II-stained one (Fig. 4d). There were always some gaps connecting the wide interior with the exterior of the

loops (Fig. 4d, arrowheads). The loops became gradually smaller toward the ceiling of the Golgi apparatus. When an optical section was just beneath the ceiling of the Golgi apparatus at the level of 6.0 μm in depth, GSA II labels were predominant, and the granular stains looked emerging from their surface (Fig. 4e, arrows). The labels of SBA-FITC were faintly seen behind the GSA II staining. The consecutive section was too dark to visualize the ceiling of the Golgi apparatus.

When the doubly stained Golgi apparatus was examined from the lateral side of a cell, it looked like a dome or a beret over a dark nucleus (Fig. 5b). When this image was rotated for -20° (Fig. 5a) and +10° (Fig. 5c) about the y-axis, it was clearly shown that the outer surface of the Golgi apparatus consisted of SBA-labeled structure and the inside was paved with GSA II-labeled one.

Figs. 5d-f and 5g-i are two examples of the doubly stained Golgi apparatus seen form the apicolateral side of a cell. Fig. 5d shows a three-dimensionally reconstructed image from 13 optical sections including 5e and f, which showed that the granular GSA II stains were found at the apical cytoplasm of the cell and inside the SBA-stained dome-like structure. The granular stains were also found as if they were crossing the gaps of the SBA-stained stacks (Fig. 5e and f, arrows). Fig. 5g shows a three-dimensionally reconstructed image from 15 optical sections including 5h and i. The GSA II-stained granular structures were found in the holes of the SBA-stained network (Figs. 5h and i, arrows) and seemed to pass through them toward the apical accumulation of the granules.

Discussion

Many studies have been attempted to reveal the three-dimensional structure of the Golgi apparatus and clarified the morphology of Golgi stacks and the relationship among them from *cis*- to *trans*-side of the apparatus (Beans, 1968; Noda, 1984; Novikoff,1970; Rambourg, 1974, 1981, 1984, 1986, 1987; Yamashina, 1994). However, the whole architecture of the Golgi apparatus is too extensive to study electron-microscopically and has still remained to be elucidated.

Mucous cells of the mouse Brunner's gland are known to have a large Golgi apparatus consisting of 7-13 stacks (Rambourg, 1987). By electron-microscopic lectin cytochemistry, we have demonstrated that a lectin SBA strongly labels 2-3 stacks at the *cis*-side of the Golgi apparatus and GSA II the successive intermediate to *trans*-stacks and the secretory granules (Suzaki, 1992): All stacks of the mouse Brunner's gland cells are, therefore, labeled by the double staining with SBA-FITC and GSA II-Texas red as distinguishing between *cis*- and other stacks. Thus, the whole architecture of the Golgi apparatus could be clarified.

The Golgi apparatus has been generally known to consist of several sets of stacks with the convex *cis*-side facing the nucleus and with the concave *trans*-side facing the apical surface of a cell (Beans, 1968; Yamashina, 1994). However, in the present study on Brunner's gland cells, it is demonstrated that the Golgi apparatus is not scattered sets of piled stacks but a dome-like structure, the outer surface of which consists of the *cis*-side network and the inner surface of which is lined with *trans*-stacks. Therefore, the whole structure of the Golgi apparatus demonstrated in this study is even opposite to what has been understood, and the dome-like structure opens toward the nucleus.

The *cis*-element has been generally considered to consist of a tubular network in various types of cells (Noda, 1984; Novikoff, 1970; Rambourg, 1986, 1987; Yamashina, 1994). In the mouse Brunner's gland cells, Rambourg et al. (1987) have reported that the *cis*-elements of the Golgi apparatus consisted of a tubular network and a subjacent perforated stack by using

conventional and a high-voltage electron microscopes. Our result by CLSM that the *cis*-stacks stained with SBA-FITC appeared as a network agrees well with these previous observations. In addition, we have clarified that the *cis*-side network forms an outer boundary of the dome-like Golgi apparatus, which is lined by a similar network and cobblestone-like structure stained with GSA II-Texas red, successively.

