Histochem Cell Biol (1998) 109:533–543

REVIEW

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The tubular network of the Golgi apparatus

Accepted: 3 November 1997

Abstract Golgi apparatus of both plant and animal cells are characterized by an extensive system of approximately 30 nm diameter peripheral tubules. The total surface area of the tubules and associated fenestrae is thought to be approximately equivalent to that of the flattened portions of cisternae. The tubules may extend for considerable distances from the stacks. The tubules are continuous with the peripheral edges of the stacked cisternae, but the way they interconnect differs across the stack. In plant cells, for example, tubules associated with the near-*cis* and mid cisternae often begin to anastomose close to the peripheral edges of the stacked cisternae, whereas the tubules of the *trans* cisternae are less likely to anastomose and are more likely to be directly continuous with the peripheral edges of the stacked cisternae. Additionally, the tubules may blend gradually into fenestrae that surround some of the stack cisternae. Because of the large surface area occupied by tubules and fenestrae, it is reasonable to suppose that these components of the Golgi apparatus play a significant role in Golgi apparatus function. Tubules clearly interconnect closely adjacent stacks of the Golgi apparatus and may represent a communication channel to synchronize stack function within the cell. A feasible hypothesis is that tubules may be a potentially static component of the Golgi apparatus in contrast to the stacked cisternal plates which may turn over continuously. The coated buds associated with tubules may represent the means whereby adjacent Golgi apparatus stacks exchange carbohydrate-processing enzymes or where resident Golgi apparatus proteins are introduced into and out of the stack during membrane flow differentiation. The limited gradation of tubules from *cis* to medial to *trans* offers additional possibilities for functional specialization of Golgi apparatus in keeping with the hypothesis that tubules are repositories of resident Golgi apparatus proteins protected from turnover during the flow differentiation of the flattened saccules of the Golgi apparatus stack.

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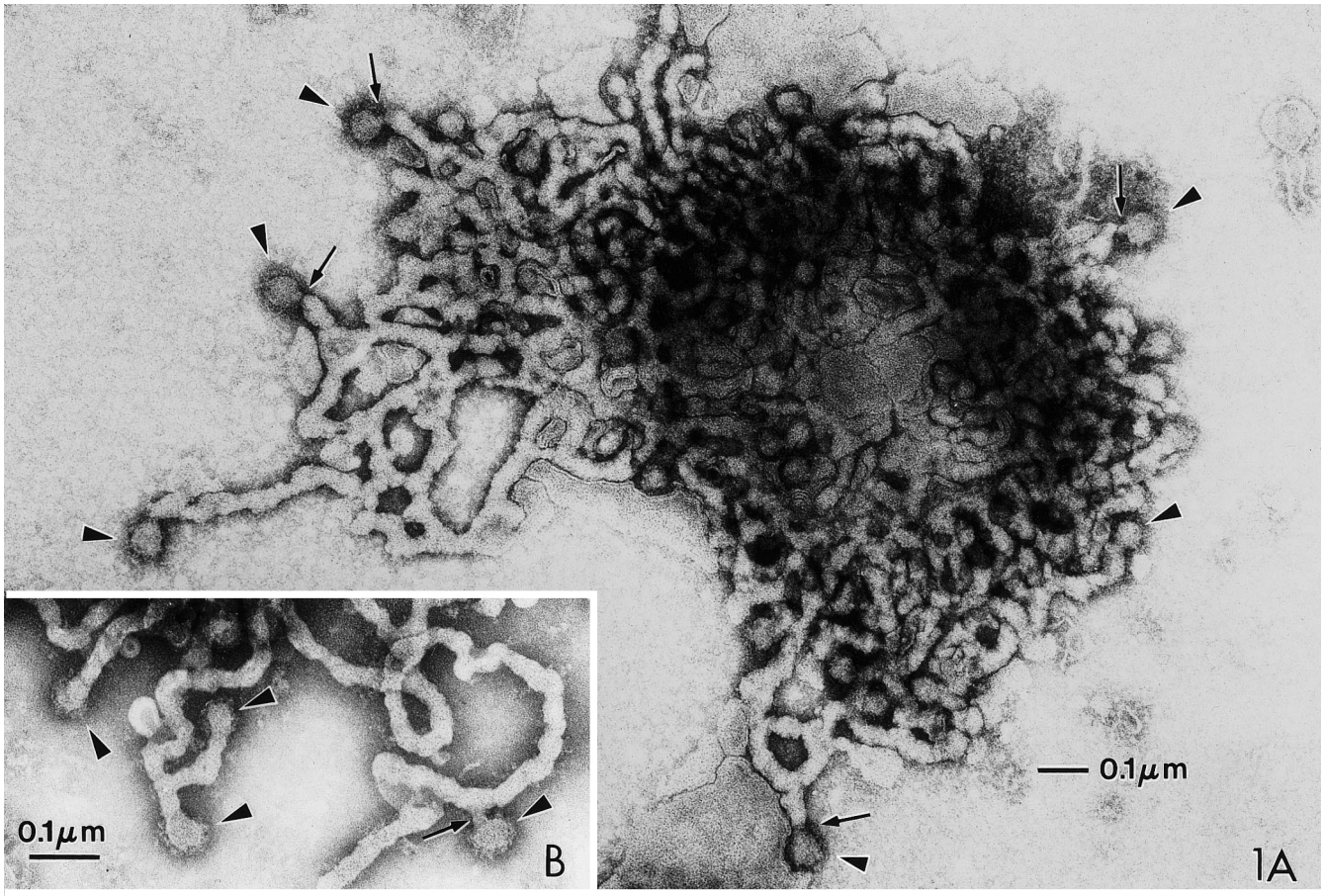
Introduction: the tubular continuum of the Golgi apparatus

Golgi apparatus organization of plant and animal cells is dominated by three general structural domains. The first is the commonly recognized central stack of flattened saccules (i.e., the flattened parts of the cisternae). The second domain is a region of fenestrae (i.e., holes or windows through the cisternae) that borders some saccules. In plant cells, the fenestrae seem to be more commonly associated with mid cisternae than with the most *cis* cisternae or with *trans* cisternae.

The third domain, and the main focus of this article, is the extensive system of tubules that are continuous with the peripheral edges of the flattened saccules either through the fenestrae or, when fenestrae are absent, directly with the central saccules of the stack. When fenestrae are present, it is common for the tubules to blend gradually into the fenestrated regions through smaller and smaller anastomoses so that a clear demarcation between the membranes delimiting fenestrae and peripheral tubules is often not possible at the structural level. When fenestrae are absent, tubules may emerge with or without anastomoses from the peripheral edges of the flattened saccules. Tubules may extend many micrometers through the cytoplasm. This system of tubules may exceed the smooth parts of the central saccules in total membrane surface and could contribute significantly to Golgi apparatus function.

