The Neuronal Endomembrane System

II. The Multiple Forms of the Golgi Apparatus cis Element¹

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Abstract

The cis element and its associated tubules and vesicles are ideally positioned to play a major role in the sorting of rough endoplasmic reticulum components destined for processing in the Golgi apparatus. Its position is also ideal for playing a major role in the assembly of the saccules which constitute the Golgi apparatus. The present study was undertaken to critically analyze the normal morphology of this Golgi apparatus component. Seventy- to 2000-nm-thick sections of bullfrog spinal ganglia, fixed by osmium impregnation as well as by conventional protocols, were studied using standard and high voltage electron microscopy. Impregnated cis elements were also reconstructed from 170-nm serial sections. These studies found that adjacent neurons within the given ganglion contain cis elements of widely different morphology. In larger neurons, a different cis element organization was also found in different regions of the same cells. Based on structural comparisons, all of the different cis element forms observed could be systematically assembled into a gradual continuum of morphological variation. This continuum was circular in a manner analogous to chromosomal variations seen in highly mitotic tissues. For the sake of discussion, five distinctly different form categories were established. Some forms contained structures that are described herein for the first time. Most notable among these is the cis tubular network, an extensive system of parallel tubules that closely apposed the regularly perforated cis saccule. Osmiophilic vesicles were found to collect in tight clusters that closely apposed certain forms of the cis element. These findings raise the possibility that the cis element and its associated structures may undergo morphological transformations as part of their normal function.

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The Golgi apparatus plays a central role in the packaging and addressing of cell products destined for the cell surface or secretion. Following these steps, the cell products in neurons are usually transported to their final sites within the neuronal processes by fast axonal transport. Direct evidence for this packaging role of the Golgi apparatus in neurons comes from ultrastructural analysis of spinal ganglia incubated in low concentrations of cobalt or monensin. Such treatments specifically block the loading of newly synthesized cell products destined for fast transport onto the fast transport machinery, the so-called initiation phase of fast axonal transport (Lavoie et al., 1979; Hammerschlag, 1980; Hammerschlag et al., 1982). Polypeptide assembly in the cell body and fast transport ongoing within the axon are unaffected by these treatments. Ultrastructural examination of treated ganglia in both cases revealed a specific and extensive disruption of Golgi apparatus morphology (Lindsey et al., 1981; Hammerschlag et al., 1982). This indicated that structural integrity of the Golgi apparatus was essential for initiation.

Histochemical investigations of bullfrog spinal ganglia exposed to these treatments were undertaken in an effort to define more carefully the actions of cobalt and monensin (Lindsey and Ellisman, 1981a, b; 1982). These studies utilized osmium impregnation, a preparative technique that selectively fills saccules, tubules, and vesicles at the cis pole⁴ of the Golgi apparatus with an electron-dense deposit. During the course of characterizing control ganglia, it was noted that the impregnation patterns were often quite different in adjacent cells seen in the same sections. Often, just one saccule in the cis-most position of the stack would be impregnated. On a regular basis, however, cells were observed in which two of the cis-most saccules were impregnated and in which only a few vesicles near the cis pole were impregnated. All of these variations were seen in cells with very light to nonexistent granular deposits within rough endoplasmic reticulum (RER). This latter point suggests that the variations were not due to differences in the level of impregnation (Lindsey and Ellisman, 1985).

Preliminary views of the three-dimensional structure of impregnated Golgi apparatus components were obtained from thick sec-

⁴ The cis pole is that side of the Golgi apparatus stack that contains the least amount of carbohydrate and often exhibits a convex appearance. The cis element consists of the cis saccule plus marginal tubules, the cis tubular network, and vesicle clusters (to be described below). The cis and trans designations (first proposed by Ehrenreich et al., 1973) currently have advantages over other nomenclature due to their specific histochemical basis and because they do not imply more than is currently known about Golgi apparatus function. Further discussion can be found in several recent reviews (Novikoff and Novikoff, 1977; Farquhar and Palade, 1981; Hermo et al., 1982).

