Review Article

DYNAMIC STRUCTURE OF GOLGI APPARATUS DEPENDING UPON FUNCTIONAL PHASES OF SECRETION

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Abstract: Ultrastructural changes in Golgi apparatus (GA) during various functional conditions were studied in the rat parotid glands, and their three dimensional configuration was analyzed with the use of computer graphics from serial section electron micrographs.

Three dimensional reconstruction confirmed that a normal GA was a connected single structure located in the supranuclear region, although it exhibited markedly complicated and reticulated branches. In contrast when host cells entered into the mitotic phase, underwent postnatal development and were inhibited of transport of secretory materials from rough endoplasmic reticulum, GA became an aggregation of small vesicles or tubules, in which cis and most likely medial, but not trans elements were included.

From these observations, we propose a new hypothesis that a GA consists of two components, basic and functional ones. The basic component having small vesicles and tubules of cis and medial natures is the most primitive feature of GA that is recognized in such cellular phases as early development, mitosis, and interruption of inflow. Basic components evolve a functional one that consists of a lamellar structure with cis, medial and trans elements. They are recognized in the usual cells at the active states. The size and overall configuration of GA changes greatly due to the amount of inflow of secretory materials depending upon the functional conditions of the cells. (J Toxicol Pathol 4: 119-128, 1991)

Key words: Golgi apparatus, Parotid gland, Development, Cell division, Brefeldin A, Secretion

1. Introduction

Golgi apparatus is extremely well-developed in secretory cells, and its entire structure changes greatly in accordance with functional phases, this being one of its major features. Morphological changes of Golgi apparatus in response to various functional phases of secretory activity are discussed in this review with special reference to acinar cells of salivary glands including our own results.

2. Morphology of Golgi apparatus

a. General picture of Golgi apparatus

The Golgi apparatus consists of stacks of 5-10 cisternae surrounded by groups of numerous vesicles and vacuoles. According to a series of studies by Rambourg et al.¹ and results of high voltage electron microscopic studies by Noda and Ogawa², the apparatus is basically one connected structure although its appearance differs considerably according to the type of cells. The overall form is that of a curve, and in simple epithelial cells including cells of various exocrine glands, it is situated in the supranuclear region with its concavity directed toward the free surface³⁻⁵. In nerve cells, the apparatus is spread out reticulately and engulfs the nucleus almost completely⁶. It encircles the nucleus as a ring in atrial muscle cells⁷. We have reconstructed a 3-dimensional image of
Golgi apparatus of a parotid acinar cell by computer graphics using electron micrographs of about 100 serial sections (Fig. 1). The apparatus was confirmed to spread out in the supranuclear region as a single connected form despite of its extremely complicated branching structure.

Golgi cisternae are divided grossly into cis, medial and trans regions, and processing enzymes for glycoprotein synthesis are distributed in order in the three regions. Glycoproteins are transported through Golgi cisternae by repeated pinching off and fusion of vesicles. During this process, their maturation is completed by sequential addition and trimming of sugar chains. Golgi cisternae can also be divided into 3 regions by the classical enzyme histochemical techniques; trans regions by TPPase, the medial ones by NADPase, and the cis ones by an osmium impregnation method (Figs. 2A and B). The osmium reaction is the application to electron microscopy of silver staining technique that was employed by C. Golgi when he had discovered the apparatus. Functional differentiation shown by Golgi cisternae according to the direction of transport of secretory materials is designated as “Golgi polarity”, and the positioning of polar Golgi apparatus in a certain area within a cell is considered to be closely related to polarity expressed by the cell (cell polarity).

Attention is being increasingly directed to the presence and functions of a special membranous system, the trans Golgi network (TGN) or trans tubular network (TTN). This network may be the exact site at which materials transported beyond Golgi apparatus are sorted. Whether TGN is actually a part of the trans region or independent organelle functionally closer to secretory granules than trans cisternae remains a controversial matter. There is evidence that it may partly overlap Novikoff’s GERL and CURL of Geuze et al., but confirmation will require further study. An obscure region in which smooth endoplasmic reticulum accumulated was occasionally detected near the Golgi apparatus under an electron microscope. This region may quite likely be the site of various functions so far not identified.