The granular stains with GSA II-Texas red were seen at any part along the inner surface of the cobblestone-like structure and in the internal space of the dome. Rambourg et al. (1987) reported in the mouse Brunner's gland cells that prosecretory granules formed at random along the *trans*-cisterns. In our previous study (Suzaki, 1992), many GSA II-labeled secretory granules were found in the internal space surrounded by the Golgi stacks and a nucleus. Therefore, the granular structures observed here by a CLSM are thought to correspond well to those prosecretory and secretory granules and are suggested to be first released into the internal space of the Golgi dome. Then, these granules are needed to be transported to the apical region of a cell where many secretory granules are accumulated. There were small and large holes in the Golgi dome observed in this study. Tanaka et al. (1986) also showed by using a scanning electron microscope that Golgi apparatus had several large holes which penetrated the whole stacks. Therefore, the granules are considered to be transported from the inside to the outside of the dome through these holes. We have also had the electron micrographs which support this idea (data not shown). Further investigations are necessary to clarify the mechanism of the transport.

The continuities of Golgi stacks are always the matter of question. Based on morphological studies by using a high voltage electron microscope or a scanning electron microscope, the Golgi stacks are becoming to be understood as one continuous reticular organelle (Noda, 1984; Novikoff, 1970; Rambourg, 1974, 1981, 1984, , 1986, 1987; Tanaka, 1991; Tanaka 1986). Cole et al. (1996) suggested the continuity of the Golgi stacks in living green fluorescent protein chimeras. Nevertheless, there have been accumulated data to show that the Golgi apparatus has at least three discrete compartments which are *cis*-, intermediate and *trans*-

compartments; and the transport between these compartments is performed by associated vesicles (Farquhar, 1985; Rothman, 1984) or by directed maturation of the stacks (Bannykh, 1997). The result by Seksek et al. (1995) that 70 nm-liposomes containing fluorescent probes fused specifically with *trans*-Golgi compartment and remained there in situ may also indicate functional discontinuity of the Golgi stacks. Our lectin cytochemical studies (1992; the present study) have also shown the different molecular compositions between *cis*- and other stacks. Further investigations are required to solve the questions (1) where Golgi stacks are continuous in structure and/or function, and (2) how discrete functions of each Golgi compartment are maintained.

In conclusion, we propose a new three-dimensional model of the Golgi apparatus as in Fig. 6: the Golgi apparatus appears as a large dome over a nucleus, the lacy structure of *cis*-stacks forms the outer boundary of the dome, and its inside is lined and paved with intermediate and *trans*-stacks, successively. Secretory granules budded from *trans*-stacks into the internal space of the Golgi dome are transported to the apical cytoplasm through the holes of the Golgi stacks.

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Figure legends

Fig. 1 By staining with SBA-FITC, the Golgi apparatuses appears as a lacy dome or beret over a dark nucleus (N). The three-dimensionally reconstructed images of the Golgi apparatuses viewed from lateral or basal side (a), apiccolateral side (b) and apical side (c) of the cells are shown. The large holes at the upper surface of the Golgi apparatus are indicated by arrows. Bars show 2.5 μm.

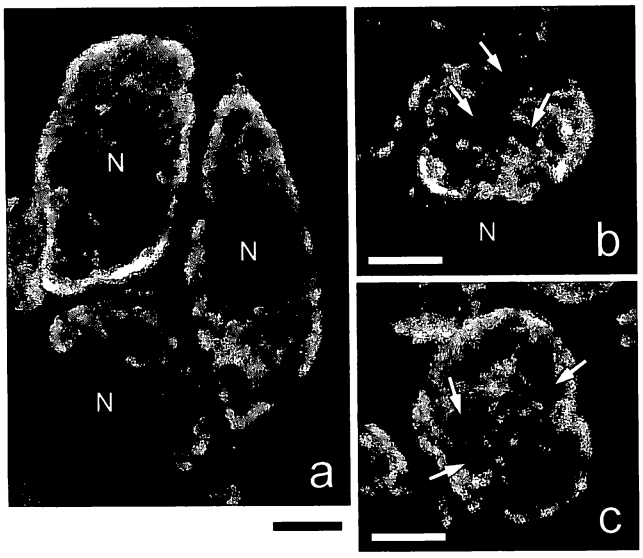
Fig. 2 A Golgi apparatus stained with SBA-FITC is reconstructed three-dimensionally (a) from 15 optical sections (0.5 μm-step) shown in b-p. The ceiling of the Golgi dome consists of lacy network (b-d). The images of g-j consist mainly of a wavy loop which has many gaps (arrowheads). The asterisks in b show Golgi apparatuses of neighboring cells. N: nucleus. Bars show 2.5 μm.

