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Functional Implications of the Golgi and Microtubular Network in Gonadotropes

Tsuyoshi Watanabe [a],* Hiroki Bochimoto [a], Daisuke Koga [b], Masahiro Hosaka [c], and Tatsuo Ushiki [b]

[a] Department of Microscopic Anatomy and Cell Biology, Asahikawa Medical University, Asahikawa, Japan;

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

[c] Laboratory of Molecular Life Sciences, Department of Biotechnology, Akita Prefectural University, Akita, Japan

*Corresponding author

Department of Microscopic Anatomy and Cell Biology, Asahikawa Medical University,

2-1-1-1 Midorigaoka-higashi, Asahikawa 078-8510, Japan

E-mail: tyshwata@asahikawa-med.ac.jp

Tel: +81 166 68 2310 / Fax: +81 166 68 2319

Abstract

In contrast to the widely accepted images of the Golgi apparatus as a cup-like shape, the Golgi in pituitary gonadotropes is organized as a spherical shape in which the outer and inner faces are cis- and trans-Golgi elements, respectively. At the center of the spherical Golgi, a pair of centrioles is situated as a microtubule-organizing center from which radiating microtubules isotropically extend toward the cell periphery. This review focuses on the significance of the characteristic organization of the Golgi and microtubule network in gonadotropes, considering the roles of microtubule-dependent membrane transport in the formation and maintenance of the Golgi structure. Because the highly symmetrical organization of the Golgi is possibly perturbed in response to experimental treatments of gonadotropes, monitoring of the Golgi structure in gonadotropes under various experimental conditions will be a novel in vivo approach to elucidate the biogenesis of the Golgi apparatus.

1. Introduction

The pituitary gonadotrope is a representative endocrine cell that synthesizes and releases two distinct peptide hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Besides these two gonadotropins, gonadotropes secrete a large amount of soluble proteins such as chromogranins and secretogranins (Chanat *et al.,* 1988; Cozzi and Zanini, 1986). Morphologically, gonadotropes contain two different types of secretory granules, small granules containing secretogranin II and large granules containing chromogranin A (Hosaka *et al.,* 2002; Watanabe *et al.,* 1998, 1993, 1991). To generate a large amount of secretory granules containing hormones and related proteins, the endomembranous organelles that constitute the exo- and endo-cytic pathways are well developed/organized in gonadotropes.

Among the endomembranous organelles, the Golgi apparatus is situated at the center of the secretory pathway and functions as an intersection of intracellular vesicular transport. The Golgi apparatus was first identified as a reticular structure in neurons using a silver impregnation method to stain the cells (Golgi, 1898a, b). Later, the intracellular structure stained by the silver impregnation method was ubiquitously observed in various cells at light microscopic levels, although the debate on the reality of the organelle lasted for a long time (for reviews on the issue including related references, see Beams and Kessel, 1968; Farquhar and Palade, 1981). Observations under an electron microscope finally settled this dispute by demonstrating the distinct membrane set that constitutes the Golgi apparatus: flattened cisterns and associated vesicles/vacuoles corresponding to the reticular structure observed at the light microscopic level (Dalton and Felix, 1954; Farquhar and Rinehart, 1954; Sjöstrand and Hanzon, 1954)

Subsequent analyses clarified that the organelle plays a pivotal role in the processing and sorting of both soluble and transmembrane proteins passing through the secretory

pathway (Farquhar and Palade, 1981, 1998; Marsh and Howell, 2002, Martinez-Alonso *et al.,* 2013). During sequential transition in the stacks of Golgi cisterns, luminal proteins and lipids are modified by the action of Golgi-resident enzymes, including glycosylation (Moremen *et al.,* 2012), sulfation (Huttner, 1988; Niehrs *et al.,* 1994), and proteolytic processing (Halban and Irminger, 1994). Upon post-translational modification and processing, the proteins are sorted at the trans-Golgi network (TGN), the exit site of the Golgi apparatus, for their appropriate destinations such as the plasma membrane via secretory tubulovesicles, secretory granules, and lysosomal/endosomal compartments (De Matteis and Luini, 2008; Farquhar and Palade, 1998; Griffiths and Simons, 1986; Mellman and Simons, 1992). On the other hand, Golgi-resident enzymes are retained in the Golgi apparatus. Membrane-bound glycosyltransferases and other modifying enzymes that function in the Golgi apparatus are excluded and/or removed from membrane carriers forwarding to the secretory pathways (Allan and Balch, 1999; Glick *et al.,* 1997; Opat *et al.,* 2001; Pelham, 2001; Puthenveedu and Linstedt, 2005; Storrie, 2005). In any case, the incessant traffic of tubulovesicular carriers that move along microtubules both in antero- and retro-grade directions largely affect the organization of the Golgi apparatus (Brownhill *et al.,* 2009; Cole and Lippincott-Schwartz, 1995).

Because the flattened cisterns of the Golgi apparatus are stacked in order forming a cup-like or hemispherical shape in exocrine cells that secrete a large amount of proteins in a polarized manner, the fundamental configuration of the Golgi apparatus has been widely accepted as a cup-like/hemispherical shape. Cisterns at the convex and concave faces of the cup-like Golgi stacks are designated as cis- and trans- cisterns that functionally face the rough endoplasmic reticulum (ER) and cell surface, respectively (Farquhar and Palade, 1981; Mellman and Simons, 1992). This organization of the Golgi stacks can explain the vectorial

movements of secretory products from the rough ER to their final destinations at the cell periphery.

On the other hand, diversity in the higher organization of the Golgi apparatus depending on the cell/tissue type has been recognized by various microscopic techniques. In the drawings of the earliest studies by light microscopy, the overall shape of the Golgi apparatus is not described as cup-like, but rather a spheroidal network, even in the original drawings of neurons by Golgi (Golgi 1898a, b). The ultrastructural characteristics and diversities of the three-dimensional architecture of the Golgi apparatus were extensively demonstrated by Rambourg and his colleagues by stereograms of a pair of tilted images from high voltage transmission electron microscopy (Clermont *et al.,* 1995; Rambourg and Clermont, 1997, 1990; Rambourg *et al.,* 1993, 1992, 1981). Through their comprehensive observation and later detailed studies with the aid of computerized tomographic reconstruction (Marsh, 2005; Marsh *et al.,* 2004; Mogelsvang *et al.,* 2004), the higher level organization of the Golgi apparatus is now accepted as a continuous ribbon-like structure in various configurations with compact and non-compact regions. Compact regions consist of piled stacks of Golgi cisterns with convex (cis-) and concave (trans-) sides that are linked to each other by a sparse tubular network defined as the non-compact region (Klumperman, 2011; Rambourg and Clermont 1990). Observations of osmium-macerated specimens under a high resolution scanning electron microscope has also revealed the diversity of the overall three-dimensional ultrastructure of the Golgi apparatus in various mammalian cells (Koga and Ushiki, 2006; Tanaka and Fukudome, 1991; Tanaka and Mitsushima, 1984; Tanaka *et al.,* 1986). However, the profound mechanisms that promote structural diversity of the Golgi apparatus in vivo remain to be solved.