Figure 1. The cis element of the Golgi apparatus as seen in sections of increasing thickness from osmium-impregnated spinal ganglia. In conventional thin sections, as shown in *a*, all components of the Golgi apparatus, including the cis saccule (CS), trans saccule (TS), and intermediate saccules (IS), are distinctly visible. Where the Golgi stack twists from being perpendicularly cross-sectioned to being semi-tangentially cut, suggestive scallops (arrowheads) and a hole (arrow) hint at the regular perforations of the cis saccules. These perforations are easily seen in 0.5- μm -thick sections (*b*). The presence of varicose tubules (VT) close to the cis saccule and to rough endoplasmic reticulum (RER) is also seen. Because the cis saccule is usually much wider than 0.5 μm , study of the cis element macrostructure is best done in very thick sections such as the 3.0- μm -thick example shown in *c*. Here it is easy to see the marginal tubule (MT) at the edge of the cis saccule. Images from the thicker sections are more readily interpreted when viewed stereoscopically (see especially Figs. 3 and 4). Also labeled are mitochondria (M). *a*, 90 nm thick, 80 KeV, magnification $\times 36,000$. *b*, 0.5 μm thick, 100 KeV, magnification $\times 13,500$. *c*, 3.0 μm thick, 1,000 KeV, magnification $\times 29,000$.

Figures 2 to 4

Figures 8 to 10

tions examined stereoscopically with the high voltage electron microscope (HVEM). In such preparations, the only structures seen are the cell nucleus and those cytoplasmic components that have become impregnated with osmium. In many of the cells, the cis saccule appeared much the same as has been reported in the pioneering osmium impregnation/HVEM studies of Rambourg and colleagues (Rambourg and Chrétien, 1970; Rambourg et al., 1974). Briefly, they showed that the cis saccule in rat spinal ganglion neuron consists of unevenly surfaced, regularly perforated membranous sheets which extensively pervade the cytoplasm. These sheets, or saccules, were interconnected by narrow band-like extensions which share the same perforated appearance as their parent saccules (Rambourg et al., 1974). Other cells in our preliminary HVEM examination, however, exhibited either the cis saccule similar to the above description, but overlaid with a previously undescribed extensive network of quasi-parallel tubules, or cytoplasm apparently devoid of the cis saccule and filled just with small vesicles and tubules. Clearly a systematic and thorough investigation of the osmophilic structures at the cis face of the Golgi apparatus in all their various forms was needed.

Many thick sections of osmium-impregnated bullfrog spinal ganglia were first examined with the HVEM to define the limits of cis element morphological variability. The next goal was to determine whether the various forms of the impregnated cis element arose due to differences in stainability or to actual differences in membrane morphology. It was also deemed important to determine the relation of impregnated structures to unimpregnated ones such as the intermediate and trans saccules of the Golgi apparatus, and RER. To accomplish this, 170-nm-thick serial sections from impregnated ganglia stained *en bloc* with lead aspartate were analyzed stereoscopically. This thickness provides an optimum balance between ease of identifying the various impregnable structures (which increases with increasing section thickness) and ease of identifying impregnation-resistant structures (which decreases with increasing section thickness). Non-impregnated structures within these sections were made visible due to the *en bloc* staining treatment.

To provide further verification for the validity of these various cis element forms, images from the 170-nm-thick sections of osmium-impregnated sections were compared to images from 170-nm-thick sections of glutaraldehyde/paraformaldehyde-fixed tissue prepared by conventional protocol. These latter images are readily comparable to conventional thin section images.

Materials and Methods

Tissue preparation and microscopy. Spinal ganglia from *Rana catesbeiana* were fixed by osmium impregnation as described by Lindsey and Ellisman (1985). Briefly, ganglia were immersed in 2% aqueous osmium tetroxide for 1 hr. Subsequently, the tissue was impregnated in the same solution for 35 to 44 hr at 37°C with one change of the solution at 16 hr. After a quick wash in distilled water, some ganglia were stained *en bloc* using lead aspartate (Walton, 1977). For comparison, other spinal ganglia were initially fixed in 1.0% glutaraldehyde, 1.0% paraformaldehyde in 0.12 M cacodylate buffer, rinsed in buffer, and then postfixed in buffered 1% OsO₄ for 1 hr. All ganglia were dehydrated in a graded ethanol series and then gradually transferred into acetone before embedding in Epon/Araldite. This procedure has been shown to yield good preservation of membranous structures within bullfrog spinal ganglion neurons (Lindsey and Ellisman, 1985).