One of the first reports of tubules was by Manton (1960) who demonstrated an extensive array of tubules associated with the Golgi apparatus stacks (dictyosomes) in the alga *Anthoceros*. The tubules were continuous with the peripheral edges of the dictyosome cisternae and were joined into a reticulum which exhibited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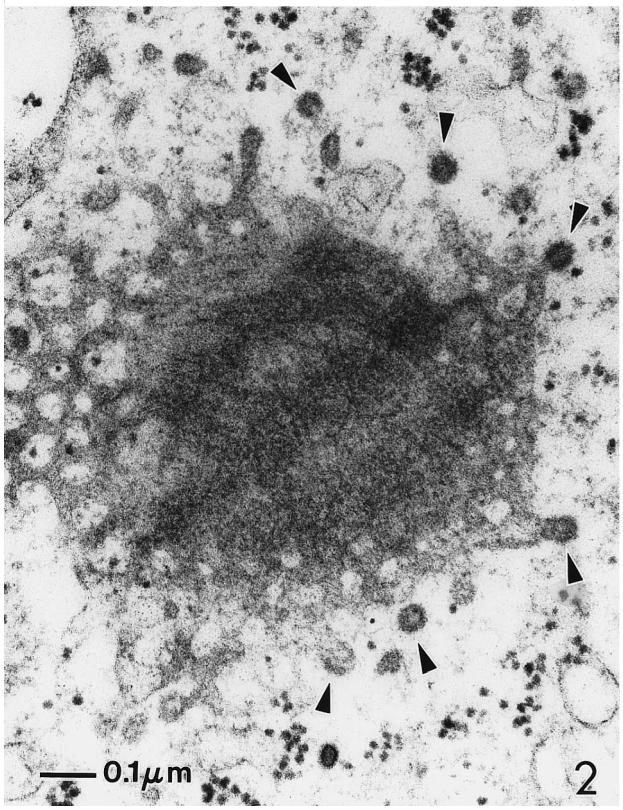
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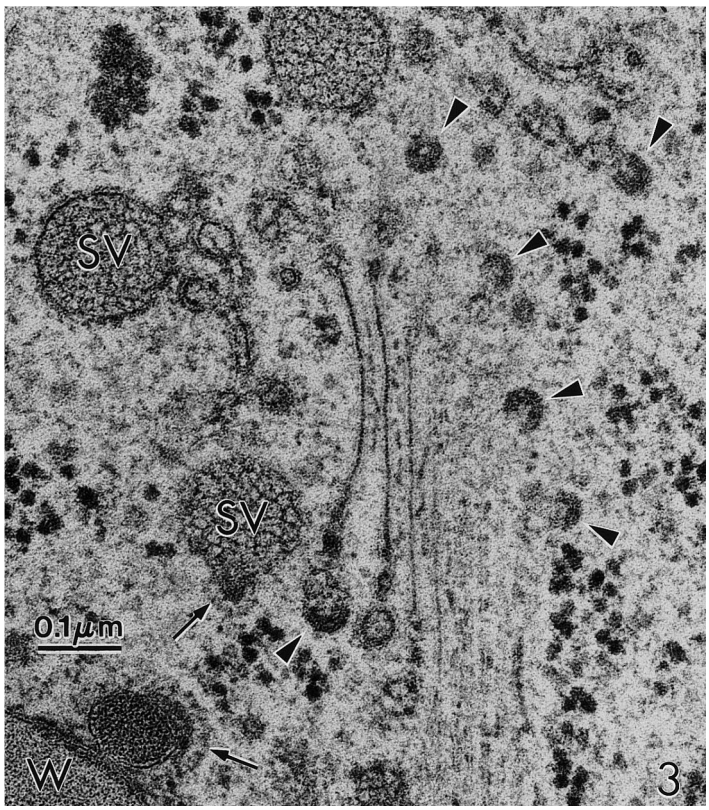
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local swellings at various distances from the unfenestrated parts of the subtending cisternae. Manton noted that the dictyosome-associated tubules were distinct from endoplasmic reticulum. These observations were made from conventional thin sections prepared for the electron microscope. Morré and coworkers also demonstrated tubules in thin sections of isolated dictyosomes that had been stabilized by glutaraldehyde (Morré et al. 1965).

A most revealing early study of tubules was that of Cunningham and coworkers of glutaraldehyde-stabilized, isolated, and negatively stained dictyosomes of onion stem (Cunningham et al. 1966). Cunningham et al. (1966) demonstrated an extensive and complex network of tubules completely engulfing the stacks and radiating outward from the stacked cisternae (Fig. 1). They also demonstrated that these tubules were continuous with the stacked cisternae (Figs. 9, 11–13) and that a vesicular bud with a uniquely coated surface was associated with them (Figs. 1–3). The tubular component of the dictyosome was verified in the same study by thin-section electron microscopy and subsequently by numerous investigators who have studied this aspect of Golgi apparatus morphology (Figs. 2, 3; Morré et al. 1965; Mollenhauer and Morré 1991; Weidman et al. 1993). Two early diagrams interpreting the relationship between the tubules and the flattened parts of the dictyosome cisternae are reproduced here (Figs. 4, 5; Mollenhauer and Morré 1966a, 1971; Morré et al. 1971). These diagrams would require only slight modifications to accurately reflect the current state of knowledge. The more modern interpretation would show mid cisternae with more fenestrae and fewer tubules and *trans* cisternae with no fenestrae and only tubules. The most *cis* cisterna, which is predominantly tubular, is not shown in Fig. 4.

◀ **Fig. 1A, B** Dictyosomes isolated from bean root, stabilized with glutaraldehyde, and negatively stained with phosphotungstic acid using the procedures described by Cunningham et al. (1966). **A** The stacked cisternae appear as a mound of tubules that anastomose and spread outward from the center of the dictyosome. The flattened parts of the cisternae are mostly hidden by the tubular parts of the dictyosome. The *cis*-most cisterna which may be predominantly tubular is thought to be absent from this dictyosome and was presumably lost during isolation. Coated buds are attached to some of the tubules (*arrowheads*). The buds may appear as swellings of tubule ends (**B**) or may be attached to the tubules by small neck-like appendages (*arrows*). The line of demarcation between the neck and tubule may be very sharp and distinct, and sometimes appears as though the two entities are separated by a membrane. In negative stain, the luminal contrast of the buds and of the connecting neck is the same but often different from that of the tubules (compare **A, B**). The buds are labile and are not observed (or recognized) without glutaraldehyde stabilization. (Micrograph reproduced from Mollenhauer and Morré 1991). **B** Further illustrations of bud morphology (*arrowheads*). These buds appear as swellings of tubule ends with luminal contents that differ only slightly from those of the tubules to which they are connected. Occasionally, buds are simply attached to the cylindrical sides of a tubule through a small neck (*arrow*). The main body of this dictyosome is off the micrograph toward the *upper left*. *Bar* 0.1 μm

Fig. 2 A plant dictyosome from daffodil trumpet fixed in glutaraldehyde and osmium tetroxide, embedded in epoxy resin, and sectioned at an angle nearly tangential to the plane of the cisternae. Tubules and buds (*arrowheads*) are clearly visible in much the same perspective as for the negatively-stained dictyosome in Fig. 1. *Bar* 0.1 μm

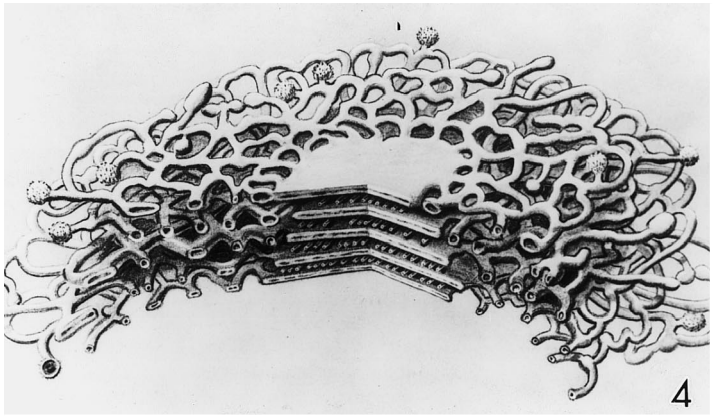
Fig. 3 A maize root tip dictyosome from the epidermis processed by freeze substitution and sectioned at nearly right angles to the plane of the cisternae. The coated buds (*arrowheads*) are easily identified by their position in the stack, their appearance, and size. The coat appears in freeze-substituted tissues as what might be described as a fuzzy unit membrane. The dictyosome is oriented so that the *cis* pole is toward the right of the micrograph. Membranes of the *cis* cisternae, like those of the endoplasmic reticulum, do not stain well when freeze substituted and are almost invisible. This staining pattern is consistent with membrane differentiation from endoplasmic reticulum-like at the *cis* face to plasma membrane-like at the *trans* face (Grove et al. 1968). The most *trans* region of a second dictyosome is visible at the *upper right* of the micrograph. Note that buds may be associated with *trans* cisternae as well as *cis* and intermediate cisternae. *SV* Secretion vesicle, *W* cell wall, *arrows* clathrin coats on secretion vesicles. *Bar* 0.1 μm

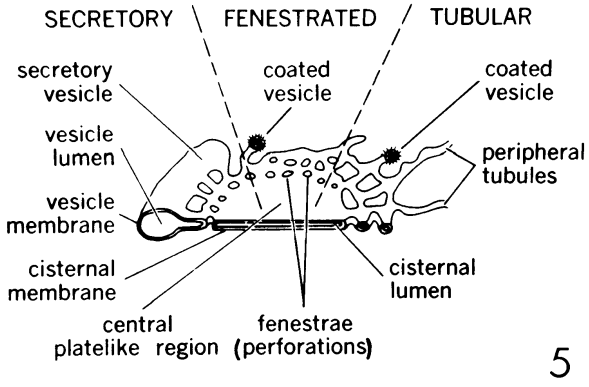
Did Golgi's internal reticulum include tubules?