To shed light on this issue, our review focuses on the structural characteristics of the Golgi apparatus and microtubule network in pituitary gonadotropes, and attempts to illustrate the significance behind the distinctive organization. We also discuss the putative crosstalk between gonadotropin-releasing hormone (GnRH) signaling and the functional molecules that maintain the Golgi organization, based on structural changes of the Golgi apparatus after stimulation of gonadotropes. In parallel, the putative links between cellular polarity and Golgi organization will be discussed by comparing the overall shape and intracellular location of the Golgi apparatus in gonadotropes with those in other endocrine and exocrine cells. The unique organization of the Golgi apparatus in pituitary gonadotropes possibly provides a clue to clarify the theoretical background behind the diversity of the overall shapes of the Golgi apparatus depending on the cell type.

2. Structural characteristics of the Golgi apparatus and microtubule network within rat pituitary gonadotropes

As shown in our recent studies, pituitary gonadotropes generally contain a Golgi apparatus with a globular shape (Figure 1A, B) in which the outer and inner surfaces are cis- (the entry side of the Golgi) and trans- (the exit side) faces, respectively (Figure 1C–E) (Koga and Ushiki, 2006; Watanabe *et al.,* 2012). Immunocytochemical localization of BiP (indicative of rough ER; Figure 1C) and γ-adaptin (indicative of vesicles generated from the TGN; Figure 1E) indicate that the pre- and post-Golgi compartments in gonadotropes are mostly segregated from each other by the spherical walls of Golgi cisterns.

The overall shape of the Golgi apparatus in gonadotropes was demonstrated at the light microscopic level about a hundred years ago by staining with the silver impregnation method, although these earlier observations have been often disregarded. The shape of the

Golgi apparatus in basophilic gonadotropes of the anterior pituitary has been described principally as round, which spherically enlarges after castration (Addison, 1917; Ellison and Wolfe, 1934; Severinghaus, 1937, 1933; Wolfe and Brown, 1942). Although the circular profile of the globular Golgi apparatus can be observed in electron micrographs of early studies focusing on pituitary gonadotropes (Farquhar and Rinehart, 1954; Kurosumi and Oota, 1968; see also chapter 4 "Gonadotropes" in Costoff, 1973), little attention has been paid to the significance of its globular shape.

To realize the significance of the globular configuration of the Golgi apparatus in pituitary gonadotropes, the intracellular organization of the microtubule network should be considered because the positioning, orientation, and overall configuration of the Golgi apparatus are largely determined by the balance of membrane input and output transported via microtubule-dependent motor proteins (Allan *et al.,* 2002; Brownhill *et al.,* 2009; Cole and Lippincott-Schwartz, 1995; Lippincott-Schwartz, 1998; Murshid and Presley, 2004; Thyberg and Moskalewski, 1999). As described in our recent study, a pair of centrioles immunolabeled with anti-γ-tubulin antibodies is situated at the center of the spheroidal Golgi apparatus as a microtubule-organizing center (MTOC) (Figure 1B, F) from which radiating microtubules extend in all directions toward the cell periphery through gaps in the spheroidal wall of the Golgi stack (Watanabe *et al.,* 2012). In addition to these radiating microtubules, relatively stable microtubules immunolabeled with anti-acetylated α-tubulin antibodies accumulate on the outer surface of the spherical Golgi (Figure 1F, G). Thus, the architecture of the microtubule network in gonadotropes appears to be much more isotropic than that of exocrine cells (Kurihara and Uchida, 1987), probably because the sites for exocytotic secretion are less strictly confined to specialized subdomain(s) on the cell surface.

Considering the contextual usage of microtubule-dependent motors and tubulovesicular membrane carriers, both the cis-trans orientation and characteristic shape of the Golgi apparatus in gonadotropes can be rationally explained. The motor proteins that drive intracellular transport carriers along the microtubules are classified into two discrete categories: plus and minus end-directed motors (Gennerich and Vale, 2009; Hirokawa, 1998; Schliwa and Woehlke, 2003). In general, dyneins move toward the minus end of microtubules and mediate the centripetal movements of tubulovesicular carriers ((Pfister *et al.,* 2005; Vallee *et al.,* 2004; Yadav and Linstedt, 2011), while kinesins (and most kinesin superfamily proteins [KIFs]) move toward the plus end of microtubules and mediate centrifugal movements (Hirokawa and Noda, 2008; Lawrence *et al.,* 2004).

These two types of microtubule-dependent motor proteins are specifically associated with their corresponding membrane carriers in pre- and post-Golgi compartments, and contribute to the well-coordinated movements of these carriers in the cell (Brownhill *et al.,* 2009; Goodson *et al.,* 1997; Hehnly and Stamnes, 2007). In the pre-Golgi compartment (Figure 2B), coat protein complex (COP)II-coated vesicles carried by dynein motors mediate anterograde transport from the ER to the Golgi (Harada *et al.,* 1998; Presley *et al.,* 1997; Watson *et al.,* 2005), while COPI-coated tubulovesicles that use kinesin/KIF motors mediate retrograde transport from the Golgi to the ER (Dorner *et al.,* 1998; Lippincott-Schwartz *et al.,* 1995; Stauber *et al.,* 2006). In the post-Golgi compartment (Figure 2C), secretory granules/tubulovesicles and newly generated primary lysosomes are likely transported by kinesin/KIFs for anterograde traffic from the Golgi (Jaulin *et al.,* 2007; Kreitzer *et al.,* 2000; Nakagawa *et al.,* 2000; Varadi *et al.,* 2002), while endocytosed tubulovesicles are likely transported mainly by dyneins for retrograde traffic from the plasma membrane to the central area of the cell near the trans-side of the Golgi (Blocker *et al.,* 1997; Burkhardt *et al.,* 1997;

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Loubery *et al.,* 2008; see also a review by Hunt and Stephens, 2011). Considering that microtubules isotropically radiate from a single MTOC at the cell center and the Golgi apparatus is formed in the middle along these radiating microtubules, this simple architecture of the microtubule network can systematically explain the peculiar polarity of the Golgi apparatus and all intracellular membrane trafficking within gonadotropes. The minus end-directed motors such as dyneins mediate the centripetal movements of the carriers, COPII-coated vesicles, and endocytosed tubulovesicles in pre- and post-Golgi compartments, while plus end-oriented motors such as kinesin/KIFs mediate the centrifugal movements of the carriers, the COPI-coated vesicles, and secretory granules/tubulovesicles in pre- and post-Golgi compartments (Figure 2).