Thin sections were grid stained with uranyl acetate and lead citrate before examination at 80 KeV on a JEM 100CX microscope equipped with a goniometric stage. Thicker sections up to 250 nm thick were sometimes grid stained and always carbon stabilized before examination at 100 KeV with the instrument mentioned above. Thick sections (1.0, 2.0, and 3.0 μ m) were examined at 1 MeV with the JEM 1000 HVEM at Boulder, Colorado. Section thicknesses of sections thinner than 200 nm were estimated based on interference color reflections. Serial sections (170 nm thick) from tissue stained *en bloc* with Walton's lead aspartate were collected on Formvar-coated slot grids, carbon stabilized, and examined at 100 KeV.

Results

Examination of the cis element as it appears in conventional thin sections and in thicker sections is highly complementary (Fig. 1). Thin sections afford close study of the relationship between the cis element and intermediate saccules but fail to give a good view of the three-dimensional structure of the Golgi apparatus. For example,

Figures 2 to 5. Stereo pairs from thick sections of osmium-impregnated spinal ganglia viewed at 1000 KeV with the HVEM.

Figure 2. Vesicle-network form of the cis element. This form consists of the cis tubular network (CTN) accompanied by numerous vesicles. Note that the cis saccule is not seen in this form type. A 2- μ m-thick section at a 4° tilt was used. Magnification $\times 12,000$.

Figure 3. Network-saccule form cis element. This form consists of expansive sheets of cis saccule (CS) apposed on one surface by networks of quasi-parallel tubules (CTN, for cis tubular network). At the edges of the cis elements, the cis tubular network sometimes collects into bundles of smooth tubules which extend into the cytoplasm (*open arrows*). The cis tubular network also makes occasional connections (*arrowhead*) with marginal tubules (MT) edging the cis saccule. The section was 2 μ m thick, at a 3° tilt. Magnification $\times 13,000$.

Figure 4. Saccule-tubule form cis element. This form consists of large, regularly perforated, sheet-like cis saccules usually edged by marginal tubules (MT). The marginal tubules are often less well defined in this form than in the network-saccule form, and in the intermediate form halfway in appearance between the network-saccule and saccule-tubule forms (e.g., Fig. 1c). The section was 3.0 μ m thick, at a 3° tilt. Magnification $\times 21,000$.

Figure 5. Saccule form of the cis element. This form consists of the cis element accompanied by vesicle clusters (VC). Note the absence of marginal tubules at the edges of the cis saccule and the absence of the cis tubular network. The section was 2 μ m thick, at a 4° tilt. Magnification $\times 13,000$.

Figures 6 to 10. Stereo pairs of Golgi apparatus profiles from osmium-impregnated spinal ganglion neurons that were *en bloc* stained with lead aspartate. All images presented here are from the same serial section series sectioned at 170 nm thickness and viewed at 100 KeV.

Figure 6. Vesicle form cis element and associated unimpregnated saccules of the Golgi stack (10° tilt). This form consists of discrete and clustered impregnated vesicles (*solid and open short arrows*, respectively) associated with several unimpregnated smooth, flat saccules. Because the only impregnated components are vesicles, this form cannot be positively identified in very thick sections viewed with the HVEM. Magnification $\times 27,000$.

Figure 7. Vesicle-network form cis element and associated Golgi stack components (10° tilt). This Golgi stack is perfectly cross-sectioned at the *top* of the image. It twists to become tangentially sectioned in the *lower part* of the image with the cis element largely contained within the thickness of the section. The cis tubular network with its characteristic branching pattern and copious accompanying vesicles is readily identified in the tangentially sectioned portion (cf. Fig. 6). In the cross-sectioned portion, the obliquely cut tubules of the cis tubular network appear as sausage-shaped profiles (*long arrows*). Discrete vesicles (*short arrows*) are also seen adjacent to the remaining smooth saccules of the Golgi stack. Magnification $\times 16,000$.

