Three-dimensional ultrastructure of the Golgi apparatus in different cells: high-resolution scanning electron microscopy of osmium-macerated tissues

Daisuke Koga and Tatsuo Ushiki

Division of Microscopic Anatomy and Bio-imaging, Department of Cellular Function, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Summary. The three-dimensional ultrastructure of the Golgi apparatus in different cells of the rat — epithelial principal cells in the epididymal duct, goblet cells in the jejunum, gonadotrophs in the pituitary gland and dorsal root ganglion cells — was studied by scanning electron microscopy (SEM) of osmium-macerated tissues. The Golgi apparatus in the epididymal principal cells took the shape of a candle flame with irregular-shaped cisterns, while those in the goblet cells of the jejunum were cup-shaped or cylindrical with flat cisterns. Gonadotrophs had a large spherical Golgi apparatus; this apparatus was composed of several concentric cisterns with large round windows through which the rough endoplasmic reticulum (rER) and mitochondria extended into the center of the globular Golgi apparatus. Dorsal root ganglion cells had several small Golgi stacks scattered in the cytoplasm. In all Golgi apparatuses of the different cells examined in the present study, the cis-most cistern was generally composed of a flattened sheet with numerous small fenestrations on its wall. On the other hand, the shape of the trans-most cistern varied by cell type; it was generally composed of tubules and/or small sheets which were sometimes connected with each other to form a rather complicated structure. The cis-most cistern and the trans-most cistern were often closely associated with the rER although no direct communication was found between them. These findings indicate that the structure of the Golgi apparatus, especially its overall shape and the ultrastructure of the trans-most cistern, varies by cell type, a point to be considered in relation to the function of the individual cells.

Introduction

The Golgi apparatus is a cell organelle first discovered by the Italian pathologist Camillo Golgi in 1898 in Purkinje cells of silver-impregnated tissues of the owl cerebellum. Since then, a large number of studies on the structure of this organelle have been performed mainly by light microscopy of silver- and osmium-impregnated specimens and transmission electron microscopy (TEM) of thin sections (Beams and King, 1933, 1934; Dalton and Fleix 1953, 1954, Beams and Kessel, 1968, Farquhar and Palade, 1981, see also a review of Dröscher, 1999; Tamaki and Yamashina, 2002). TEM studies, together with autoradiography, histochemistry, and/or immunohistochemistry, have especially revealed that the Golgi apparatus is a stack of flattened membranous cisterns that plays an important role in the posttranslational modification, sorting, and packaging of proteins within cells. The Golgi apparatus also has polarity because the forming face (cis-face) and maturing face (trans-face) are present in its cisterns. Accordingly, the Golgi cisterns are generally classified into three categories: the cis-most cistern, medial cisterns, and trans-most cistern.

The three-dimensional ultrastructure of the Golgi apparatus has been also studied in relation to its polarity and function, especially by Rambourg and Clermont.
and their colleagues: They observed many different cell types by the combined use of conventional and high-voltage TEM of thin and/or thick sections treated with various staining methods including Ur-Pb-Cu staining, cytochemistry, and osmium impregnation (Rambourget al., 1974, 1979, 1984, 1987, 1988, 1992; Rambourg and Clermont, 1986, 1990, 1992; Clermont et al., 1995).

More recently, three-dimensional reconstructions made by high-voltage EM tomography have been also applied in investigations of the structure of the Golgi apparatus in three dimensions (Ladinsky et al., 1999, Marsh et al., 2001, 2004; Mogelsvang et al., 2004; Trucco et al., 2004; Marsh, 2005). Results of these studies emphasize on the complexity of the three-dimensional ultrastructure of the Golgi apparatus.

Despite the series of TEM studies introduced above, few studies on the Golgi apparatus have been performed by scanning electron microscopy (SEM) — even though SEM has a long focal depth and is suitable for observing specimens three-dimensionally — mainly because cytoplasmic proteins obstruct the observation of intracellular structures in conventionally prepared SEM specimens. To overcome this difficulty, Tanaka and his colleagues invented the osmium-maceration method (i.e., the osmium-DMSO-osmium method, and aldehyde- osmium-DMSO-osmium method), which enables direct SEM observation of membraneous cell organelles within cells by removing cytoplasmic soluble proteins with a diluted OsO₄ solution (Tanaka and Naguro, 1981; Tanaka and Mitsushima, 1984). Using these techniques, the Golgi apparatus in some cells was visualized more directly and three-dimensionally than that observed by TEM, though only a few studies have been published to date (Tanaka and Naguro, 1981; Tanaka et al., 1986; Tanaka and Fukudome, 1991, Ho et al., 1999).