In addition to the trafficking lines within gonadotropes, the symmetrical globular shape of the Golgi apparatus in gonadotropes can also be explained based on the isotropic architecture of the microtubule network. As discussed above, Golgi cisterns are likely formed at the position where the membrane input and output to and from the Golgi apparatus are balanced (Cole and Lippincott-Schwartz, 1995; Lippincott-Schwartz, 1998). Geometrically, such balanced positions in gonadotropes are likely to be arrayed spherically at a certain distance from the single central MTOC. Thus, the cis-trans orientation and globular shape of the Golgi apparatus in gonadotropes can be regarded as an ideal model for poorly polarized endocrine cells.

Because the characteristic structure of the Golgi apparatus in gonadotropes largely depends on the microtubule architecture as discussed above, the organization of the Golgi is possibly altered by compounds that affect microtubule dynamics. Based on our preliminary observations, the most rapid morphological change in gonadotropes treated with colchicine, which disturbs microtubule dynamics by tightly binding to the intradimeric α–β interface of

tubulin heterodimers (Stanton *et al.,* 2011), is the appearance of atypical vacuoles in the peripheral region of cells (unpublished data). These vacuoles are widely observed in pituitary endocrine cells including gonadotropes at 30–60 minutes after intraperitoneal injection of rats with colchicine. Moreover, there is accumulation of specific marker proteins for the pre-Golgi compartment, such as ERGIC-53 (Schindler *et al.,* 1993; for a review, see also Appenzeller-Herzog and Hauri, 2006) and p23 (Rojo *et al.,* 1997; Hosaka *et al.,* 2007; for a review, see also Emery *et al.,* 1999), on their membrane. In contrast, the lateral integrity of the Golgi apparatus is preserved as the spherical configuration at this time point, but the Golgi cisterns gradually shrink after a certain delay and finally break into small dispersed stacks at 8 hours after treatment. These findings suggest that the kinetics of microtubule-dependent transport carriers are considerably different in pre- and post-Golgi compartments. The trafficking processes in the pre-Golgi compartment appear to be much more sensitive to colchicine treatment than those in the post-Golgi compartment. Although the mechanisms behind this phenomenon should be investigated further, the spatial segregation of pre- and post-Golgi compartments and the spherical wall of Golgi stacks in gonadotropes may help us to divide the influence of colchicine into two different steps in pre- and post-Golgi compartments. The distinct intracellular organization of gonadotropes is potentially beneficial for further analyses of vesicular transport processes along microtubules in vivo.

3. Influences of extrinsic stimuli to the GnRH receptor on the organization of the Golgi in pituitary gonadotropes

The functional states of pituitary gonadotropes are largely regulated by GnRH released from hypothalamic neurons into the hypothalamic-hypophyseal portal vascular system. GnRH

specifically binds to the corresponding GnRH receptor expressed on pituitary gonadotropes, and subsequently stimulates cells to synthesize and release secretory products including two gonadotropins (for reviews see: Bliss *et al.,* 2010; Naor, 2009; Ruf *et al.,* 2003). Castration of experimental animals increases the intrinsic GnRH level released from hypothalamic neurons by impairing the negative feedback control of gonadal sex steroids (Pielecka and Moenter, 2006). Although chronic enlargement of the Golgi apparatus in pituitary gonadotropes of castrated animals has been reported (Addison, 1917; Ellison and Wolfe, 1934; Farquhar and Rinehart, 1954; Severinghaus, 1933; Wolfe and Brown, 1942), the immediate alteration of the Golgi structure after castration has been overlooked to date. The morphological effects of synthetic GnRH agonists on the Golgi apparatus in gonadotropes have been also scarcely examined until our most recent study (Bochimoto et al., 2013).

GnRH agonists generated by substitution of amino acid residues of intrinsic GnRH initially augment the intracellular signaling events downstream of its receptor (Conn and Crowley, 1994; Millar *et al.,* 2004). Because sustained treatment with powerful GnRH agonists such as buserelin (Brogden *et al.,* 1990) and leuprorelin (Plosker and Brogden, 1994) can paradoxically suppress synthesis and secretion of gonadotropins by receptor desensitization (McArdle *et al.,* 2002; Naor, 2009), depot formulations of these GnRH agonists have been clinically used to deplete gonadal sex steroids in patients suffering from sex steroid-dependent diseases such as metastatic prostate cancer (Conn and Crowley, 1994; Engel and Schally, 2007). However, depot formulations of GnRH agonists potentially induce a flare effect caused by their own strong stimulatory effects on gonadotropes and the subsequent transient increase of serum sex steroids at the beginning of treatment (Conn and Crowley, 1994). Although gonadotropes are a direct target of these agonists, the

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ultrastructural changes in pituitary gonadotropes under the influence of GnRH agonists have been poorly analyzed until recently.

The configuration and ultrastructure of the Golgi apparatus in pituitary gonadotropes are largely affected by treatment with the depot formulation of the GnRH agonist leuprorelin. The ball-like shape of the Golgi apparatus in gonadotropes of control rats (Figure 3A, B) is transiently disrupted into a mini-stack from 1 day after administration of the leuprorelin depot formulation (Figure 3C, D), although the Golgi apparatus later coalesces again into a globular shape by 7 days of treatment (Bochimoto *et al*., 2013). Because similar changes in the Golgi apparatus are observed shortly after castration (Koga *et al*., unpublished observation), this transient disruption of the Golgi apparatus possibly occurs in response to intensive stimulation of the GnRH receptor.