b. Development of Golgi apparatus
Rat parotid gland undergoes rapid differentiation immediately after birth, giving rise to an acino–tubular structure within 1 week and acinar cells take on the form characteristic of exocrine cells. Morphological changes of Golgi apparatus can be followed during the course of development (Fig. 3A–D). The apparatus first appears as a group of small vesicle, which show positive reaction to osmium impregnation but are devoid of TPPase activity, in the most undifferentiated cells of immediately after birth. On the 2nd day, a few straight cisternae are present in the vesicular aggregation. On about the 3rd day, they have gradually become curved through increase in the number of layers, and TPPase activity comes to be expressed for the first time, thus establishing the trans region. At the same time, osmium positive components become situated...
opposite to the trans cisternae to form the cis region, thus indicating that the internal polarity of Golgi apparatus has been established. After the Golgi apparatus has taken its position in the supranuclear area, secretory granules begin to form\textsuperscript{22}. The basic structure of Golgi apparatus is complete at this time. It continues to enlarge with the functional development. It has been shown by electron microscopic observation that secretory function may possibly start following completion of the structure. The process of development seems the same as that of reconstruction of Golgi apparatus in dividing cells as is discussed in the following.

3. Changes in Golgi apparatus during cell division

During cell division, Golgi apparatus has been shown to break up into fragments which subsequently disseminate throughout the cytoplasm. However, most observations in this regard have been made on cultured cells\textsuperscript{23–28}, since an adequate experimental system for examining various stages of cell division in a normal mature cell in a tissue has not been available except for regeneration of the liver following partial hepatectomy\textsuperscript{29–32}. In rodent salivary glands, the repeated administration of β-adrenergic agonist, isoproterenol (IPR) induces mitotic proliferation of acinar cells raising labeling index of [\textsuperscript{3}H]-thymidine by several per cent. Consequently, changes in Golgi apparatus in dividing parotid acinar cells could be examined at various stages of mitosis following the IPR treatment\textsuperscript{33}. Immediately after acinar cells have entered the mitotic stage, the Golgi apparatus comes to consist of a large number of groups of small vacuoles scattered throughout the cytoplasm (Figs. 4 and 5). The vacuoles showed osmium reaction even though TPPase activity had disappeared, thus indicating the cis elements to be still present. The rapid
reorganization of these vacuoles occurs in daughter cells at the terminal stage of mitosis, followed by reconstruction of the typical stacked structure of Golgi apparatus. As will be discussed later, similar changes were observed following the administration of anti-microtubular agents. This is because the mitotic form of Golgi apparatus may have been induced by mobilization of microtubules for the transfer of chromosomes. Golgi apparatus in the mitotic stage can also be viewed as an involuted form due to interruption of transport to it from RER34,35. This fragmentation should serve the purpose of equal partition of Golgi apparatus into daughter cells26.

4. Morphological changes in Golgi apparatus by secretory inhibitors

Several drugs that affect the secretory function cause morphological changes in the Golgi apparatus. These drugs and their effects are outlined as follows.

a. Anti-microtubular agents

Depolymerizers of microtubules such as colchicine, vinblastine, and nocodazole break up the stacked structure of Golgi apparatus into fragments and scatter them throughout the cytoplasm of various cells86. This effect is intensified with duration of the anti-microtubular action87.
Antimicrotubular agents have been shown to cause extensive morphological change in the apparatus in the acinar cells of salivary glands \(^{37}\) and exocrine cells of the pancreas \(^{38}\), and the apparatus has been found closely related to microtubules by immunohistochemical technique \(^{39,40}\). Fragments of the Golgi apparatus produced by nocodazole treatment accumulate again along microtubules immediately after washing out of the drug \(^{41}\). Microtubules would thus appear essential to the maintenance of the structure and functions of the apparatus and for its positioning at the proper site in a cell. Golgi proteins such as 58-KD \(^{42}\) and 110-KD \(^{43}\) are considered to be binding sites for microtubules although details have yet to be confirmed.

b. Monensin

A monovalent cation ionophore, monensin, interferes with protein transport in Golgi apparatus without having any effect on protein
Fig. 6. Parotid acinar cells following treatment with BFA. **A**: BFA inhibited the export of protein from RER to Golgi, causing secretory materials to accumulate in cisternal spaces of RER. In contrast, Golgi apparatus changed into aggregation of small vesicles and tubules (basic components). ×5,800. **B**: High power view of a Golgi apparatus of BFA treated acinar cells. ×20,000. **C**: Golgi apparatus of BFA-treated acinar cells following prolonged incubation in osmium tetroxide to make cis regions observable. Basic components are found to contain cis elements. ×17,700.