The aim of the present study was to observe the three-dimensional ultrastructure of the Golgi apparatus in various cells by high resolution SEM of the osmium-macerated tissues. It first shows the three-dimensional ultrastructure of the Golgi apparatus in different cells, which includes epithelial principal cells in the epididymal duct, goblet cells in the jejunum, gonadotrophs in the pituitary gland, and dorsal root ganglion cells. The results are then discussed with special attentions to: 1) the shape of the Golgi apparatus in different cells; 2) the stack of the Golgi cisterns; 3) the ultrastructure of the cis-most cistern; 4) the ultrastructure of the trans-most cistern; and 5) connections between the rough endoplasmic reticulum (rER) and the Golgi stack.

### Materials and Methods

Adult male Wistar rats, 8 to 10 weeks of age, were used in the present study. The samples were prepared as follows based on an osmium-maceration method by Tanaka and Mitsushima (1984). After deep anesthesia with pentobarbital sodium (Nembtal, Dainippon Pharma Co, Osaka), the animals were perfused through the left ventricle with physiological saline followed by a mixture of 0.5% glutaraldehyde and 0.5% paraformaldehyde in a 0.1M phosphate buffer (pH 7.4). After perfusion fixation, the epididymis, jejunum, and pituitary gland were removed and cut into small pieces about 2 mm × 2 mm × 5 mm. Lumber spinal ganglia with small segments of spinal roots and nerve were also removed. All specimens were further fixed with 1% OsO₄ in a 0.1M phosphate buffer for 2 h at 4°C. After the samples were rinsed in a 0.1M phosphate buffer for 1 h, they were immersed in 25% and 50% dimethyl sulfoxide (DMSO) in distilled water for 30 min each. They were then frozen on a metal plate which had been previously chilled with liquid nitrogen and cracked into two pieces with a screwdriver and a hammer. The freeze-cracked pieces were immediately replaced into a 50% DMSO solution for thawing and rinsed in a 0.1M phosphate buffer for 1 h. For cell maceration, specimens were immersed in 0.1% OsO₄ in a 0.1M phosphate buffer for 72 h at 20–22°C under light; the solution was renewed every 24 h to prevent blackening of the solution. The macerated specimens were further fixed by immersed in 1% OsO₄ in a 0.1 M phosphate buffer for 1 h, washed in the phosphate buffer for 1 h, and treated with 1% tannic acid in the phosphate buffer for 2 h, rinsed in the buffer for 1 h, and placed in 1% OsO₄ in a 0.1M phosphate buffer for 1 h (i.e., conductive staining). The samples were dehydrated through a graded ethanol series, transferred to isoamyl acetate, and dried in a critical point dryer (HCP-2, Hitachi Kokki Co. Ltd, Japan) using liquid CO₂. Dried samples were mounted onto a metal plate with silver paste and coated with platinum-palladium, at a thickness of about 5 nm in an ion-sputter coater (E1010, Hitachi, Tokyo). They were observed in an in-lens-type field emission SEM (S-5000 Hitachi).
Results

The epithelial principal cell in the rat epididymis

The Golgi apparatus of the epithelial principal cell in the rat epididymal duct was located in the supranuclear region and was shaped like a candle flame (Fig. 1a,b). The stacks of cisterns in the Golgi apparatus were basically arranged in a U- or cup-shape with an opening towards the apical portion; they were often complexly folded inside the "U" and/or extended additional stacks in it. Secretory granules, the rER, and mitochondria were observed in the flame-shaped Golgi apparatus (Fig. 1b).

At high magnification, the polarity of the Golgi apparatus could be identified because the cis-most cistern and tran-most cistern had the characteristic features indicated by previous investigators (Fig. 1c); the cis-most cistern was located in the outer side of the U-shaped Golgi apparatus, and the trans-most cistern was in the inner side of the "U". The medial cisterns usually possessed eight layers.