Similar disorganization of the Golgi apparatus has been well analyzed and documented in cells during the mitotic phase of the cell cycle (Persico *et al.,* 2009). The process of disassembly of the Golgi apparatus during mitosis is principally divided into two successive but distinct steps: unlinking of the Golgi ribbon and fragmentation of the Golgi cisterns into tubulovesicles. During late G2 phase preceding mitosis, highly organized Golgi ribbons are severed into isolated small pieces of the Golgi stack because of dispersion of non-compact regions interlinking the solid Golgi stacks of compact regions (unlinking of the Golgi ribbon). Following the unlinking of the Golgi ribbon, each isolated stack of cisterns is further broken down into tubulovesicles during mitosis (fragmentation of the Golgi stacks). Disorganization of the Golgi apparatus in acutely stimulated gonadotropes resembles the unlinking of the Golgi ribbon described above.

In the molecular mechanisms of the first unlinking step during mitotic changes in the Golgi apparatus, phosphorylation of Golgi-associated proteins plays a crucial role (Preisinger

and Barr, 2005). Unphosphorylated Golgi reassembly stacking proteins (GRASPs) are able to laterally tether membranes of adjacent compact Golgi stacks by their trans-dimerization (Feinstein and Linstedt, 2008; Puthenveedu *et al.,* 2006) and thus contribute to formation and maintenance of the overall organization of the Golgi apparatus as a ribbon (Lowe, 2011; Vinke *et al.,* 2011). However, GRASPs are potential substrates of various kinases such as mitotic kinases Cdk1-cyclin B and polo-like kinase 1 (Lin *et al.,* 2000; Wang *et al.,* 2003), and extracellular-activated protein kinases (ERKs) (Jesch *et al.,* 2001). Phosphorylated GRASPs are forced to dissociate from adjacent partner GRASPs, resulting in the breakdown of the link between two adjacent units of the Golgi stacks (Feinstein and Linstedt, 2008; Xiang and Wang, 2010).

GnRH released from hypothalamic neurons into hypothalamic-pituitary portal circulation can successively provoke intracellular signaling events within pituitary gonadotropes, including activation of kinases in MAPK signaling cascades (Bliss *et al.,* 2010; Naor, 2009). Thus, Golgi-associated proteins including GRASPs are possibly phosphorylated by activated kinase(s), resulting in the scattering of isolated small Golgi stacks throughout gonadotropes as observed in the acute phase of GnRH agonist depot treatment. A recent study demonstrated that novel protein kinases C (nPKC) delta and epsilon, which activate ERKs in response to GnRH, are partially colocalized with GRASP65 in the Golgi apparatus of gonadotrope-derived cell lines, and are translocated to the perinuclear zone and plasma membrane, respectively, after GnRH stimulation (Dobkin-Bekman *et al.,* 2010). These findings apparently support a putative link between GnRH signaling and the molecules that regulate the structural integrity of the Golgi apparatus in gonadotropes.

In contrast to the disorganization of the Golgi apparatus during mitosis, the scattered Golgi stacks in the acute stimulatory phase converge again into a well-organized spherical

configuration by 1 week after treatment (Bochimoto *et al*., 2013). The initial effect of stimulation of the GnRH receptor is gradually alleviated by adaptive responses occurring downstream of the GnRH signaling cascade(s) (McArdle *et al.,* 2002; Naor, 2009). Thus, the transient increase of signals downstream of the GnRH receptor is probably not sufficient to provoke further fragmentation of Golgi cisterns following the unlinking of Golgi ribbons.

Although it should be further clarified which signaling molecules are activated downstream of the GnRH receptor and are responsible for transient disorganization of the Golgi apparatus, perturbation of rat gonadotropes by treatment with depot formulations of GnRH agonists possibly provide intriguing animal models to analyze the morpho-functional relationship of the Golgi apparatus. Because of its highly symmetrical organization, disassembly and/or deformation of the Golgi apparatus can be sensitively detected in pituitary gonadotropes under these experimental conditions.

4. Diversity of the global organization of the Golgi and microtubule network
4-1. Diversity of the Golgi organization of pituitary endocrine cells

In addition to gonadotropes, there are at least four more kinds of endocrine cells in the anterior pituitary: somatotropes, mammotropes, corticotropes, and thyrotropes. Although each type of endocrine cell synthesizes and secretes its own peptide hormone(s) in a regulated manner, the size, shape, and intracellular location of the Golgi apparatus vary depending on the cell type (Costoff, 1973; Severinghaus, 1937, 1933). Thyrotropes possess a Golgi apparatus with a large spheroidal shape comparable with that in gonadotropes, while somatotropes and mammotropes contain a much smaller Golgi apparatus. Compared with gonadotropes and thyrotropes, the shape of the Golgi apparatus is considerably distorted in most mammotropes, corticotropes, and some somatotropes. Despite its diverse size and shape,

the Golgi apparatus in pituitary endocrine cells is commonly enclosed in a certain amount of cytoplasm as demonstrated in mammotropes by Rambourg and his colleagues (Rambourg *et al.,* 1992). Furthermore, the MTOC is often situated in cytoplasm enclosed by Golgi ribbons.

Assuming that formation of the Golgi apparatus is based on the balance of input and output of membrane transport along microtubules and that the microtubule network is isotropically organized from a single central MTOC, which is identical to that in gonadotropes as discussed above, the reason why the Golgi apparatus of some pituitary endocrine cells is not symmetrically globular but is rather distorted is unknown. One possible explanation is that the Golgi shape correlates to the cell shape itself. Both the overall configuration of the Golgi apparatus and the cell contour of rat corticotropes are mostly irregular in the physiological state. In this case, intracellular organization of the other cytoskeletal elements such as actin and intermediate filaments possibly affect both the Golgi and cell shape.

Another possibility is that distortion of the overall shape of the Golgi apparatus is because of its proximity to the nucleus. Because the MTOC in most mammotropes and some somatotropes appears to be too close to the nucleus, the Golgi apparatus in these cells is greatly deformed by pressing against the nucleus (Figure 4A). In contrast, the Golgi apparatus in gonadotropes and thyrotropes is enough apart from the nucleus, and could be isotropically expanded (Figure 4B). According to our preliminary evaluation of the spatial distances between the nucleus and MTOC in each cell type by laser confocal microscopy, the average distances of these two structures in somatotropes and mammotropes are significantly smaller than those in gonadotropes and thyrotropes (unpublished data). As a result, the overall shape of the Golgi apparatus in mammotropes and somatotropes does not often appear to be ball-like, but rather cap-like on the nucleus. Although the mechanism that determines

the positioning of the MTOC should be further clarified, the intracellular location of the MTOC likely contributes to the diversity of both the intracellular positioning and organization of the Golgi apparatus.