Fig. 3 GSA II-Texas red staining exhibits dome-like Golgi apparatus in the supranuclear region. a: The accumulated secretory granules (G) are also stained in the apical region of a cell. The cell membrane is weekly stained (arrowheads). The nuclei (N) appear dark without staining. L: acinar lumen. b: An enlarged picture of a Golgi apparatus over a dark nucleus (N) reveals the cobblestone-like patterns (arrows). Bars show 2.5 μm.

Fig. 4 A dome-like Golgi apparatus, doubly stained with SBA-FITC and GSA II-Texas red, is viewed from the base of the cell. The Golgi apparatus reconstructed from 15 serial sections is shown as a stereo pair (a). The relationship of these optical sections (a-e) is schematically drawn in f. Both SBA and GSA II stainings show lacy network in b and c. In d, two parallel loops are shown with gaps (arrowheads) connecting the interior with the exterior of the dome. In e, granular structures (arrows) are shown on the inner surface of the ceiling of the dome. N: nucleus. Bar shows 2.5 μm.

Fig. 5 A Golgi apparatus doubly stained with SBA-FITC and GSA II-Texas red is viewed from the lateral side of the cell (b) and is rotated for -20° (a) and +10° (c) about the y-axis in order to observe its outer and inner surfaces. The outer surface of the dome- or beret-like Golgi apparatus is formed by lacy network of the SBA stain, and the inner surface is paved by cobblestone-like structures labeled with GSA II. Figs. d-f and g-i are two examples of the Golgi apparatus viewed from the apicolateral side of the cells. Three-dimensionally reconstructed image (d and g) and the serial two constituents (e, f and h, i, respectively) are shown. The *cis*-stacks stained with SBA-FITC look an anastmosing network. The granular structures stained with GSA II-Texas red are seen in a group at the apical portion of a cell (G in d) and inside the region surrounded by SBA stains. In addition, some of granular structures are found within the holes of SBA-stained network (arrows). N: nucleus. L: acinar lumen. Bars shows 2.5 μm.