When the Golgi apparatus was viewed from the cis-side, the cis-most cistern appeared like a continuous sheet with numerous round fenestrations (Fig. 2a). The size of the fenestrations was rather uniform at about 30 nm, though there were also some larger fenestrations up to 150 nm. The rER with a tubular or irregular shape was often located close to the cis-most cistern though no direct connection between the two structures was observed. On the other hand, the trans-most cistern was composed of tubular membranes of about 70 nm which were connected with each other roughly two dimensionally (Fig. 2b). The trans-most cistern also often had spherical swellings, suggesting the formation of secretory vesicles. The rER forming the tubular shape was closely associated with the trans-most cistern, but no continuity was observed between them. Some medial cisterns facing the trans-most cistern had small (ca. 50 nm) fenestrations similar to those found in the cis-most cistern. The other medial cisterns were observed as sheet-like structures with only a few large windows for connecting the cis and trans sides of the Golgi apparatus.

The goblet cell in the rat jejunum

The Golgi apparatus of the goblet cells in the jejunum was located in the upper portion of the nucleus and assumed a cylindrical or cupshape (Fig. 3a). The "cup" of the Golgi apparatus was usually filled with secretory granules, which were also abundant in the apical portion of the cell. The rER and mitochondria were also found inside the cup (Fig. 3b).

The stack of the Golgi cisterns consisted of the cis-most cistern, medial cisterns (usually six layers), and trans-most cistern (Fig. 3c); the cis-most cistern was located in the outer side of the cup and the trans-most cistern was in the inner side. The cis-most cistern was composed of both tubules and sheets which were connected with each other two-dimensionally (Fig. 4a). The sheet of the cisterns had a number of fenestrations (ca. 30 nm in diameter) on its surface. Although the cis-most cistern had a few spherical structures suggesting the fusion of transport vesicles, numerous vesicular structures were attached to the medial cistern next to it (Fig. 4a). The lamellae of the rER were present in the vicinity of the cis-most cistern, but no communication was found between the rER and the cis-most cistern. On the other hand, the trans-most cistern was composed of several small sheets with tubules (Fig. 4b). These sheets were often fringed with vesicular swellings. The medial cistern next to the trans-most cistern also acquired several vesicular structures on the trans-side surface. The rER was in contact with the trans-most cistern, but there was no direct communication with them.

The gonadotroph in the rat pituitary gland

The Golgi apparatus of the gonadotroph in the pituitary gland appeared spherical, occupying the middle of the cell (Fig. 5a, b, 6a). The Golgi apparatus consisted of several Golgi cisterns piled up concentrically. Inside the sphere of the Golgi apparatus were a number of secretory granules, the rER, and mitochondria.

At higher magnifications, the cis-most cistern was present in the outer surface of the spherical Golgi apparatus, and the trans-most cistern was in its inner surface (Fig. 5b, c). Medial cisterns amounted to three in the layers. The cis-most cistern was observed as a flat sheet with numerous fenestrations and several large windows. The size of fenestrations was roughly 30 nm; they were distributed evenly throughout the cistern. On the other hand, large windows had diameters of 200–500 nm. Beneath these windows were others of the underlying medial cisterns, resulting in the formation of a passage connecting the inside with the outside of the sphere of the Golgi apparatus (Fig. 6b). Tubules of the rER, mitochondria, and secretory granules were observed in these windows. The trans-most cistern of the gonadotroph was composed of several sheets which were connected with each other by anastomosed tubules. The rER with a tubular shape was often in contact with the trans-most cistern. However, no direct communication was observed between them (Fig. 6c).
Fig. 1. Legend on the opposite page.
Fig. 1. Scanning electron micrographs of the Golgi apparatus in the epithelial principal cells of the rat epididymal duct. 

a: Low magnification of epididymal epithelial principal cells. The Golgi apparatus (colored green) is located in the upper portion of the nucleus (colored red). The shape of the Golgi apparatus looks like a candle flame. The long axis of the apparatus is about 10 μm, the short axis about 6 μm. The cytoplasm in the center of the epithelial cell is colored blue. AP: apical portion of the epithelial cell, cis: cis-side of the Golgi apparatus, trans: trans-side of the apparatus. Bar: 3 μm.