4-2. Comparison of exocrine acinar cells and the acinus

In contrast to the endocrine cells discussed above, the organization of the Golgi apparatus in polarized exocrine cells is widely accepted as a cup-like or hemispherical shape (Koga and Ushiki, 2006; Motta *et al.,* 1997). In the intracellular architecture of the microtubule network of typical polarized epithelial cells, the MTOC is localized in the apical cytoplasm away from the nucleus (Kano *et al.,* 2001), and the minus and plus ends of microtubules principally direct to the apical and basal surfaces of the cell, respectively (Kano *et al.,* 2001; Meads and Schroer, 1995; Müsch, 2004; see also Figure 4C). On these microtubules along the longitudinal axis of the cell, motor proteins drive diverse tubulovesicular carriers to their proper destinations, resulting in establishment of cellular polarity on the cell surface (Drubin and Nelson, 1996; Müsch, 2004).

The Golgi apparatus in polarized exocrine cells is possibly formed at an appropriate position along the coherent and directional array of microtubules extending from the apical to basal regions of the cytoplasm. As a result, the slightly curved cisterns of the Golgi apparatus are stacked perpendicularly to the longitudinal axis of the polarized acinar cells, and the convex and concave faces of the Golgi stacks face the rough ER and apical plasma membrane, respectively (Farquhar and Palade, 1998, 1981).

Although the overall shape of the Golgi apparatus in individual exocrine acinar cells is quite different from that in pituitary gonadotropes, the merged Golgi structure of a whole exocrine acinus can be regarded as a sphere. The secretory lumen, around which MTOCs are

located in the apical cytoplasm of acinar cells (Kano *et al.,* 2001; Motta *et al.,* 1997), is situated at the center of the "spherical" organization of the integrated Golgi apparatus of the acinus (Figure 4D). From the MTOCs in the apical area at the center of the acinus, microtubules extend radially along the longitudinal axis of acinar cells toward the periphery of the acinus. Thus, the overall topology of the Golgi and microtubule network of a whole acinus are apparently analogous to that in a single gonadotrope (Figure 4B, D).

In summary, the overall shape of the Golgi apparatus in poorly polarized endocrine cells is theoretically a symmetrical sphere as observed in gonadotropes when the MTOC is located at the putative center of the cell with enough distance from both the nucleus and cell surface (Figure 4B). When the MTOC closely approaches the nucleus, the Golgi apparatus exposes its trans-face to the nucleus as a cap-like shape on the nucleus (Figure 4A). On the other hand, when the position of the MTOC is deviated from the putative center toward the apical cytoplasm of the cell, the Golgi apparatus possibly becomes a cup-like shape in which the trans-face is toward the apical membrane (Figure 4C). In any case, intracellular positioning of the MTOC, especially its spatial relationship with the nucleus and apical surface, is probably essential to determine not only the cellular polarity but also the overall shape and organization of the Golgi apparatus. The ball-like shape of the Golgi apparatus in pituitary gonadotropes can be regarded as the ideal shape in poorly polarized endocrine cells, and will be a good starting point to consider the theoretical background for the diversity of Golgi organization in various differentiated cells in vivo.

5. Concluding remarks and perspectives

The Golgi apparatus in pituitary gonadotropes is spherical and its trans-side is facing the inside of the sphere, where the MTOC is situated in the cell. Although the spherical shape

and cis/trans direction of the Golgi apparatus are apparently peculiar, the symmetrical organization is rather ideal for poorly polarized endocrine cells, considering the orientation and isotropic architecture of the microtubule network in the cell and the accumulated findings on the contextual usage of microtubule-dependent motors and transporting membrane carriers around the Golgi apparatus (Allan *et al.,* 2002; Murshid and Presley, 2004; Thyberg and Moskalewski, 1999).

The highly symmetrical organization is also advantageous for experimental purposes because the anomalous shift from the symmetrical shape can be sensitively detected under experimental conditions. Because the functional states of gonadotropes in experimental animals can be reproducibly modulated by established methods such as castration and administration of GnRH analogues or sex steroids, monitoring the structural changes of the Golgi apparatus in gonadotropes after experimental perturbation of the hypothalamic-pituitary-gonadal axis may provide a clue to clarify the mechanisms that regulate biogenesis and maintenance of the Golgi apparatus.

On the other hand, the close relationship between the intracellular location of the MTOC and overall organization of the Golgi apparatus indicates the significance of MTOC positioning in the cell (de Forges *et al.,* 2012; Sütterlin and Colanzi, 2010; Yadav and Linstedt, 2011). Elucidation of the mechanisms that regulate the positioning of the MTOC in well-differentiated cells will also provide a clue to the theoretical background behind the diversity of the shape and organization of the Golgi apparatus in vivo.

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Figure legends

Figure 1. Organization of the Golgi and microtubular network in pituitary gonadotropes of

male rats. (A, B) Intracellular localizations of the Golgi apparatus (immunolabeled with an

anti-GM130 antibody; green pseudocolor), the MTOC (immunolabeled with an anti-γ-tubulin

(γ-Tub) antibody; red), and the nucleus (stained with DAPI; blue) in a thick section of an

anterior pituitary gland (A). Gonadotropes were identified by immunolabeling with an

anti-LH antibody (white pseudocolor). A three-dimensionally reconstructed image of the

gonadotrope indicated by a white square in (A) is demonstrated in a stereo pair at a higher

magnification (B; the immunopositive signal for LH is omitted in this panel; see also

supplementary movie S1 and S2). Bars = 10 μm. (C–E) Immunocytochemical localizations of

BiP (C; green), GM130 (D; green), and γ-adaptin (E; green) in three adjacent semi-thin

sections (thickness: 1μm each) of rat anterior pituitary gland. These semi-thin sections were

simultaneously immunostained with anti-LH (blue) and anti-TGN38 (red) antibodies. Bar =

10 μm. (F, G) Intracellular localizations of the Golgi apparatus (immunolabeled with an

anti-TGN38 antibody; green), the MTOC (immunolabeled with an anti-γ-tubulin (γ-Tub)

antibody; red), realatively stable microtubules (immunolabeled with an anti-acetylated

α-tubulin (Ac-α-Tub); white), and the nucleus (stained with DAPI; blue) in a thick section of

an anterior pituitary gland (F). An optical slice at the equatorial position (F) and a pair of

three-dimensionally reconstructed images (G) of the spherical Golgi apparatus are shown (the

signal for TGN38 staining is omitted in G; see also supplementary movie S3 and S4). Bar =

10 μm. For detailed Materials and Methods, see Watanabe et al. (2012).