Figure 8. Network-saccule form cis element and associated Golgi stack components (10° tilt). The cis tubular network is designated with *long arrows* and the cis saccule with *arrowheads*. Stereo examination of the tangentially sectioned Golgi stack in the *lower right* of the image clearly illustrates the cis tubular network overlying the cis saccule. The Golgi stack in the *upper left* of the image shows that the cis tubular network and cis saccule can appear as two impregnated saccules at the cis pole of the cross-sectioned stack. Also note the unimpregnated saccules present at the trans pole in this form type. Magnification $\times 20,000$.

Figure 9. Saccule-tubule form cis element and associated Golgi stack components (10° tilt). Here the cis saccule appears without the cis tubular network. The intermediate and trans saccules are not clearly seen in this figure because the Golgi stacks are all semi-tangentially sectioned. The stout marginal tubule (*curved arrow*) lies at the edge of the cis element. A connection between the cis saccule and the marginal tubule is contained within this section. Also seen is the proximal portion of a varicose tubule anastomosing with the cis saccule (*asterisk*). Magnification of $\times 23,000$.

Figure 10. Saccule form cis element and associated Golgi stack components (10° tilt). The stack is perfectly cross-sectioned in the *upper portion* of the image and tangentially sectioned in the *lower portion*. The absence of marginal tubules from the edge of the lightly impregnated cis saccule (*arrowhead*) and the appearance of impregnated vesicle clusters near the cis element (*open short arrow*) verify this form type. In cross-section, the cis saccule is closely apposed by several smooth, flat saccules similar in appearance to those associated with the vesicle form cis element (cf. Fig. 9). Magnification $\times 22,000$.

the thin sections do not allow ready appreciation of a 50- to 60-nm-wide tubule-like component of the cis element that is easily seen in thicker sections. This tubule-like component is distinct and yet regularly anastomotic with the body of the cis saccule, and was often seen to course along one or more edges of the saccule (see Fig. 1c). This "marginal tubule" extended along the ribbon bridges and continued around the margin of adjacent cis saccules. Once identified, however, this tubule can be acknowledged in thin sections as probably corresponding to one of the "vesicular" profiles seen at the edge of the conventional view of the cis element. Several forms of the cis elements were seen in 2.0- and 3.0- μ m-thick sections examined with the HVEM. The descriptions that follow apply directly to the larger "light" or "B" cells of the ganglion (Lieberman, 1976). In the smaller "dark" or "A" cells, the cis saccules appeared more ribbon-like than in the larger cells. Although not systematically studied as in the large cells, variations in small cell cis elements similar to those of the large cells were observed during the course of the investigation.

HVEM analysis of the cis element forms

1. The vesicle-network form

The first form of the cis element to be considered is called the "vesicle-network" form to reflect its dominant components; i.e., vesicles and the "cis tubular network," a cis element component described here for the first time. It is shown in Figure 2. The cis tubular network is a system of short to moderately long, coplanar, membranous tubules. Often, two or more tubules will run side by side and describe curving patterns. In some of the other cis element forms, it lies close to the cis side of the cis saccule. In the present vesicle-network form of the cis element, however, the cis saccule is not seen. This allows an unobstructed view of the cis tubular network. Substantially more vesicles were seen to accompany the cis tubular network in this form than with any other form. Occasionally, single smooth tubules extended from the network patches to end a short distance away in the cytoplasm.

2. The network-saccule form

The "network-saccule" form of the cis element was similar to the vesicle-network form described above except that the cis saccule is also seen (Fig. 3). The cis saccule here is often edged by short segments of the marginal tubules that were described above. Occasionally, the cis tubular network was seen to anastomose with the marginal tubules. In some cases, coverage of the cis saccule by the cis tubular network was very extensive with the network forming intricately curving, parallel patterns as well as cytoplasmic extensions of parallel tubules. These parallel tubule extensions anastomosed with the cis tubular networks of adjacent saccules (Fig. 3). In other

cases, only patches of the cis tubular network were seen over the cis saccules. These network patches usually appeared near the edge of the saccule and formed continuities with the marginal tubules. Sometimes, as seen here, the cis tubular network was impregnated more deeply than the cis saccule.

2. The saccule-tubule form

The third form to be considered is called the "saccule-tubule" form to indicate the presence of the cis saccule accompanied by marginal tubules (Fig. 4). Here, the cis saccule appeared as unevenly surfaced, regularly perforated sheet-like saccules. Although there was considerable variation, they were usually less than 2 μ m across. Overall, the cis saccule appeared more extensive in this form than in any of the other forms. Very few impregnated vesicles were seen in close association with this form of the cis element.