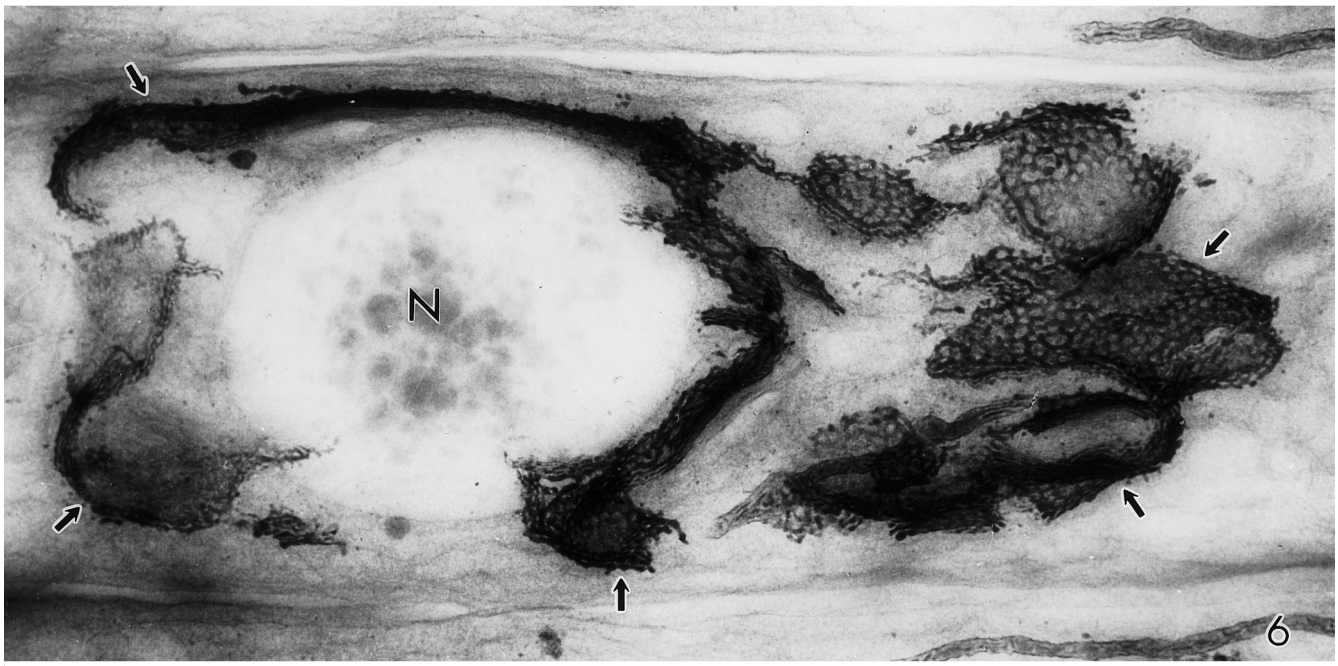
A reticular part of the Golgi apparatus was implicit even in work prior to electron microscopy. These included a continuous membrane system that interconnected the stacks (dictyosomes or osmiophilic platelets) (Bourne and Tewari 1964) although the details of the interconnecting membrane system could not be resolved. It was also suggested that components of endoplasmic reticulum were responsible for Golgi's reticulum (see Bourne and Tewari 1964) although verification of that suggestion was beyond the magnification and resolution available with the light microscope.

Early electron micrographs did not immediately resolve the origins of Golgi's internal reticulum in part because the volume of tissue accessed by electron microscopy was small and, in part, because no reticulum per se was observed. However, the electron microscope made it possible to visualize structural detail within the internal membranous systems of the cell and to point the way toward defining those components of the cell that might have given rise to Golgi's reticulum.

Intuitively, one would link the endoplasmic reticulum to Golgi's reticulum since it was the only continuous and extended membranous system in the cell that could be identified by cytologists that was positionally associated with what the early Golgi apparatus-specific stains identified as the Golgi apparatus. Recent studies, however, indicate that there are at least two other membranous components of the cell that could be candidates for a part of Golgi's reticulum. One of these is composed of the tubular constituents associated with the peripheral edges of the stacked cisternae of the Golgi apparatus and is the subject of this report. It is of interest, perhaps, to note that cross-sections of these tubules were observed even in the very earliest electron microscope studies, but were often incorrectly identified as small Golgi apparatus vesicles. The second component is the network of mem-







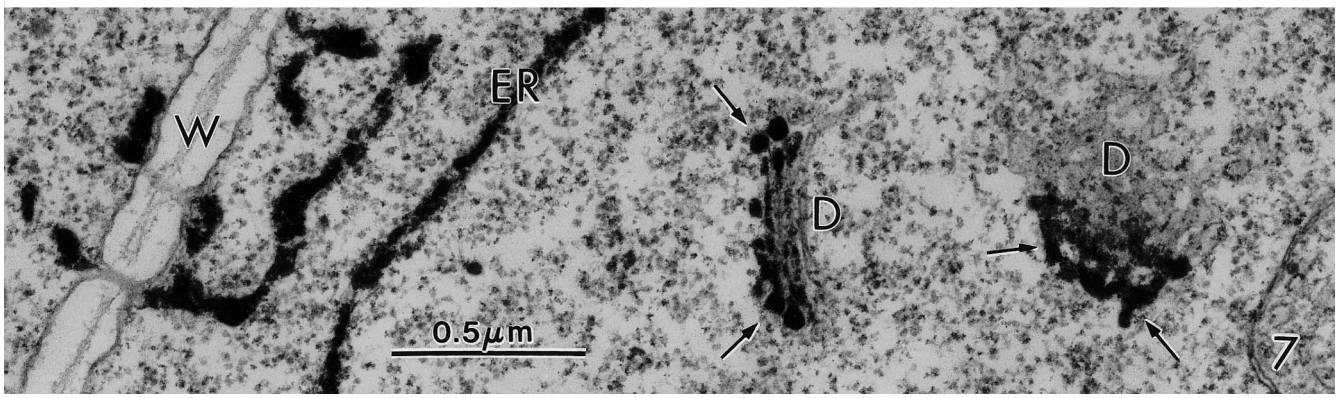


Fig. 4 Diagrammatic interpretation of a plant dictyosome from Mollenhauer and Morré (1966a). The *cis* to *trans* polarity is depicted from *top* to *bottom*. In light of subsequent findings, the mid cisternae should have fewer tubules and more fenestrae than illustrated here whereas the *trans* cisternae should have longer tubules, almost no fenestrae, and fewer anastomoses. However, the diagram does convey a complexity and tubular content in keeping with current interpretations of dictyosome structure. Note that the predominantly tubular *cis*-most cisterna is not illustrated

Fig. 5 composite diagram illustrating the three major configurations of tubules as applied to a plant dictyosome. *Left* Secretory vesicles are attached to stack cisternae by tubules. *Center* Coated buds and fenestrae representative of mid-stack cisternae. *Right* Coated buds and longer tubules that may interconnect adjacent stacks. Diagram reproduced from Mollenhauer and Morré (1971)

Fig. 6 Golgi apparatus from multifid gland of the snail *Helix pomatia*, treated so as to precipitate osmium tetroxide, thick sectioned (1 μm), and examined in an electron microscope operating at 2.5 meV accelerating potential. The Golgi apparatus, which courses through much of the cell, is outlined by *arrows*. The tubular constituent is substantial and appears to interconnect the stacks to form a single large organelle. *N* Nucleus. Courtesy of P. Favard, N. Carasso, and L. Ovtracht, CNRS, Saclay, France

Fig. 7 A maize root tip cell treated so as to precipitate osmium tetroxide (osmium impregnation). Dictyosome tubules (*arrows*) and endoplasmic reticulum (*ER*) contain heavy osmium deposits. The central parts of the dictyosomes (*D*) lack heavy deposits. *W* Cell wall. *Bar* 0.5 μm

branes and vesicles associated with the *trans* poles of the dictyosome stacks and commonly referred to here as the *trans*-Golgi apparatus network (TGN) (Griffiths and Simons 1986; Geuze and Morré 1991) although it is recognized that not all such networks are equivalent either functionally or biochemically (for example, see Pesacreta and Lucas 1985; Griffing 1991).