Figure 2. Schematic representation of the organization of the Golgi and microtubular

network within pituitary gonadotropes (A). The contextual usage of microtubule-dependent

motors and membrane carriers in pre- and post-Golgi compartments is demonstrated in (B) and (C), respectively.

Figure 3. Effects of the GnRH agonist leuprorelin on the organization of the Golgi in gonadotropes. (A, C) Thick cryosections (10 μm) of anterior pituitary glands prepared from control rats (A) and at 1 day (C; Leu_1d) after receiving a depot formulation of leuprorelin were immunostained simultaneously with mouse monoclonal anti-GM130 (Alexa Fluor 594; red pseudocolor), sheep polyclonal anti-TGN38 (Alexa Fluor 488; green), and rabbit polyclonal anti-LH (Pacific Blue; blue) antibodies. Bars = 1 μm. (B, D) Osmium-macerated pituitary tissues of rats under the experimental condition described above were also viewed under a scanning electron microscope. In support of the immunocytochemical findings (A, C), typical gonadotropes of non-treated control rats contain a ball-like Golgi apparatus (B; green), whereas gonadotropes of leuprorelin-treated rats contain numerous isolated Golgi mini-stacks scattered in the cytoplasm. Blue: nucleus. For detailed Materials and Methods, see Bochimoto *et al.,* (2013). Bars = 1 μm.

Figure 4. The putative relationship between positioning of the MTOC and overall shape of the Golgi apparatus in pituitary endocrine (A, B) and exocrine acinar (C) cells. In addition to the Golgi organization of individual acinar cells (C), that of a whole acinus is also shown (D). Note that the trans-side of the Golgi apparatus is not always facing the plasma membrane, but rather faces the MTOC in cells.

Supplementary Movie S1. The series of the original data (thickness of each optical section: 0.33 μm) for three-dimensional reconstruction of the gonadotrope demonstrated in Figure 1B

are additionally shown in a tomographic movie form. The z-axial distance of each optically sliced section from the bottom of the specimen is indicated in the left-lower corner of the movie clip. Pseudocolors are assigned similarly to those in Figure 1A (white pseudocolor: anti-LH to identify the gonadotrope; red: anti-γ-tubulin for the MTOC; green: anti-GM130 for the Golgi apparatus; blue: DAPI for the nucleus).
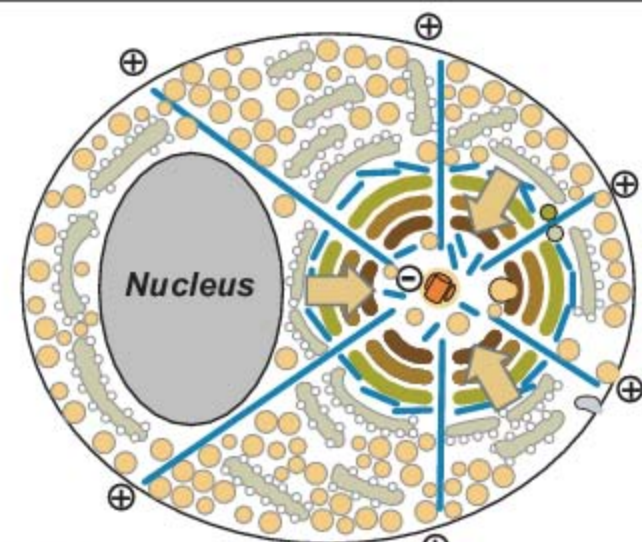
Supplementary Movie S2. 3D-rotation movie of the gonadotrope demonstrated in Figure 1B. Note that the MTOC immunolabeled with an anti-γ-tubulin antobody (red) is situated at the center of the sperical Golgi (immunostained with an anti-GM130 antibody; green) in the gonadotrope (immunostained with an anti-LH antibody; white).

Supplementary Movie S3. The series of the original data (thickness of each optical section: 0.33 μm) for three-dimensional reconstruction of the cells demonstrated in Figure 1G are additionally shown in a tomographic movie form. The z-axial distance of each optically sliced section from the bottom of the specimen is indicated in the left-lower corner of the movie clip. Pseudocolors are assigned similarly to those in Figure 1F (white pseudocolor: anti-acetylated α-tubulin for relatively stable bundles of microtubules; red: anti-γ-tubulin for the MTOC; green: anti-TGN38 for the Golgi apparatus; blue: DAPI for the nucleus).

Supplementary Movie S4. 3D-rotation movie of the cells demonstrated in Figure 1G. Note that relatively stable microtubules (immunolabeled with an anti-acetylated α-tubulin antibody; white) are radiating from the MTOC (immunolabeled with an anti-γ-tubulin antobody; red) situated at the center of the sperical Golgi apparatus (immunostained with an

anti-TGN38 antibody; green). Some bundles of the microtubules cover also tangentially the

spherical Golgi.

Panel A: γ-Tub.(R), GM130(G), DAPI(B), LH(W)

Panel B: (boxed region from A, magnified)

Panel C: TGN38(R), BiP(G), LH(B)

Panel D: TGN38(R), GM130(G), LH(B)

Panel E: TGN38(R), γ-Adaptin(G), LH(B)

Panel F: γ-Tub.(R), TGN38(G), DAPI(B), Ac-α-Tub.(W)