The drug causes dilation accompanied by vacuolation of the osmium-negative cisternae, the degree being greater for trans elements where secretory proteins have accumulated in vacuoles that have not been transported further and terminal glycosylation is also inhibited. However, TPPase activity is still present in the dilated cisternae. These effects of monensin are explained in relation to pH increase within Golgi cisternae.
c. **Brefeldin A (BFA)**

BFA is a macrolide antibiotic with unique activity to inhibit the transport of secretory materials from RER to the Golgi apparatus. This action may possibly cause the complete disappearance of the Golgi apparatus and the redistribution of enzymes present not only in cis and medial cisternae but trans cisternae as well over ER. This effect can be detected in the presence of an inhibitor of protein synthesis, since the preexisting Golgi membrane may be returned to ER. The tubular structure in the cis region becomes a route for the above redistribution, and this retrograde movement is mediated by microtubules. Recently, detachment of the above stated 110-KD protein from Golgi membrane has been shown to be the first action exerted by BFA. The 110-KD protein may be a coat protein (beta coat protein) differing from clathrin, and present in non-clathrin coated vesicles that assist transport between ER and Golgi or within Golgi cisternae. Thus, BFA action is becoming an important key to disclose the mechanism of transport between ER and Golgi apparatus.

Following the administration of BFA to parotid acinar cells, stacked cisternae of Golgi apparatus were noted to disappear and RER to be greatly dilated, thus filling the cytoplasm. However, clusters of small vesicles about 70 nm in diameter could be seen among RER and many of which showed osmium reaction although no 

### 5. Secretory function and Configuration of the Golgi apparatus

This function in secretory cells is amplified greatly in the acino-tubular structure. During acinar development, extensive cell proliferation of epithelial layers occurs to form bundles of epithelial cells in a direction toward connective tissue with repeated branching at the tip, followed by gradual arrangement of the cells at the terminus into single layer surrounding a lumen. As this takes place, the Golgi apparatus under successive cell division is certainly being distributed continuously to daughter cells as groups of small vesicles containing cis elements, the basic component of the Golgi apparatus. The synthesis of secretory proteins is accompanied by reduction in the mitotic rate and prolongation of the G1 stage, and at the same time, increased transport into basic components of Golgi apparatus leads to establishment of the cisternal structure. In this process, trans cisternae evolve accompanied by the commencement of secretory granule formation to accumulate gradually in the cytoplasm. This means that functional components of the apparatus are added to basic components. After inflow from ER has been inhibited by the mitosis or the administration of BFA, the functional components immediately become involuted, so that only basic components remain. The basic components are assumed to be connected together into a single mass by some mechanism of microtubular action, and the administration of anti-microtubular agents caused their disruption. However, it appeared that functional...
components remained as long as inflow from RER continued even following disruption to disseminate the basic components by anti-microtubular agents. In such a case, the synthesis of secretory proteins continues to occur even in fragmented Golgi apparatus, resulting in the continuation of secretory granule formation though at a lesser degree of efficiency. Golgi apparatus usually observed under an electron microscope would thus appear exactly as its acting feature with the basic components and functional one together, and the magnitude of functioning would be in proportion to the overall size of Golgi cisternae.

Degraded membrane components are concluded to derive from Golgi apparatus, since they always form aggregated masses. Thus, one significant feature of the Golgi membrane is the presence of mechanism responsible for connecting these masses into aggregates. Though the order of sugar chain binding to glycoproteins is not recorded on genes, some mechanism by which related enzymes are arranged in order should be present to produce a sugar chain arrangement having a certain order. The real significance of greatly integrated structure expressed by the Golgi apparatus should lie in this functional demand.

Acknowledgements: The authors thank Mr. O. Katsumata for his technical assistance, and also thank the personnel of the Electron Microscopy Center, Kitasato University School of Medicine. A part of this study was supported by a grant from the Japanese Ministry of Education, Science and Culture (No. 03264218).

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