It is important to understand that our modern acceptance and understanding of Golgi's reticulum was largely dependent on the development of fixation procedures that preserved the constituents of the cells, and on cytochemical stains such as osmium tetroxide, potassium permanganate, and uranyl acetate that rendered the cellular constituents visible in the electron microscope. It was fortuitous that osmium tetroxide could also be adapted to mimic the silver deposition procedures used by Golgi. Precipitation stains using osmium, or mixtures of osmium and zinc (Juniper et al. 1982; Harris and Oparka 1983; Rambourg et al. 1987; Clermont et al. 1994), added a third dimension to the general architecture of the Golgi apparatus, especially when used in conjunction with high-voltage electron microscopy and thick sections (Fig. 6; Favard et al. 1971; Rambourg et al. 1987; Ladinsky et al. 1994). These procedures mimicked the black reaction originally used in the discovery of the Golgi apparatus (Golgi, 1898; Beams and Kessel 1968; Infarerra and Carrozza 1975; Whaley 1975). The procedure generally marked several constituents of the cell usually including parts of the Golgi apparatus stack, parts of a tubular system associated with the stack, the endoplasmic reticulum, and even the cristae and bounding membranes of some mitochondria (Fig. 7). In plants, deposits were often found in plastids.

The intensity of the precipitates, as well as the cellular components that stained, varied considerably even in the same kinds of cells or even in adjacent cells. Nonetheless, the results clearly confirmed that the Golgi apparatus was, indeed, an organelle that was often distributed throughout much of the cell (Favard 1969; Favard et al. 1971; Tanaka et al. 1986; Rambourg et al. 1987; Tai and Albrecht 1992). The organelle consisted of one or more stacks of flattened saccules, and tubular elements that were continuous with the peripheries of the saccules. To what extent endoplasmic reticulum and Golgi apparatus exhibited direct membrane continuity could not be determined unequivocally by the osmium precipitation procedures.

The black reaction and other heavy metal precipitation stains are now known to react most intensely with the *cis*-oriented tubular network of the Golgi apparatus, the most *cis* parts of the stacked cisternae, and with the tubules of the *cis* half of the stack (Figs. 6, 7). These stains also may react with the endoplasmic reticulum. Therefore, it would seem reasonable to assume that the major part of Golgi's internal reticulum was the tubular constituents of the *cis* poles of the stacked cisternae along with some of the *cis*-most cisternae and a portion of the endoplasmic reticulum. Because of a general lack of reactivity of the TGN with silver and osmium precipitation markers (at least in animal cells; see Griffing 1991), it is doubtful that these structures were included in the images observed by Camillo Golgi.

Universality of tubules

Three classes of tubular constituents have been recognized as being continuous with, or derived from, the stack cisternae of the Golgi apparatus. These are the *cis* cisternae (which are predominantly or entirely tubular), the peripheral fenestrae and tubules of the other stacked cisternae, and the structures that make up the various TGNs. Although the most *cis* cisternae can be isolated with the stacks and have the coated buds typical of other stack cisternae, they also react with fixatives and markers in much the same way as does endoplasmic reticulum. Thus, the most *cis* cisternae appear to be intermediates between endoplasmic reticulum and Golgi apparatus.

On the other hand, the tubules of the TGN are morphologically distinct from the other tubular constituents of the Golgi apparatus, are not continuous with the stack cisternae (Griffiths and Simons 1986; Geuze and Morré 1991), and are not isolated as components of the Golgi apparatus stacks. Moreover, there are at least two kinds of (clathrin?) coated vesicles associated with the TGN (Mollenhauer et al. 1991), neither of which are present on the *cis* cisternae or peripheral tubules of the stack. In plant cells, the TGN initially lies within the zone of exclusion surrounding all dictyosomes and may be derived from the *trans*-most cisternae of the stacks (Mollenhauer et al. 1991).

Tubular *cis* cisternae and peripheral tubules of Golgi apparatus cisternae appear to be conspicuous parts of all dictyosomes. Both plant and animal dictyosomes have tubules of approximately the same size and character (Mollenhauer et al. 1967; compare Figs. 1–3 with Fig. 8). In both, the tubules may be configured as fenestrae or extended networks and both have coated buds attached to, or continuous with, some of the tubules. The totality of tubules within cells may be quite striking as suggested by simple observations of isolated dictyosomes (Fig. 1). Calculations comparing the surface areas of the flattened parts of cisternae to the surface areas of their tubular constituents confirm these impressions (Mollenhauer and Morré 1990). In these experiments, membrane areas of tubules including some fenestrae and the flattened parts of stack cisternae, were calculated for plant dictyosomes. Isolated cisternae were stabilized with glutaraldehyde and then negatively stained. The cisternae were released from the stack for visualization using chaotropic agents as described (Mollenhauer et al. 1973). Cisternae from the *cis*, mid, and *trans* parts of the stack were analyzed separately to determine surface areas of the tubules and fenestrae and of the flattened parts. These data were combined to determine the average ratio of flattened saccule area to tubule and fenestrae area for a typical plant dictyosome as 0.74.