Panel G

A

Nucleus
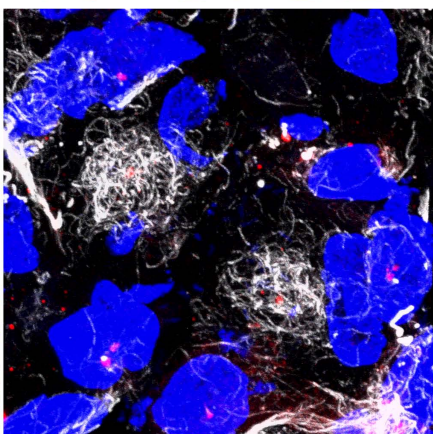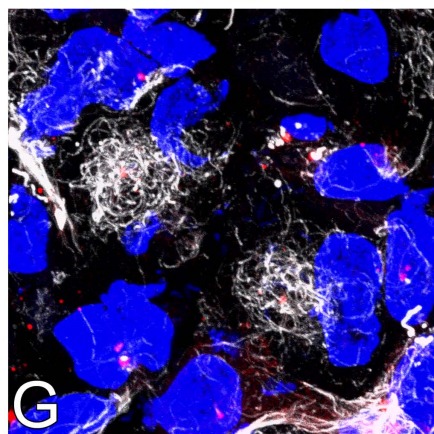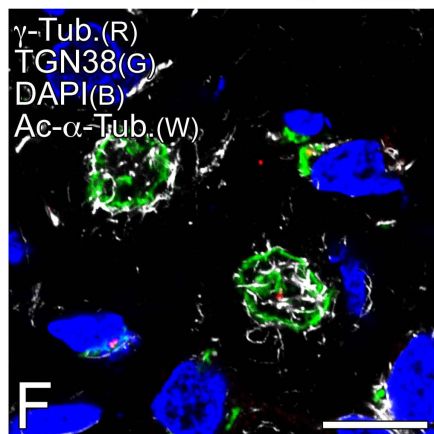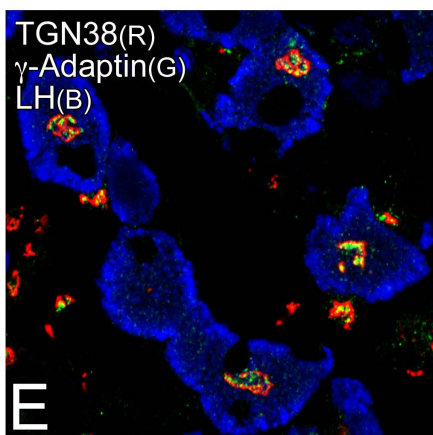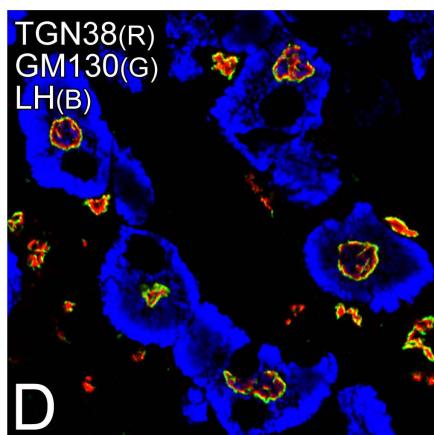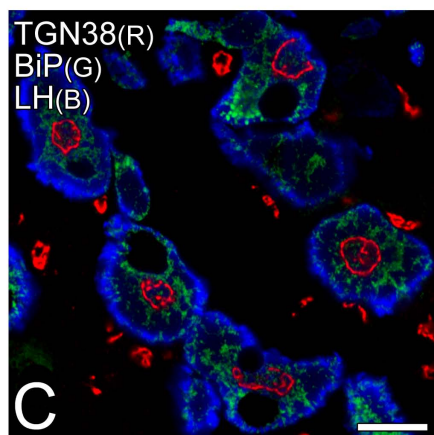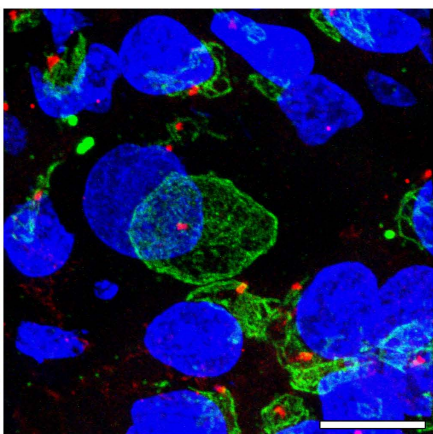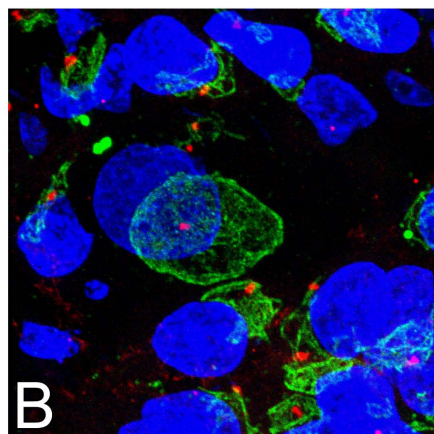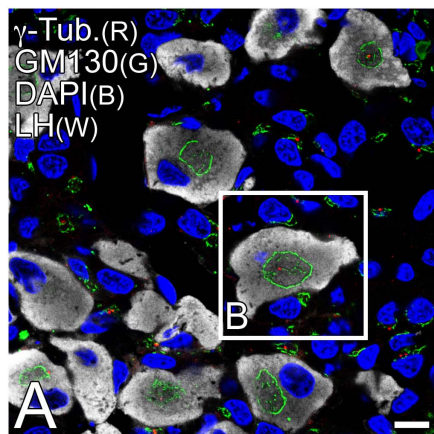
⊕— ⊖ microtubule

⊖ minus end of microtubule
⊕ plus end of microtubule

dynein-dependent movement
kinesin/KIFs -dependent movement

MTOC / centrosome (γ tubulin positive)