b: Closer view of the Golgi apparatus (colored green) fractured along its longitudinal axis. The Golgi apparatus consists of several cisterns which are piled up in layers. Note the Golgi cisterns extending towards the inside of the flame-shaped Golgi apparatus (arrows). The rER (r-ER) and secretory granules (arrowheads) are observed in the Golgi apparatus. Bar: 1 μm.

c: High magnification SEM image. The cis- and trans-sides of the Golgi apparatus can be identified because of their characteristic shapes confirmed in previous electron microscopic studies. The cis-most cistern (C) is located in the outside, and the trans-most cistern (T) is in the inside of the Golgi apparatus. Note medial cisterns (numbered 1–8). Bar: 500 nm

Fig. 2. SEM images of the Golgi apparatus in the epithelial principal cells of the epididymal duct showing its cis surface (a) and trans surface (b). a: The cis-most cistern (greenish blue) is observed as a flat sheet with numerous fenestrations (arrows). The size of the fenestrations is rather uniform at about 30 nm. yellow: medial cisterns. Bar: 500 nm. b: The trans-most cistern (red) is composed of anastomosed tubules. These tubules have round swellings (arrowheads), suggesting the formation of secretory vesicles. The rER (purple) is closely associated with the trans-most cistern, although no continuity is found between them. Note that a medial cistern (asterisks) facing the trans-most cistern also has fenestrations. yellow: medial cisterns. Arrows: secretory granules. Bar: 500 nm
Fig. 3. Legend on the opposite page.
**Fig. 3.** SEM images of the Golgi apparatus in the goblet cell of the jejunum. **a:** Low magnification of the epithelium, where a goblet cell (blue) has been fractured along its long axis. The Golgi apparatus (green) is located in the upper region of the nucleus (red). The shape of the Golgi apparatus is cylindrical or cup-shaped, measuring about 8–10 μm along the long axis and about 3–4 μm along the short axis. Note secretory granules (Sg) accumulating at the apical portion of the cell. MV: microvilli, cis: cis-side of the Golgi apparatus, trans: trans-side of the apparatus. Bar: 3 μm. **b:** Closer view of a cup-shaped Golgi apparatus (green) in the goblet cell. The Golgi apparatus consists of several sheet-like cisterns which are piled up in layers. Secretory granules (orange), rER (r-ER) and mitochondria (M) are present in the inside of the cup of the Golgi apparatus. Arrows indicate openings which connect the inside of the cup with the outside. Apical secretory granules (asterisk) are bigger than those inside the Golgi apparatus. Note the nucleus (red) in the lower left of the micrograph. cis: cis-side of the Golgi apparatus, trans: trans-side of the apparatus. Bar: 1.5 μm. **c:** High magnification SEM image showing a Golgi stack. The cis-most cistern (C) is found in the outer surface of the cup-shaped Golgi apparatus. Note medial cisterns (numbered 1–6). r-ER: rER, M: mitochondria, Sg: secretory granules. Bar: 500 nm