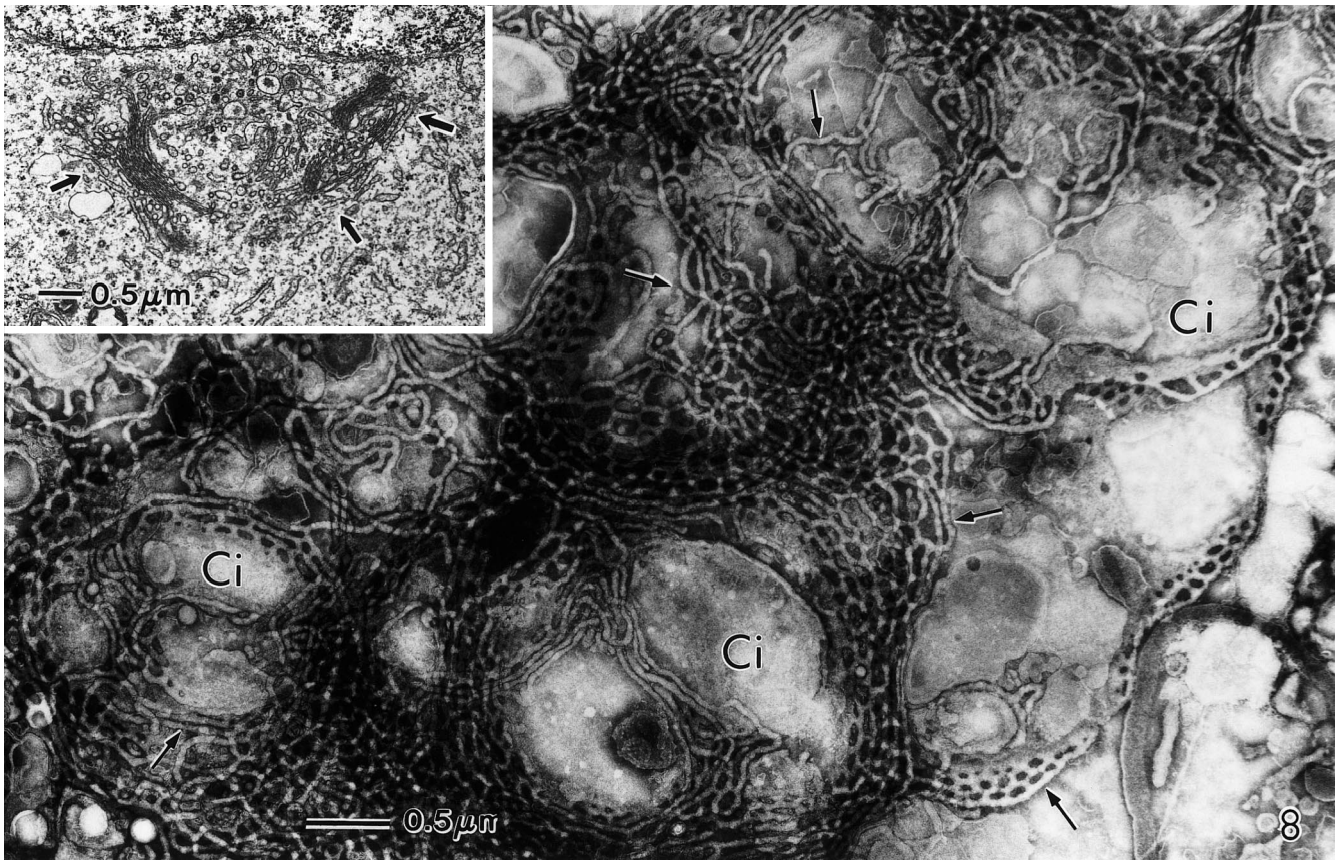


Fig. 8 Portion of a Golgi apparatus from the seminiferous tubule of the rat testis negatively stained with phosphotungstic acid (PTA). The Golgi apparatus was not stabilized with glutaraldehyde and the addition of PTA resulted in partial unstacking. Flattened saccules (i.e., cisternae; *Ci*), tubules (*arrows*), and fenestrae are revealed and have the same general configuration as do cisternae of other animal and plant Golgi apparatus. Since the preparations were not stabilized by glutaraldehyde, coated buds were not visible. (Micrograph reproduced from Mollenhauer and Morré 1991). *Bar* 0.5 μm. *Inset* Section of a rat germ cell illustrating the organization of the Golgi apparatus from a region considered to be similar to that of Fig. 8. *Bar* 0.5 μm

The coated buds

The coated buds associated with the dictyosome tubules were first described by Cunningham et al. (1966) and later verified by numerous studies dealing with Golgi apparatus morphology (for example, Mollenhauer and Morré 1991). They were first studied systematically by Orci et al. (1986) who suggested that they were the non-clathrin-coated buds and vesicles associated with the transport of protein through the Golgi apparatus stacks. Coated buds appear to be a component of all Golgi apparatus. Subsequent studies have shown the buds to be coated by the now well-characterized coatomer proteins (Bannykh and Balch 1998; Bannykh et al. 1996).

In glutaraldehyde-stabilized, negatively stained preparations, the buds appear as small blebs with dense, matlike coatings on their outer surfaces (Fig. 1). The blebs (or at least the nap) are labile, but can be stabilized with glutaraldehyde. Blebs may be configured as swellings of tubule ends (Fig. 1B) or as nearly complete vesicles attached to the tubules by short protuberances or thin necks (Fig. 1). In some instances, the attachment site between the neck and the tubule is demarcated by what appears to be a membrane (Fig. 1). In these instances, the contents of the blebs do not appear to be continuous with those of the tubules.

Comparable buds can be identified in sections of tissues fixed by glutaraldehyde and osmium tetroxide using either conventional procedures (Fig. 2) or freeze substitution (Fig. 3). Buds preserved by freeze substitution are particularly interesting in that the dense nap, so clearly visible in negatively stained preparations, now appear as dense, fuzzy, unit-type membranes continuous with the membranes of the less intensely stained tubules and stack cisternae (compare Figs. 1, 3). The blebs are most easily identified with the cisternae of the *cis* half of the stacks (Fig. 3) although similar kinds of blebs have been observed on *trans* cisternae, TGN, and forming secretory vesicles (Mollenhauer et al. 1991). There may also be considerable size and density variations of the blebs depending, apparently, on their stage of formation and location within the cell.

Free vesicles with bud characteristics have not been observed either in negatively stained preparations or in Golgi apparatus fractions (Morré and Keenan 1994). Neither have accumulations of bud-derived vesicles been demonstrated in sectioned material. Thus, if free vesicles

are formed, they either have a very limited life or they lose their coats and can no longer be recognized.

Relationship of tubules to the stacked cisternae

Tubules continuous with the peripheral edges of stack cisternae are unique and have many characteristics that distinguish them from the cisternae to which they are connected. For example, they often react differently to cytochemical stains and markers (Figs. 6, 7) and, at least in plants, seem to show less structural differentiation across the stack than do the flattened parts of the cisternae. In studies of isolated plant Golgi apparatus, the tubular constituents of dictyosome cisternae appear to be more stable after isolation than the flattened parts of the cisternae to which they are attached (Mollenhauer and Morré 1991). In these studies, plant cells were homogenized in the presence of a chaotropic agent which resulted in rapid dictyosome unstacking (Mollenhauer et al. 1973). The cisternae were then stabilized with glutaraldehyde at various intervals following homogenization, reisolated, and examined in the electron microscope. The sequence of changes after Golgi apparatus isolation was documented by Mollenhauer and Morré, (1991) (see Figs. 6A–D in Mollenhauer and Morré 1991). Briefly, there was a plaque on the flattened surfaces of some cisternae immediately after isolation. These plaques had been interpreted as part of the bonding material between adjacent cisternae, i.e., the substances that maintain stack integrity (Mollenhauer et al. 1973; Mollenhauer and Morré 1991). As the cisternae degraded, the plaques disappeared leaving cisternae with areas of partial degeneration similar in size and shape to the plaques previously observed. As degeneration of the cisternae continued, the entire central parts of the cisternae were lost leaving only peripheral tubules.

Interrelationship between tubules and other cellular constituents

Intuitively, one would think that a major function of the tubules would be to interconnect dictyosomes or to connect dictyosomes with segments of endoplasmic reticulum. Numerous reports suggest continuities between endoplasmic reticulum and Golgi apparatus (e.g., Juniper et al. 1982; Harris and Oparka 1983; Tanaka et al. 1986; Clermont et al. 1994; and references therein). Such interconnections are most often associated with protein-secreting cells and are less common (or less visible) where non-proteinaceous cell wall materials are being secreted (Juniper et al. 1982). However, it is very difficult to establish unequivocally that direct tubular connections between the Golgi apparatus and endoplasmic reticulum exist, especially when the stacks and endoplasmic reticulum are dispersed as in plant cells. Therefore, most estimates of tubule interassociations rely on fortuitous sections (Fig. 10) or on specialized procedures such as isolated and negatively stained preparations, precipitate stains, thick sections, and 3-D reconstructions. Unfortunately, such approaches are sometimes misleading because of the harsh preparative procedures necessary or because of the loss of resolution that occurs when viewing preparations such as thick sections where structures may overlap.

A morphological transition of tubules between dictyosomes and endoplasmic reticulum has been demonstrated in negatively stained plant cells although micrographs of this event are extremely rare (for example, Fig. 7 of Mollenhauer and Morré 1976). We have also demonstrated that direct connections between endoplasmic reticulum and Golgi apparatus cisternae can be established in plant dictyosomes if the cells are maintained at a lower than normal growing temperature (Mollenhauer et al. 1975). One interpretation would be that the low-temperature-induced connections are some manifestation of what at higher temperatures would be an intermittent association or a connection mediated by a small vesicle intermediate.

In cells where stacks are closely adjacent to one another, it can be shown very clearly that tubules do interconnect the cisternae of one Golgi apparatus stack with those of adjacent stacks (Fig. 10; Clermont et al. 1994; Bracker et al. 1996). Such connections do not appear to be random but, rather, maintain a polarity equivalent to the cisterna to which they are attached; that is, tubules tend to interconnect adjacent cisternae at the same level within the stack. Tubules probably also interconnect more distant stacks (Fig. 11), but this has been very difficult to demonstrate because the tubules tend to branch and change directions within the plane of a thin section. A potentially interesting observation is the presence of small flat saccules (Figs. 11, 12), termed by us junctional cisternae, which seem to act as intermediate connecting points for tubules. These are often observed in negatively stained isolated cisternae. Figures 11–13 also illustrate asymmetries commonly observed in isolated dictyosomes and cisternae. Connections of tubules between cisternae within the same stack have also been reported (Clermont et al. 1994).