4. The saccule form

The next form of the cis element to be described is the "saccule form." It consists of the cis saccule either surrounded by occasional fragments of marginal tubules or else not margined at all (Fig. 5). Usually the cis saccule was less intensely impregnated here than in the network-saccule or the saccule-tubule forms. Although individual vesicles were fairly common, the most distinctive characteristic was the presence of vesicle clusters closely apposed to the cis pole of the cis saccule. Because cis element forms intermediate between certain pairs of the form types described below were observed, they could all be collectively organized into a gradual continuum of morphological variation. The order of this continuum is: vesicle-network, network-saccule, saccule-tubule, saccule.

Thin section appearance of the various forms of the cis element. Serial 170-nm-thick sections of impregnated ganglia that had been stained *en bloc* with lead aspartate were studied to address the possibility that the different forms seen in the HVEM image could arise from differences in the staining pattern without a concomitant change in the actual membrane form of the cis element. These sections were thin enough to allow easy identification of the usual structures seen in thin section images, and yet sufficiently thick to contain enough of the cis element so that positive identification of form type usually could be made. On the rare occasions when ambiguities did arise, these were readily resolved by examination of the structure involved as it appeared in several serial sections on either side of the one in question. In addition to linking the thick- and thin-section images of the form types described above, a fifth cis element form category was identified and will be presented first.

5. The vesicle form

The "vesicle" form of the cis element consisted of condensed clusters of impregnated vesicles closely apposed to the unimpreg-

Figures 11 to 15. The various forms of the cis element as they appear in ganglion cells conventionally fixed with aldehydes followed by osmium. These 170-nm-thick sections were grid-stained with lead to enhance the contrast of membranes and were viewed at 80 KeV.

Figure 11. Cross-sectioned Golgi stack with several vesicle clusters (*open short arrows*) as well as many discrete vesicles at the cis pole. The saccule nearest to the cis pole appears smooth and flat. Because of these characteristics, the cis element of this Golgi stack is the vesicle form previously illustrated in Figure 9. Magnification $\times 35,000$.

Figure 12. Cross-sectioned to tangentially sectioned Golgi stack with sausage-shaped profiles (*long arrows*) and vesicles (*short arrows*) apposed to three smooth, flat saccules. These attributes indicate that the cis element of this Golgi stack is the vesicle-network form previously illustrated in Figure 10. Magnification $\times 35,000$.

Figure 13. Cross-sectioned Golgi stacks in which the cis saccule, identified by its regularly spaced openings (*small arrowheads*; lower Golgi stack), is apposed on one side by three smooth, flat saccules. On the other side it is apposed by occasional sausage-shaped profiles (*long arrows*) which constitute a fifth layer to the Golgi stack. When the plane of section is not co-linear with the cis saccule openings, as is the case in the upper Golgi stack, regularly spaced notches can be a useful clue to identify this saccule (*large arrowheads*). The characteristics of this cis element indicate that it is the network-saccule form (see Fig. 11 for comparison). Magnification $\times 29,000$.

Figure 14. Cross-sectioned Golgi stack in which the regularly perforated cis saccule (*arrowheads*) is only apposed on one side by three smooth, flat saccules. The other side is free from closely associated membranous structures. Thus, the cis element here is the saccule-tubule form (see Fig. 12 for comparison). Magnification $\times 29,000$.

Figure 15. Cross-sectioned Golgi stack with regularly perforated cis saccule (*arrowheads*) apposed on one side by three smooth, flat saccules and accompanied on the other side by a vesicle cluster (*open arrow*). These characteristics indicate that this cis element is the saccule form previously illustrated in Figure 13. Magnification $\times 34,000$.