**Fig. 4.** The Golgi apparatus observed from the cis-side (**a**) and trans-side (**b**). **a:** The cis-most cistern (greenish blue) is composed of both tubules (arrowheads) and fenestrated sheets (stars) which are connected with each other two-dimensionally. Note numerous vesicles (arrows) attaching to the medial cisterns (yellow). r-ER: rER. Bar: 500 nm. **b:** The trans-most cistern (red) consists of several small round sheets with tubules. Tubules of the rER (purple) are in close contact with the trans-most cistern, but no continuity is observed between them. Bar: 500 nm
Fig. 5. The Golgi apparatus in the gonadotroph of the rat pituitary gland. a: Low magnification of a gonadotroph. A spherical Golgi apparatus (green) is present in the center of this cell. The diameter of the Golgi apparatus is about 6 μm. red: nucleus; BC: blood capillary; cis: cis-side of the Golgi apparatus; trans: trans-side of the Golgi apparatus. Bar: 3 μm. b: Closer view of the Golgi apparatus. It is composed of Golgi cisterns piling up concentrically to form a spherical apparatus. There are windows (arrows) which allow communication between the inside and the outside of the Golgi apparatus. Note secretory granules (arrowheads), rER (r-ER) and mitochondria (M) inside the Golgi apparatus. cis: cis-side of the Golgi apparatus; trans: trans-side of the Golgi apparatus. Bar: 1 μm. c: The cis-most cistern (C) is found in the outer surface of the spherical Golgi apparatus, and the trans-most cistern (T) is in the inner surface of the apparatus. Note the medial cisterns (numbered 1–3). Bar: 500 nm
Fig. 6. The Golgi apparatus observed from its cis-side (a, b) and trans-side (c). a: The Golgi apparatus is observed as a sphere with several windows about 200–500 nm in diameter. cis: cis-side of the Golgi apparatus. The boxed area with a red broken line is enlarged and shown in b. Bar: 1 μm. b: The cis-most cistern (blue) has numerous fenestrations (arrows) which are distributed evenly throughout the cistern. Note also several large windows (asterisks) which apparently facilitate communication between the inside and the outside of the Golgi apparatus because the underlying medial cisterns (yellow) also have similar windows in the same portion. r-ER: rER, Sg: secretory granules. Bar: 300 nm. c: The trans-most cistern (red) is composed of several sheets which are connected with each other by anastomosed tubules. The tubular extension of the rER (purple) is closely associated with the trans-most cistern, although no connection is found between the two structures. yellow: medial cistern, green: mitochondrion. Bar: 500 nm
Fig. 7. Low magnification of the spinal ganglion cell. Many small Golgi stacks (green) are scattered throughout the cytoplasm. The sizes of the Golgi apparatus are about 1 to 2 μm. red: nucleus. Bar: 6 μm

Fig. 8. The Golgi apparatus in the spinal ganglion cells. a: In a closer view, each Golgi stack is composed of several cisterns piled up in layers. The rER (t-ER) and mitochondria (M) are observed around the Golgi apparatus. Bar: 1 μm. b: High magnification of a Golgi stack. This stack has five medial cisterns (numbered 1–5) with a typical cis-most cistern (C) and trans-most cistern (T). r-ER: tER; M: mitochondrion. Bar: 500 nm
Fig. 8. Legend on the opposite page.
The spinal ganglion cell

The spinal ganglion cell possessed a number of small Golgi stacks which were scattered throughout the cytoplasm (Fig. 7, 8a). Each Golgi stack was composed of cisterns piled up in layers; it usually had five layers of the medial cisterns in addition to the cis-most cistern and the trans-most cistern (Fig. 8b). The trans-side of each Golgi stack had a tendency to face toward the nucleus.

The cis-most cistern was like a sheet with numerous fenestrations, about 30 nm in diameter (Fig. 9a). The medial cisterns in this Golgi apparatus were also observed as fenestrated sheets although the size and the number of the pores were smaller than those in the cis-most cistern. On the other hand, the trans-most cistern took the shape of a lacework with larger fenestrations (ca. 100 nm in diameter) and sometimes appeared to be composed of anastomosed tubular structures. Some tubules of the rER were very closely associated with the trans-most cistern, but no connection was found between the two (Fig. 9b).

Discussion

The present study has documented the three-dimensional ultrastructure of the Golgi apparatus in different cells by high resolution SEM of osmium-macerated tissues. The osmium maceration method used here was effective for removing the cytoplasmic matrix from cells, thus enabling the direct visualization of the Golgi apparatus in three dimensions. In this study, we chose four different types of cells — the epididymal cell, the goblet cell, the gonadotroph and the ganglion cell — for observing the Golgi apparatus by SEM as these cells are known to have a well-developed Golgi apparatus with different functions and have been repeatedly studied by previous TEM researchers (e.g., epididymal cell: Dalton and Flex, 1954; Hermo et al., 1991; goblet cell: Neutra and Leblond, 1966; gonadotroph: Inoue and Kurosuni, 1977, 1989; dorsal root ganglion cell: Novikoff et al., 1971; Rambourg and Clermont, 1984, 1986). The findings obtained in
Fig. 10. Schematic drawings of the Golgi apparatus in different cells. A: The Golgi apparatus of the epididymal epithelial cell is located in the upper portion of the nucleus and takes the shape of a chandelier. The trans-cisterns appear in the inner side of this apparatus in red. B: In the intestinal goblet cell, the Golgi apparatus is located in the upper region of the nucleus, and its shape is similar to a cup. The trans-cistern is found in the inner side of this structure. C: The gonadotroph has a spherical Golgi apparatus, which is located in the center of the cell. The trans-most cistern is present in the inner surface of this spherical Golgi apparatus. D: The spinal ganglion cell has many small Golgi stacks which are scattered throughout the cytoplasm. The trans-most cistern of each Golgi stack has a tendency to face the nucleus.