Fig. 6 A schematic drawing of the Golgi apparatus in the mouse Brunner's gland cell. N: nucleus, C: lacy network of the *cis*-stacks, T: *trans*-stacks, H: hole, G: granule.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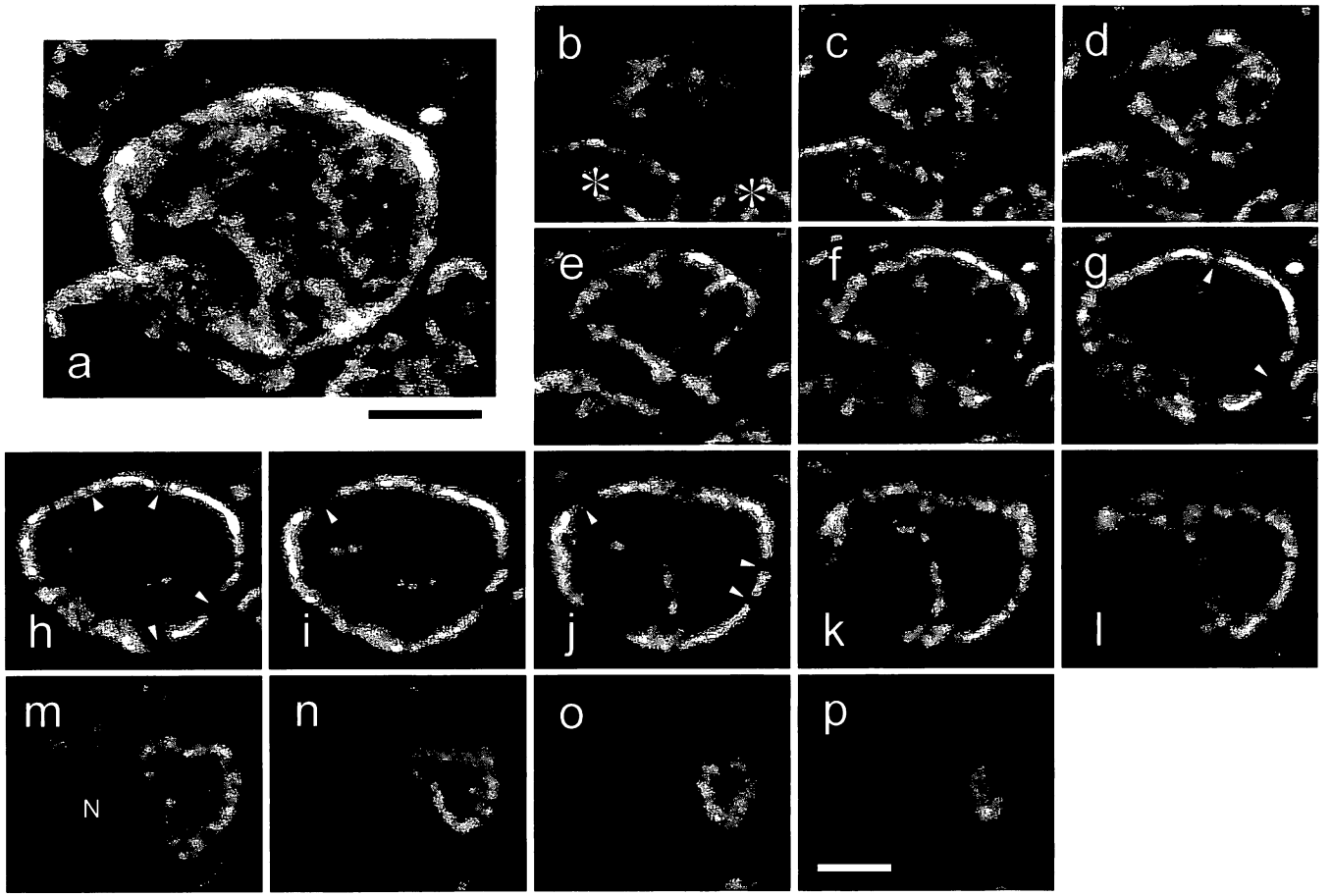
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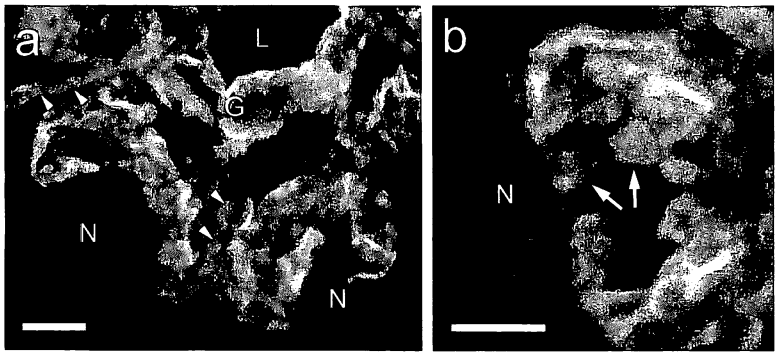