Tubules are also clearly a major feature of the TGN (Geuze and Morré 1991). At least part of the TGN lies within the zone of exclusion (Mollenhauer and Morré 1978) at the *trans* poles of the dictyosomes and is often closely aligned with the Golgi apparatus (Mollenhauer and Morré 1994; Mollenhauer et al. 1991). In plant cells, the TGNs appear to be derived from the *trans*-most cisternae shortly after the cisternae or cisternal remnants are sloughed from the stack when secretory vesicle formation is complete (Mollenhauer et al. 1991). These sloughed cisternae form arrays of tubules and coated vesicles that appear similar to the partially coated reticula of plants (Pesacreta and Lucas 1985) and TGNs of animal cells (Griffiths and Simons 1986; see also Griffing 1991). When compared to dictyosome tubules, the post-Golgi apparatus structures are generally more variable in size than the Golgi apparatus tubules and are commonly

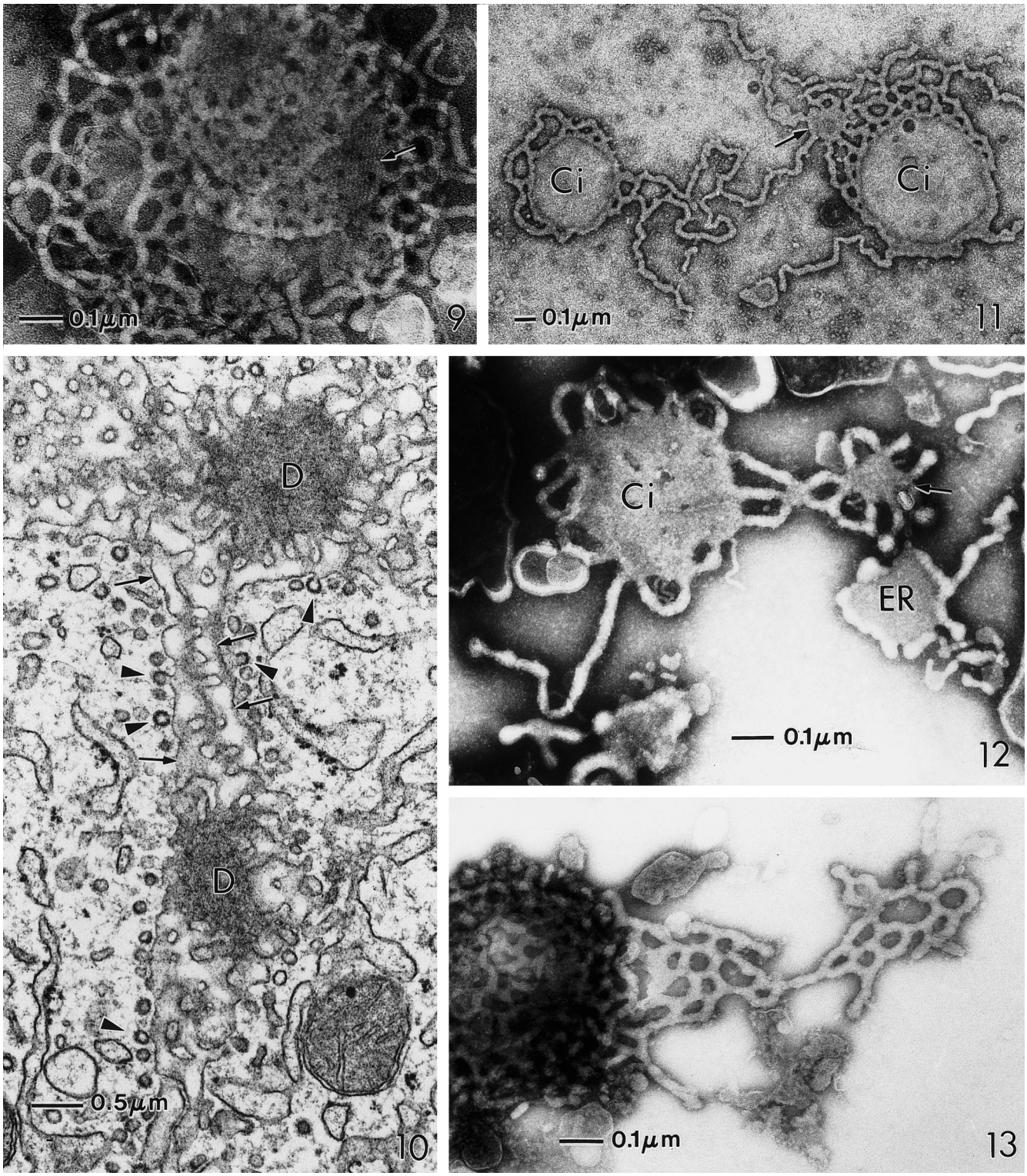


Fig. 9 A plant (radish root) dictyosome (i.e., stack) isolated without glutaraldehyde stabilization. Unstacking had begun so that portions of all component cisternae are partially revealed in the same positional relationships as in the original stack. The *cis* pole of the dictyosome is toward the upper edge of the micrograph and the *trans* pole toward the bottom edge of the micrograph. Note that the peripheries of the mid cisternae are mostly fenestrated whereas the peripheries of the *trans* cisternae are mostly tubular. Coated buds are not visible because the dictyosome was not stabilized with glutaraldehyde during isolation. *Arrow* indicates a mat of intercisternal filaments characteristic of the *trans* half of plant dictyosomes. (Micrograph reproduced from Mollenhauer and Morré 1966b). *Bar* 0.1 μm

Fig. 10 A section from rat epididymis showing two dictyosomes (*D*) connected by tubules (*arrows*). A few of the coated buds associated with the interconnecting tubules are indicated at the *arrowheads*. *Bar* 0.5 μm

Fig. 11 Two bean (*Phaseolus vulgaris*) root tip dictyosome cisternae (*Ci*) prepared by a smear or spread technique (Mollenhauer et al. 1973) and simultaneously negatively stained with phosphotungstic acid. The cisternae are connected via peripheral tubules through a small subcisterna or junctional complex (*arrow*). Junctional cisternae like this are common in these preparations. Golgi apparatus in plant roots are frequently seen to be asymmetric. *Bar* 0.1 μm

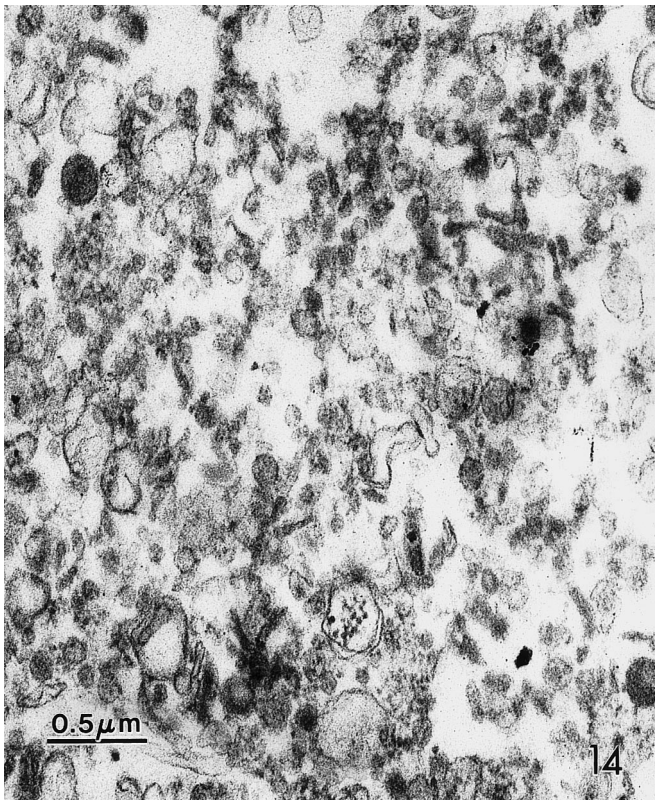


Fig. 14 Purified fraction of Golgi apparatus tubules isolated by a procedure involving use of chaotropic agents to disrupt low-density lipoprotein-depleted Golgi apparatus fractions isolated from rat liver into tubule- and plate-like cisternal fractions. The tubule fraction illustrated in this micrograph, like the SII microsome fraction of Dallner (1978), could not be aggregated by divalent cations and was found to be enriched in nonaprenyl-4-hydroxybenzoate transferase. Unpublished data of D.J. Morré, T.W. Keenan, and G. Dallner. *Bar* 0.5 μm

associated with clathrin-coated vesicles which are absent from the tubules.