The shape of the Golgi apparatus in different cells

The entire shape of the Golgi apparatus has been mainly studied by light microscopy of silver- and/or osmium-impregnated specimens (e.g., Penfield, 1920; Beams and King, 1933, 1934; Lieberman, 1969) because TEM of ultrathin sections is inadequate to investigate the three-dimensionally complicated shape of the Golgi apparatus without reconstruction. On the other hand, SEM of osmium-macerated tissues is very useful not only for observing the ultrastructure of the Golgi stacks but also for studying the overall shape of the Golgi apparatus, as has also already been shown by Tanaka’s group (Tanaka et al., 1986, 1991). Thus, the present study has three-dimensionally shown the shape of the Golgi apparatus clearly in different cells with the osmium-maceration method for SEM and confirmed that the Golgi apparatus varies in shape depending on the cell types (Fig. 10).

Both the epididymal epithelial principal cell and intestinal goblet cell are exocrine cells: the former is involved in the secretion of a variety of substances such as ions, small organic substances and glycoproteins, while the latter produce many mucous granules. The present study has shown that the Golgi apparatus in these cells is roughly cup-shaped with the trans-side inside, although the shape in the epididymal principal cells is rather
complicated. The slight variance in structure between the two might be related to their specific functions including the secretion of different contents and production activities, but further studies shall have to elucidate this matter.

The present study has clearly demonstrated that the gonadotroph has a spherical Golgi apparatus. Similar findings were reported by Tanaka and his colleagues (Tanaka et al., 1986; Tanaka and Fukudome, 1991) in their SEM studies in anterior pituitary cells and tracheal epithelial cells, though they did not specify the type of cell in the pituitary gland nor the tracheal epithelium. The present study has further shown the polarity of the spherical Golgi apparatus in the gonadotroph: the cis-side is located in the outer surface of the sphere, while the trans-side is in the inner surface. This finding indicates that secretory granules in the gonadotroph are produced inside the spherical Golgi apparatus and are transported from the inside to the outside probably through large windows of the Golgi sphere by a certain mechanism.

The Golgi apparatus in the spinal ganglion cells differs considerably from the other Golgi apparatuses examined in the present study in that many small Golgi stacks are scattered throughout the cytoplasm. In their high-voltage TEM studies of osmium-impregnated thick sections, Rambourg and Clermont (1986) showed that the the Golgi stacks were connected with each other via small anastomosing tubules to form a large perinuclear network as a whole. However, we could not observe such connecting tubules in the present study. Further studies using SEM of osmium-macerated tissues might be useful for clarifying this point.

**The stack of the Golgi cisterns**

The stack of the Golgi apparatus consists of the cis-most cistern, the medial cisterns, and the trans-most cistern. The present study has clearly demonstrated that the shape of both the cis-most and trans-most cisterns is unique in comparison with that of the medial Golgi cisterns. This will be discussed later.
The present study has shown that the number of the medial cisterns of the Golgi stack differs by cell type. Some previous investigators stated that a direct connection was observed between medial cisterns in certain cell types (spermatid: Clermont et al., 1994; lacrimal gland cell: Tanaka et al., 1986, Tanaka and Fukudome, 1991). However, we could not observe connections between cisterns of the Golgi apparatus in the present study. Whether the direct connection between Golgi cisterns is present in certain cells remains unclear. On the other hand, recent reconstruction studies using EM tomography of thick sections demonstrated tubular connections appearing between successive medial cisterns in nocodazole-treated NRK cells (Trucco et al., 2004). Marsh et al. (2004) also reported in their EM tomography studies that direct continuities appeared between cisterns of the Golgi apparatus in glucose-stimulated mouse islet beta cells. These findings suggest that direct communication between cisterns is produced when the cells are stimulated in certain conditions. Clarification of this matter awaits future studies.