Figures 11–15

Figure 16. Low magnification image of the osmium-impregnated cis element as seen in a 3.0- μ m-thick section viewed with the HVEM. Note that, as you progress from left to right, the form type of the cis element changes from the vesicle-network (a) to the network-saccule form (b) to the saccule-tubule form (c). Magnification $\times 21,000$.

nated intermediate saccules of the Golgi stack (Fig. 6). Many discrete vesicles were also seen apposed to the cis-most intermediate saccule. This form could not be positively identified in thick sections viewed with the HVEM because it was impossible to see the unimpregnated intermediate saccules. Since this form of the cis element shares characteristics with the saccule form (well developed vesicle clusters) and with the vesicle-network form (absence of the cis saccule), it can link the linear continuum of form variation into a circle.

The other forms of the cis element were all readily identified based on their appearance in tangentially sectioned Golgi apparatus profiles (Figs. 7 to 10). Often, it was possible to follow the tangentially sectioned Golgi stacks as they twisted within the section to become cross-sectioned. As an alternative, the tangentially sectioned Golgi cis element could be followed as it progressed through serial sections until it became cross-sectioned. The various cis element components as they appeared in cross-section were thus easily identified by careful comparison between the tangentially and cross-sectioned images. This is detailed for each of the cis element forms in the legend to Figures 7 to 10. Images obtained from these sections clearly showed that the different forms of the cis element reflected actual differences in cis element structure. This point is particularly well made in the case of the saccule-tubule form. As shown in Figures 1a and 9, when just the cis saccule was impregnated, unimpregnated tubule or sausage-shaped profiles characteristic of the cis tubular network (see Fig. 7) were not found adjacent to the cis side of the cis saccule.

Appearance of the various cis element forms in conventionally prepared thin sections

Sections (170 nm thick) of ganglia fixed in aldehydes followed by osmium were compared with the 170-nm-thick sections from osmium-impregnated ganglia to establish how the various cis element forms appeared in conventional preparations. Images from the 170-nm-thick/aldehyde sections were quite similar to those from 90-nm-thick/aldehyde sections (see Fig. 1b in Lindsey and Ellisman, 1985). Definitive components such as vesicle clusters, sausage-shaped profiles, and cis saccule perforations were readily identified in the aldehyde-fixed tissues (Figs. 11 to 15). This permitted unambiguous assignment of the observed Golgi apparatus profiles to the various cis element form categories described above.

The osmiophilic vesicles seen near the cis element

Osmiophilic vesicles were often seen closely apposed to most forms of the cis element. Discrete and clustered vesicles were most common in association with the vesicle form and the vesicle-network form cis elements (Figs. 2 and 5). Clusters were seen in lesser numbers in association with the network-saccule form and the saccule form (Figs. 3 and 5). Often the vesicle clusters were very tightly packed. Although still present, the density of vesicles near the cis element was lowest in the saccule-tubule form (Fig. 4). A summary of the presence of vesicles and vesicle clusters in association with the various forms of the cis element is diagrammed in Figure 16.

The presence of different cis element forms within the same neuron

A major consideration is the possibility that the different cis element forms seen in adjacent spinal ganglion neurons may reflect different types of neurons. This possibility, however, is not likely as different "forms" of the cis element are found within the same neurons. This is readily apparent in thick sections viewed with the HVEM (Fig. 16). By tracking Golgi apparatus profiles as they progress through serial 170-nm-thick sections it was possible to demonstrate that the different forms were part of the same Golgi apparatus. Examination of these morphological transitions of the cis element greatly aided in the ordering of the cis element types within the continuum diagrammed in Figure 17. In the small spinal ganglion neurons, such as is shown in Figure 2 of the first paper of this series (Lindsey and Ellisman, 1985), the appearance of the cis element in different regions of the cells is much more homogeneous than the appearance of those found in large neurons.

Discussion

The cis element of the Golgi apparatus appears in multiple forms. These forms can be ordered on relative similarities so as to describe a gradual and circular continuum of morphological variation. Present within these variations are new structures including the cis tubular network and the marginal tubule.

The various forms of the cis element collectively are intriguing. One possible explanation for these variations is that they reflect different stages in the maturation and eventual turnover of the Golgi

Figure 17. Diagram illustrating the various forms of the cis element. Because intermediate forms between these stages were observed, they comprise a circular continuum of variation. Note that the various components of the cis element are present at different stages of the continuum. For example, cis elements with well developed cis saccules (CS) and marginal tubules (MT) are rarely accompanied by vesicle clusters (VC). Reflecting their dominant components, these forms are called: the vesicle form (ves.), the vesicle-network form (ves.-net.), the network-saccule form (net.-sac.), the saccule-tubule form (sac.-tub.), and the saccule form (sac.). Other labeled structures include: the cis tubule network (CTN), varicose tubules (VT), smooth tubules (ST), and discrete vesicles (V).