Isolation of tubule-enriched fractions

The first reported isolation of Golgi apparatus tubules (Ovtracht et al. 1973) began with intact isolated Golgi apparatus from rat liver (Morré 1971). Lysosomal extracts or crude Taka-diastase preparations were used to unstack the Golgi apparatus followed by disruption by means of repeated excursions through a fine-bore pasteur pipette. The unstacked and separated Golgi apparatus components were then subjected to discontinuous sucrose density gradient centrifugation and were analyzed by electron microscope morphometry. Fraction II, collected just above and just below the 0.9/1.2-M sucrose interface, was enriched in the lipoprotein-filled tubules of the rat liver Golgi apparatus. Attempts to separate the peripheral tubules not containing lipoprotein particles from fractions just containing the cisternal plates has, thus far, seemed to require the use of chaotropic agents to dissolve the plates and release the tubules (Malhotra et al. 1989). We have utilized such procedures successfully in preliminary experiments to prepare tubule-enriched fractions both from rat liver and from Golgi apparatus isolated from rat liver (Fig. 14; D.J. Morré and T.W. Keenan, unpublished results).

◀ **Figs. 12, 13** Plant dictyosome cisternae from cauliflower inflorescence isolated and stabilized with glutaraldehyde and then negatively stained with phosphotungstic acid to illustrate junctional cisternae (*arrow* Fig. 12) and cisternal asymmetry. *Ci* Cisterna, ER endoplasmic reticulum. *Bar* 0.1 μm

Function of Golgi apparatus tubules

There is as yet no hard evidence to identify tubule functions primarily because so little is known about their biochemistry or their molecular configuration. Therefore, one can only make assumptions based on the mostly morphological evidence currently available. It has also been shown that, in plant cells, dictyosome function is synchronized even when Golgi apparatus function changes (Mollenhauer 1965). It is also clear, at least in our studies, that all dictyosomes within a cell always have the same general morphology suggesting again that the components of the Golgi apparatus (i.e., the dictyosomes or stacks) are either physically or functionally interconnected (Mollenhauer and Morré 1965; Favard 1969). At least one potential form of interconnection is via the peripheral tubules of the dictyosomes. It is abundantly clear that tubules interconnect cisternae of closely adjacent stacks within a Golgi apparatus and, perhaps, all dictyosomes of the Golgi apparatus. Thus, tubules might be one of the mechanisms that synchronize stack function within a Golgi apparatus.

In some cells, tubules also interconnect forming secretory vesicles with the central plates of the stacks and, presumably, act as transition elements between the two components of the Golgi apparatus (Mollenhauer and Morré 1966a,b). Transitions between stacked cisternae and tubules can also be demonstrated simply by noting differences in their morphology. For example, ultrastructural evidence, including cytochemical markers and membrane characteristics, clearly demonstrates regional transitions between the tubules and the central plates of the stacks. Tubules may also act as transition elements between the Golgi apparatus stack and the endoplasmic reticulum. Such transitions sometimes appear to occur via what appear to be specialized junctional complexes (Mollenhauer et al. 1975; Mollenhauer and Morré 1976) or small junctional cisternae similar to those of Figs. 11, 12.

There is little information as to what biochemical activities or metabolic functions might be associated with Golgi apparatus tubules. Golgi apparatus contain high concentrations of ubiquinone (Nyquist et al.. 1970; Zambrano et al. 1975; Kalen et al. 1987) and of ubiquinone biosynthetic enzymes, for example, the enzymes that transfer solanesol pyrophosphate to 4-hydroxybenzoate

(nonaprenyl-4-hydroxybenzoate transferase) (Teclebrhan et al. 1995). The latter authors have suggested that ubiquinone is synthesized sequentially in the endoplasmic reticulum-Golgi apparatus system and is, thereafter, translocated to other cellular membranes.

Dallner (1978) described an unusual microsomal fraction which could not be aggregated by divalent cations. This is a property shared with Golgi apparatus tubule fractions such as those isolated from Golgi apparatus rat liver by chaotropic disruption (Fig. 14; D. J. Morré, unpublished results). The unusual microsome fraction, designated by Dallner and colleagues as SII microsomes, have been assumed to represent some compartment of the Golgi apparatus and are enriched in both enzymes of ubiquinone and sterol biosynthesis (Teclebrhan et al. 1995). Nonaprenyl-4-hydroxybenzoate transferase which transfers the solanesol side chain to the precursor ring during ubiquinone biosynthesis is concentrated in this fraction. Following condensation, a number of additional reactions take place, which convert the 4-hydroxybenzoate moiety to a methoxy- and methyl-substituted benzoquinone ring. These reactions also have been suggested to take place in the Golgi apparatus (Teclebrhan et al. 1995). A major role for Golgi apparatus tubules in the biosynthesis and transport of isoprenoid compounds including, but not restricted to, ubiquinone and cholesterol would not be unexpected based upon these preliminary findings.

Tubules may also account for cholesterol or quinone transport and targeting, for example, the selective transfer of ubiquinone to mitochondria and of plastoquinone to the chloroplast in plants where both ubiquinone and plastoquinone appear to be synthesized in the endoplasmic reticulum-Golgi apparatus system (Swiezewaska et al. 1993; Osowska-Rogers et al. 1994).

Measurements of the flux of membrane constituents through the Golgi apparatus, inhibitor studies, and direct light and electron microscopic observations in favorable cell types, reveal a pattern of events suggestive of cisternal turnover (Morré 1987; Mollenhauer and Morré 1991). In actively secreting cells, the plate-like portions of Golgi apparatus cisternae appear to be formed from transition vesicles at the *cis* face of the Golgi apparatus and discharged as secretory vesicles plus a cisternal remnant at the *trans* face at a rate of one every 3–4 min (Morré 1987). With a total transit time through the Golgi apparatus of 15–20 min and with new cisternae being formed as existing cisternae are lost, turnover of tubules in parallel to that of plate-like portions of cisternae would be difficult to reconcile. Rather, the tubules might be envisioned as representing a relatively more permanent feature of the cytoplasm. An exception might be tubules comprising the *cis* Golgi network. These, presumably, represent an intermediate or precursor stage in the formation of the cisternal plate. One mechanism compatible with cisternal maturation and turnover would be where the tubular connections to the plate-like portions of the cisternae are broken and reformed as cisternae are displaced one after the other across the stack. Different types of tubules and different types of tubule attachments characterize each of the several cisternal positions (*cis*, mid, *trans*) in the stack in keeping with this concept. As a result, the tubular system might serve as a repository for resident Golgi apparatus proteins, such as glycoprotein-processing enzymes, that could be added to or removed from the cisternal plates as needed during cisternal maturation.

However, despite a greater appreciation of the existence of Golgi apparatus tubules, their functions are still far from being resolved. Other than morphology, there are no convenient markers for Golgi apparatus tubules or any reliable means of differentiating Golgi apparatus tubules from tubules of endoplasmic reticulum or from tubular elements potentially derived from other cell structures, especially in isolates of cell constituents.