The ultrastructure of the cis-most cistern

In previous TEM studies of thin and thick sections, the cis-most cistern was referred to as the cis tubular network because it appeared to be composed of anastomosing tubules (Rambourg et al., 1979, 1987 1988; Rambourg and Clermont., 1986). However, we have demonstrated that the cis-most cistern generally takes the shape of a sheet with numerous fenestrations, although there are also tubular structures in some cell types (Fig. 11).

The present study has also shown the presence of large windows in addition to small fenestrations in the sheet of the cis-most cistern. It is interesting that underlying medial cisterns also have similar windows in the same position, resulting in the formation of a tunnel for connecting the inside of the Golgi apparatus with the outside. Similar findings were observed by some TEM and SEM investigators (Tanaka et al., 1986, Ichikawa and Ichikawa, 1987; Tanaka and Fukudome, 1991). These windows are apparently used as a passage for various cell organelles (such as the rER and mitochondria) and vesicles.
The ultrastructure of the trans-most cistern

The present study has documented that the shape of the trans-most cistern depends on the cell type, although it is generally composed of tubules and/or small plates (Fig. 12). The trans-most cistern of the epidymal principal cell is composed of tubular structures connecting with each other two dimensionally. It is interesting that these tubular structures often had spherical swellings, suggesting the formation of secretory vesicles. Similar structures were observed by Hermo et al. (1991), who demonstrated by TEM that nodular and dilated regions were present in the trans-most cistern of the same cell type. On the other hand, the trans-most cistern of the goblet cell consists of several small sheets with short tubules and/or vesicles. In the gonadotroph, the trans-most cistern is composed of several sheets and anastomosed tubules which are connected with each other two-dimensionally; vesicular swellings are only located in the tubular regions. The trans-most cistern in the spinal ganglion cell takes the shape of a lacework which consists of anastomosed tubular structures. The variety of the shape of the trans-most cistern in different cells is probably related to the function of the individual cells. Indeed, in their TEM studies on the Golgi apparatus of various cell types, Clermont et al. (1995) stated that the structure of the trans-most cistern, or the trans-Golgi network, varies considerably from one cell type to another, being extensive in cells not showing typical secretory granules but having an extensive lysosomal system, while in secretory cells showing small or large secretory granules the cistern is either small or even entirely absent. Studies on the trans-most cistern of the same cell types in different conditions might also provide useful information on this subject.

Connections between the rER and the Golgi stack

In their TEM studies on the Golgi apparatus in the small neurons of dorsal root ganglia, Novikoff et al. (1971) stated that the trans-most cistern was continuous with the rER and related to the formation of lysosomes and secretory granules. Thus, they coined the term "GERL" for this structure because this Golgi-associated rER produces lysosomes. On the other hand, contradictory results were reported by later TEM researchers, who considered that the trans-most cistern to be closely associated with the rER but not in communication with each other (Inoue and Kurosuni, 1977, 1989; Rambourg et al., 1986, 1988; Hermo et al., 1991). The present study has shown that no direct communication is found between the trans-most cistern and the rER in the cells studied, though the two structures are either closely associated or in contact with each other. The three-dimensional observation of the Golgi apparatus in the small type of the spinal ganglion cells might be important because the cells observed by Novikoff and his colleagues were of this type.

Interestingly, there are also some reports on the connection between the cis-most cistern and the rER (Lindsey and Ellisman, 1985; Tanaka et al., 1986; Tanaka and Fukudome, 1991). However, we were unable to observe any connection between the cis-most cistern and rER in the present study.

In conclusion, the present study has documented the structure of the Golgi apparatus in different cell types and has shown that the structure varies by cell type. The variety in the ultrastructure of the cis- and trans-most cistern in different cell types has been also clearly observed in our SEM observations. In the future, a combination of SEM and histochemical techniques may serve to clarify the three-dimensional ultrastructure of the Golgi apparatus in relation to its function.