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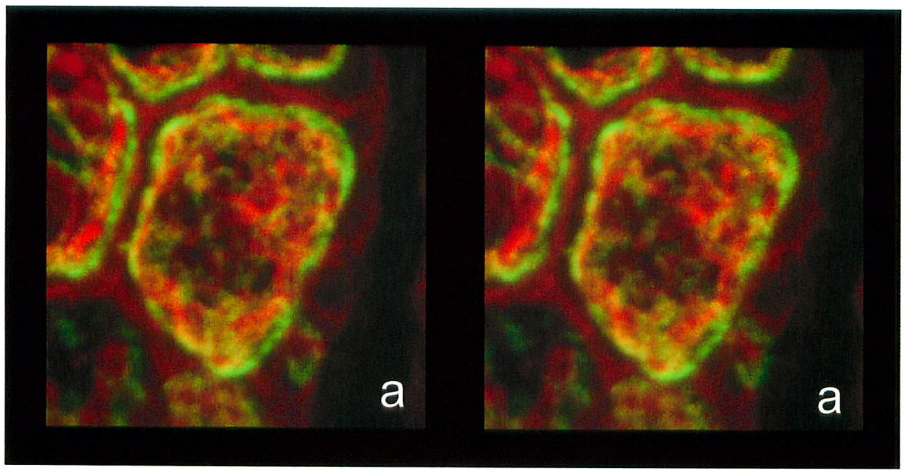
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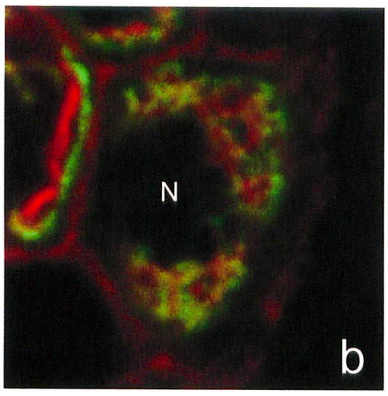
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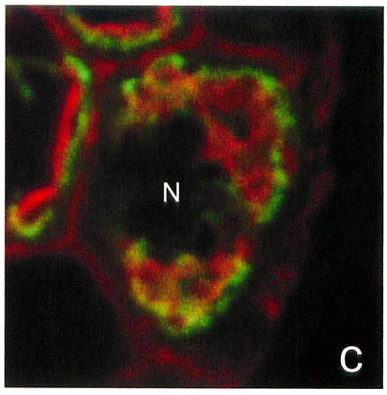
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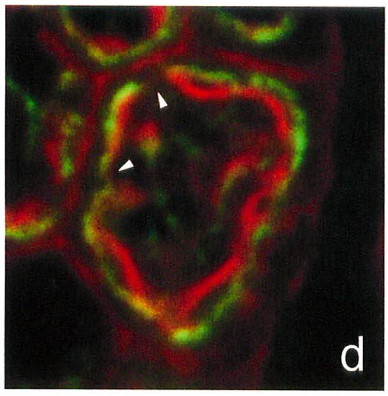