References

Bannykh SI, Balch WE (1998) Selective transport of cargo between the endoplasmic reticulum and Golgi compartments. Histochem Cell Biol 109:463–475

Bannykh SI, Rowe T, Balch WE (1996) The organization of endoplasmic reticulum complexes. J Cell Biol 135:19–35

Beams HW, Kessel RG (1968) The Golgi apparatus: structure and function. Int Rev Cytol 23:209–276

Bourne GH, Tewari HB (1964) Mitochondria and the Golgi complex. In: Bourne GH (ed) Cytology and cell physiology. Academic Press, New York, pp 377–421

Bracker CE, Morré DJ, Grove SN (1996) Structure, differentiation, and multiplication of Golgi apparatus in fungal hyphae. Protoplasma 194:250–274

Clermont Y, Rambourg A, Hermo L (1994) Connections between the various elements of the *cis*- and mid-compartments of the Golgi apparatus of early rat spermatids. Anat Rec 240: 469–480

Cunningham WP, Morré DJ, Mollenhauer HH (1966) Structure of isolated Golgi apparatus revealed by negative staining. J Cell Biol 28:169–179

Dallner G (1978) Isolation of microsomal subfractions by use of density gradients. Methods Enzymol 52:71–83

Favard P (1969) The Golgi apparatus. In: Lima de Faria A (ed) Handbook of molecular cytology. North Holland Publishing, Amsterdam, pp 1–24

Favard P, Ovtracht L, Carasso N (1971) Observations de spécimens biologiques en microscopie électronique a hauté tension. I. Coupes épaisses. J Microsc (Paris) 12:301–316

Geuze JH, Morré DJ (1991) Trans Golgi reticulum. J Electron Microsc Tech 17:24–34

Golgi C (1898) Sur la structure des cellules nerveuses. Arch Ital Biol 30:60–71

Griffing LR (1991) Comparisons of Golgi structure and dynamics in plant and animal cells. J Electron Microsc Tech 17:179–199

Griffiths G, Simons K (1986) The *trans* Golgi network: sorting at the exit site of the Golgi complex. Science 234:438–443

Grove SN, Bracker CE, Morré DJ (1968) Cytomembrane differentiation in the endoplasmic reticulum-Golgi apparatus-vesicle complex. Science 161:171–273

Harris N, Oparka KJ (1983) Connections between dictyosomes and GERL in cotyledons of Mung bean (*Vigna radiata* L.). Protoplasma 114:93–102

Infarerra C, Carrozza G (1975) The discovery of the Golgi apparatus by the black reaction and its present fine structural visualization. In: Santini (ed) Golgi centennial symposium proceedings, Raven Press, New York, pp 13–38

Juniper BE, Hawes CR, Horne JC (1982) The relationship between the dictyosomes and the forms of endoplasmic reticu-

lum in plant cells with different export programs. Bot Gaz (Chicago) 143:135–145
Kalen A, Norling B, Applekvist EL, Dallner G (1987) Ubiquinone biosynthesis by microsomal fraction from rat liver. Biochim Biophys Acta 926:70–78
Ladinsky MS, Kremer JR, Fucinitti PS, McIntosh JR, Howell KE (1994) HVEM tomography of the trans-Golgi network: structural insights and identification of a lace-like vesicle coat. J Cell Biol 127:29–38
Malhotra V, Sarafini T, Orci L, Shepard JC, Rothman JE (1989) Purification of a novel class of coated vesicles mediating biosynthetic protein transport through the Golgi stack. Cell 58: 329–336
Manton I (1960) On a reticular derivative from Golgi bodies in the meristem of *Anthoceros*. J Biochem Biophys Cytol 8:221–231
Mollenhauer HH (1965) Transition forms of Golgi apparatus secretion vesicles. J Ultrastruct Res 12:439–446
Mollenhauer HH, Morré DJ (1965) Interassociation of dictyosomes to form plant cell Golgi apparatus. J Cell Biol 27:68A
Mollenhauer HH, Morré DJ (1966a) Golgi apparatus and plant secretion. Annu Rev Plant Physiol 17:27–46
Mollenhauer HH, Morré DJ (1966b) Tubular connections between dictyosomes and forming secretory vesicles in plant Golgi apparatus. J Cell Biol 29:373–376
Mollenhauer HH, Morré DJ (1971) Golgi apparatus. In: Lapedes DN (ed) Encyclopedia of science and technology, 3rd edn. McGraw-Hill, New York, pp 262–264
Mollenhauer HH, Morré DJ (1976) Transition elements between endoplasmic reticulum and Golgi apparatus in plant cells. Cytobiologie 13:297–306
Mollenhauer HH, Morré DJ (1978) Structural compartmentation of the cytosol: zones of exclusion, zones of adhesion, cytoskeletal and intercisternal elements. In: Roodyn DB (ed.) Subcellular biochemistry. Plenum, New York, pp 327–359
Mollenhauer HH, Morré DJ (1990) Dictyosome tubules, a conspicuous membranous system of the plant cell. Proc. 12th Int Congr Electron Microsc. San Francisco Press, San Francisco, pp 702–703
Mollenhauer HH, Morré DJ (1991) Perspectives on Golgi apparatus form and function. J Electron Microsc Tech 17:2–14
Mollenhauer HH, Morré DJ (1994) Structure of Golgi apparatus. Protoplasma 189:14–28
Mollenhauer HH, Morré DJ, Bergman L (1967) Homology of form in plant and animal Golgi apparatus. Anat Rec 158: 313–318
Mollenhauer HH, Morré DJ, Totten C (1973) Intercisternal substances of the Golgi apparatus, unstacking of plant dictyosomes using chaotropic agents. Protoplasma 78:443–459
Mollenhauer HH, Morré DJ, Van Der Woude WJ (1975) Endoplasmic reticulum-Golgi apparatus associations in maize root tips. Mikroskopie 31:257–272
Mollenhauer HH, Morré DJ, Griffing LR (1991) Post Golgi apparatus structures and membrane removal in plants. Protoplasma 162:55–60
Morré DJ (1971) Isolation of Golgi apparatus. Methods Enzymol 22:130–148
Morré DJ (1987) The Golgi apparatus. Int Rev Cytol (suppl) 17:211–253
Morré DJ, Keenan TW (1994) Golgi apparatus buds – vesicles or coated ends of tubules? Protoplasma 179:1–4
Morré DJ, Mollenhauer HH, Chambers JE (1965) Glutaraldehyde stabilization as an aid to Golgi apparatus isolation. Exp Cell Res 38:672–675
Morré DJ, Mollenhauer HH, Bracker CE (1971) Origin and continuity of Golgi apparatus. In: Reinert J, Urspring H (eds) Origin and continuity of cell organelles. Springer, Berlin Heidelberg New York, pp 88–126
Nyquist SE, Barr R, Morré DJ (1970) Ubiquinone from rat liver Golgi apparatus fractions. Biochim Biophys Acta 208: 532–534
Orci L, Glick BS, Rothman JE (1986) A new type of coated vesicular carrier that appears not to contain clathrin: its possible role in protein transport within the Golgi stack. Cell 46:171–184
Osowska-Rogers S, Swiezewska E, Anderson B, Callner G (1994) The endoplasmic reticulum-Golgi system is a major site of plastoquinone synthesis in spinach leaves. Biochem Biophys Res Commun 205:714–721
Ovtracht L, Morré DJ, Cheetham RD, Mollenhauer HH (1973) Subfractionation of Golgi apparatus from rat liver: method and morphology. J Microscopie 18:87–102
Pesacreta TC, Lucas WJ (1985) Presence of a partially coated reticulum in angiosperms. Protoplasma 1225:173–184
Rambourg A, Clermont Y, Hermo L, Segretain D (1987) Tridimension architecture of the Golgi apparatus and its components in mucus cells of Brunner's Gland. Am J Anat 179: 95–107
Swiezewaska E, Dallner G, Andersson B, Ernster L (1993) Biosynthesis of ubiquinone and plastoquinone in the endoplasmic reticulum-Golgi membranes of spinach leaves. J Biol Chem 268:1494–1499
Tai S, Albrecht RM (1992) The 3-D architecture of the Golgi complex of chicken pituitary cells. Acta Microsc 1:74–86
Tanaka K, Mitsushima A, Kashima Y (1986) Three-dimensional architecture of the Golgi complex observed by high resolution scanning electron microscopy. J Submicrosc Cytol Pathol 18:1–9
Teclebrhan H, Jakobsson-Borin A, Brunk U, Dallner G (1995) Relationship between the endoplasmic reticulum-Golgi membrane system and ubiquinone biosynthesis. Biochim Biophys Acta 1256:157–165
Weidman P, Roth R, Heuser J (1993) Golgi membrane dynamics imaged by freeze etch electron microscopy: views of different membrane coatings involved in tubulation versus vesiculation. Cell 75:123–133.
Whaley WG (1975) The Golgi apparatus. In: Alfert et al. M. (eds) Cell biology monographs. Springer, Berlin Heidelberg New York, pp 216–221
Zambrano FS, Fleischer S, Fleischer B (1975) Lipid composition of the Golgi apparatus of rat kidney and liver in comparison with other subcellular organelles. Biochim Biophys Acta 380: 357–359