References


Dröscher A: From the "apparato reticolare interno" to "the Golgi": 100 years of Golgi apparatus research.
Farquhar MG, Palade GE: The Golgi apparatus (complex)-
Hermo L, Rmbourg A, Clermont Y: Golgi apparatus
of epithelial principal cells of the epididymal initial
segment of the rat: structure, relationship with
endoplasmic reticulum, and role in the formation of
Ho HC, Tang C-Y, Suarez SS: Three-dimensional
structure of the Golgi apparatus in mouse spermatozoids:
a scanning electron microscopic study. Anat Rec 256:
Ichikawa M, Ichikawa A: The fine structure of sublingual
gland acinar cells of the Mongolian gerbil, Meriones
unguiculatus, processed by rapid freezing followed by
Inoue K, Kurosumi K: Cytochemical and three-
dimensional studies on Golgi apparatus and GERL
of rat anterior pituitary cells by transmission electron
Inoue K, Kurosumi K: Ultrastructural observation of the trans-Golgi associated plate-like cisterna in
the secretory cells of the rat anterior pituitary gland
with special reference to the intracisternal skeleton. Anat Rec
Ladinsky MS, Mastronarde DN, McIntosh JR, Howell
KE, Staehelin LA: Golgi structure in three dimensions:
functional insights from the normal rat kidney cell. J
Lieberman AR: Light- and electron microscope
observations on the Golgi apparatus of normal and
axotomized primary sensory neurons. J Anat 104: 309-
Lindsey JD, Ellisman MH: The neuronal endomembrane
system. 1. Direct links between rough endoplasmic
reticulum and the cis element of the Golgi apparatus. J
Marsh B: Lessons from tomographic studies of the
mammalian Golgi. Biochim Biophys Acta 1744: 273-
Marsh BJ, Mastronarde DN, Buttle KF, Howell KE,
McIntosh JR: Organelar relationships in the Golgi
region of the pancreatic beta cell line, HIT-T15,
visualized by high resolution electron tomography.
Marsh BJ, Volkman N, McIntosh JR, Howell KE: Direct
continuities between cisternae at different levels of
the Golgi complex in glucose-stimulated mouse islet beta
Mogelvang S, Marsh BJ, Ladinsky MS, Howell KE:
Predicting function from structure: 3D structure studies of
Neutra M, Leblond CP: Synthesis of the carbohydrate
of mucus in the Golgi complex as shown by electron
microscope radioautography of goblet cells from rats
(1966).
Novikoff PM, Novikoff AB, Quintana N, Hauw Jean-
Jacques: Golgi apparatus, GERL, and lysosomes of
neurons in rat dorsal root ganglia, studied by thick
section and thin section cytochemistry. J Cell Biol 50:
859-886 (1971).
Penfield W G: Alterations of the Golgi apparatus in nerve
Rambourg A, Clermont Y: Tridimensional structure of the
Golgi apparatus in type A ganglion cells of the rat. Am
Rambourg A, Clermont Y: Three-dimensional electron
Rambourg A, Clermont Y: Three-dimensional structure of
cytidine monophosphatase reactive trans-Golgi
elements in spinal ganglion cells of the rat. Am J Anat
Rambourg A, Clermont Y, Marraud A: Three-dimensional
structure of the osmium-impregnated Golgi apparatus
as seen in the high voltage electron microscope. Am J
Rambourg A, Clermont Y, Hermo L: Three-dimensional
architecture of the Golgi apparatus in sertoli cells of the
Rambourg A, Segretain D, Clermont Y: Tridimensional
architecture of the Golgi apparatus in the atrial muscle
Rambourg A, Clermont Y, Hermo L, Segretain D:
Tridimensional architecture of the Golgi apparatus and
its components in mucous cells of Brunner's glands of
Rambourg A, Clermont Y, Hermo L: Formation of
secretion granules in the Gplgi apparatus of pancreatic
Rambourg A, Clermont Y, Chretien M, Olivier
L: Formation of secretory granules in the Golgi
apparatus of prolactin cells in the rat pituitary gland: a
Tamaki H, Yamashina S: The stack of the Golgi aparaat.
Tanaka K, Fukudome H: Three-dimensional organization
of the Golgi complex observed by scanning electron