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apparatus membranes. Tracer experiments following plasma surface components which bind cationized ferritin or dextran have led to the conclusion that the membrane surrounding secretory granules is recycled between the trans face of the Golgi apparatus and the cell surface (Farquhar, 1981). Kinetic studies of certain glandular cells have found that the difference in turnover between secretory and membrane proteins can be as large as 100-fold (Meldolesi and Borgese, 1981). However, the latter study indicates that, following an extended period of functional service, the Golgi membranes are turned over.

Evidence suggesting that Golgi saccules are turned over as a unit has been reviewed by Morr et al. (1979). By far the most compelling evidence comes from cinematographic studies of scale formation in the marine haptophycean algae *Pleurochrysis scherffeli* (Brown, 1969). These scales, which are visible in the light microscope, surround the outside of the algal cell with a sort of exoskeleton. This study showed the dynamic progression of developing scales from the cis toward the trans pole of the Golgi stack. This migration occurred as the scales were assembled within the lumen of the Golgi apparatus saccules as has been shown by electron microscopy. The mature scales within the swollen trans saccule characteristic of these cells moved to the plasma membrane where the scale was expelled by a process similar in appearance to exocytosis. Evidence suggesting that similar saccule turnover occurs in higher

vertebrate cells comes from studies of the urothelial cells lining the ureter. The urothelium consists of thickened plaques composed of a hexagonal lattice of dodecameric subunits. These subunits are located on the membrane's luminal surface and are separated by thinner unstructured narrow bands (Hicks et al., 1974). To a large extent, these plaques appear to be assembled within the saccules of the urothelial Golgi apparatus (Hicks, 1966; Porter et al., 1967). The mature plaques are delivered to the cell surfaces as single intact cisternae or saccules ("fusiform vesicles") released from the trans face of the Golgi apparatus. The elaboration of the plaques appears to be progressive across the stacked cisternae. If the saccules of the Golgi stack undergo unitized turnover as the above studies indicate, then it is likely that the saccules are assembled as units at the cis pole of the Golgi stack. Thus, it is quite plausible that vesicle clusters, the cis tubular network, marginal tubules, and the regularly perforated cis saccule represent successive organizational membrane transformations and that the cis element cycles through its various forms during the assembly of each new Golgi saccule. Alternatively, or in addition, these structures may be involved in sorting RER products destined for the Golgi apparatus from RER components that remain in the RER (see Rothman, 1982).

The most striking new structure is the cis tubular network. Because of the confluence of the network with the marginal tubule system, these structures are probably functionally related. Still, they should

be considered separate structures since marginal tubules often appear in the absence of the network (as in the saccule-tubule form *cis* element). At present, little can be directly said of the network's possible functions. Perhaps this structure might play a role in the formation of the *cis* saccule as discussed above. In addition, the network along with marginal tubules and interconnecting tubules may play roles in sorting and preprocessing of RER products destined for processing in the Golgi stack. These functions have been suggested previously for the "boulevard peripherique," a system of tubules first seen at the edge of negatively stained isolated liver Golgi saccules (Morré and Ovtracht, 1977). Although the *cis* element tubule system may be involved in functions similar to those of the boulevard peripherique, secretory vesicles are not seen to bud from the former as they are from the latter.

The presence of many vesicles in certain forms of the *cis* element suggest that, in addition to varicose tubules and smooth tubules, they also play an important role in the movement of material to or from the *cis* element. Particularly interesting are the tightly packed vesicle clusters. Such clusters may be in a transition state with structures such as the *cis* tubular network.

In conclusion, the *cis* element of the Golgi apparatus appears in different forms. A possible interpretation of these different structural forms is that they represent static images of a dynamically changing structure. If one accepts this possibility, these forms can be ordered into a circular continuum. This perspective is consistent with a model of normal Golgi apparatus function in which the *cis* element regularly transforms its structure according to the sequence of this continuum.

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