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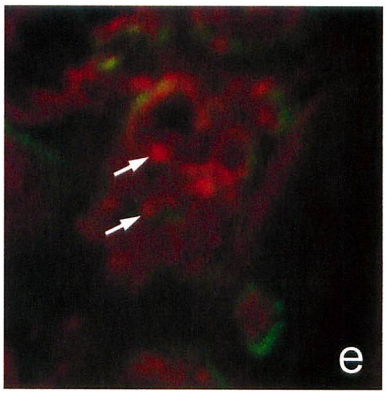
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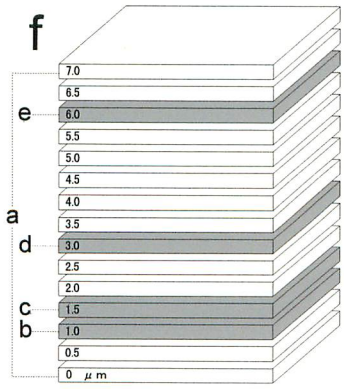
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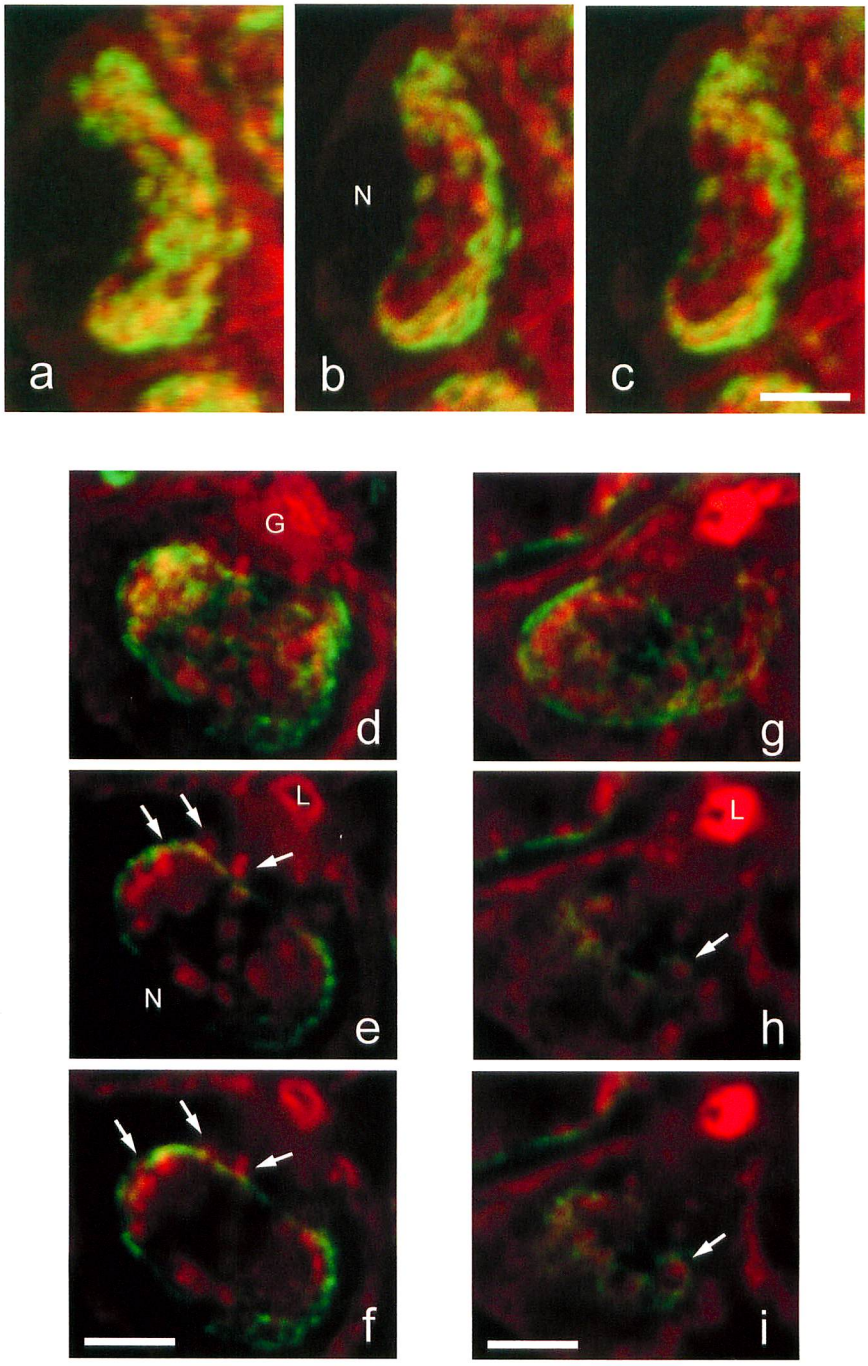
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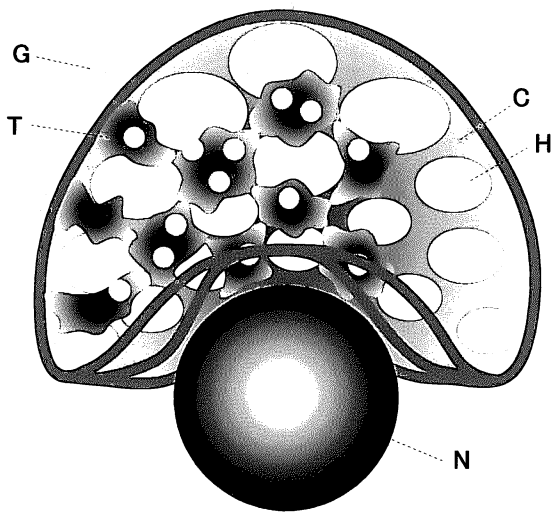
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