cis   trans

Golgi apparatus

rER: rough endoplasmic reticulum

flow of secretory proteins in the Golgi

B

membrane traffic
in the pre-Golgi compartment

COP I vesicle
COP II vesicle

C

secretory granule
primary lysosome
endosome

membrane traffic
in the post-Golgi compartment
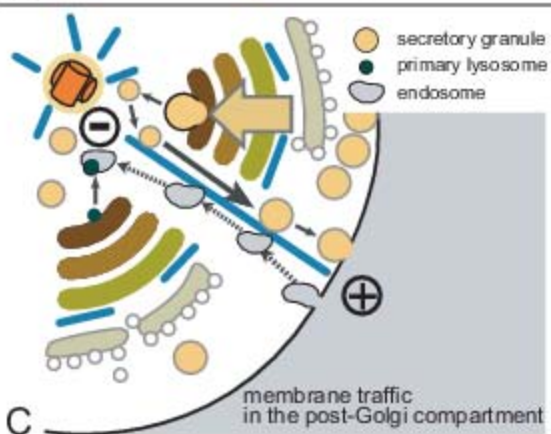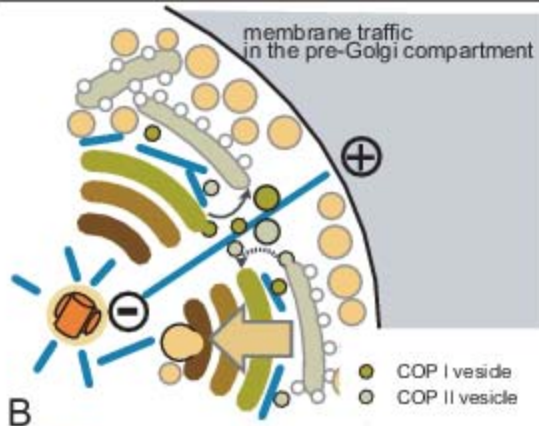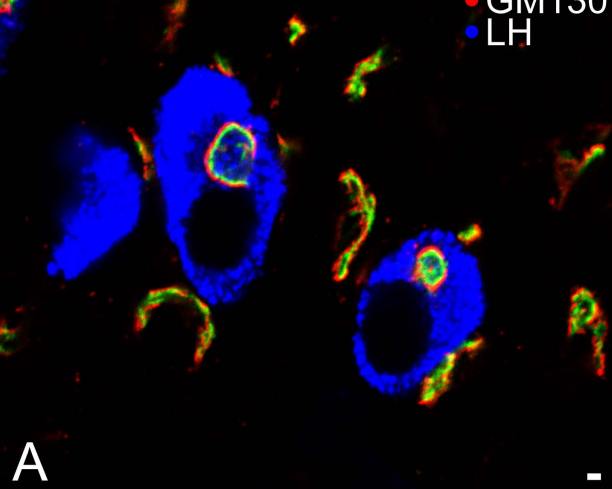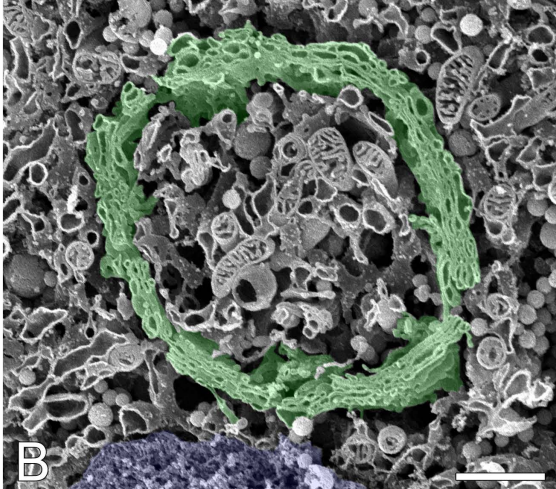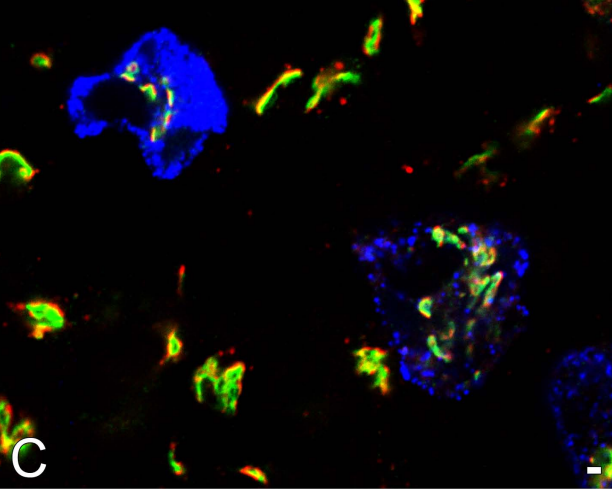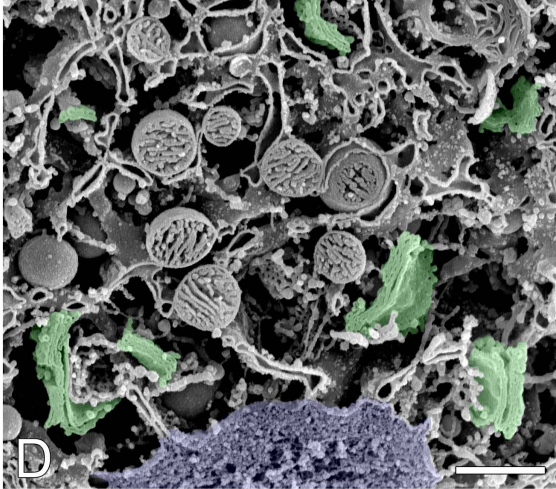
poorly polarized endocrine cell
(mammotrope / somatotrope)

Cap-like shape Golgi
on the nucleus
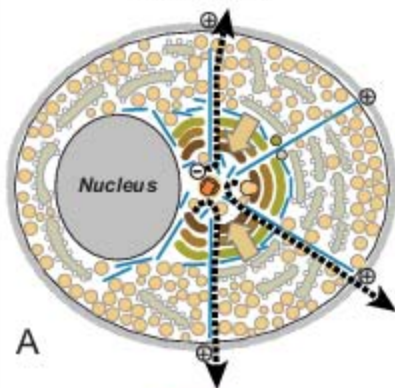
poorly polarized endocrine cell
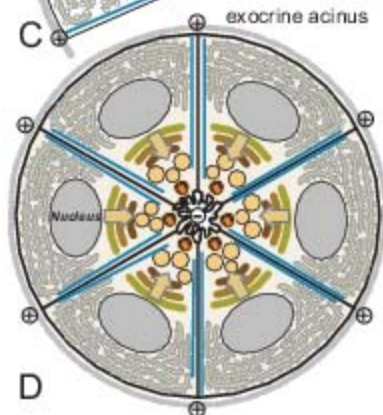(gonadotrope / thyrotrope)

Ball-like shape Golgi
at the center
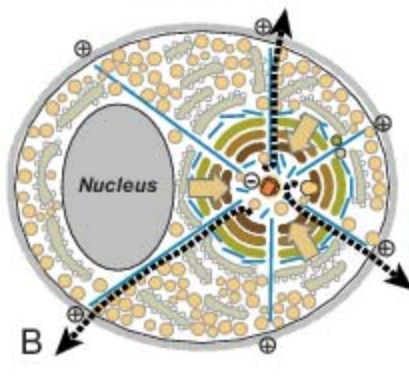
polarized exocrine acinar cell
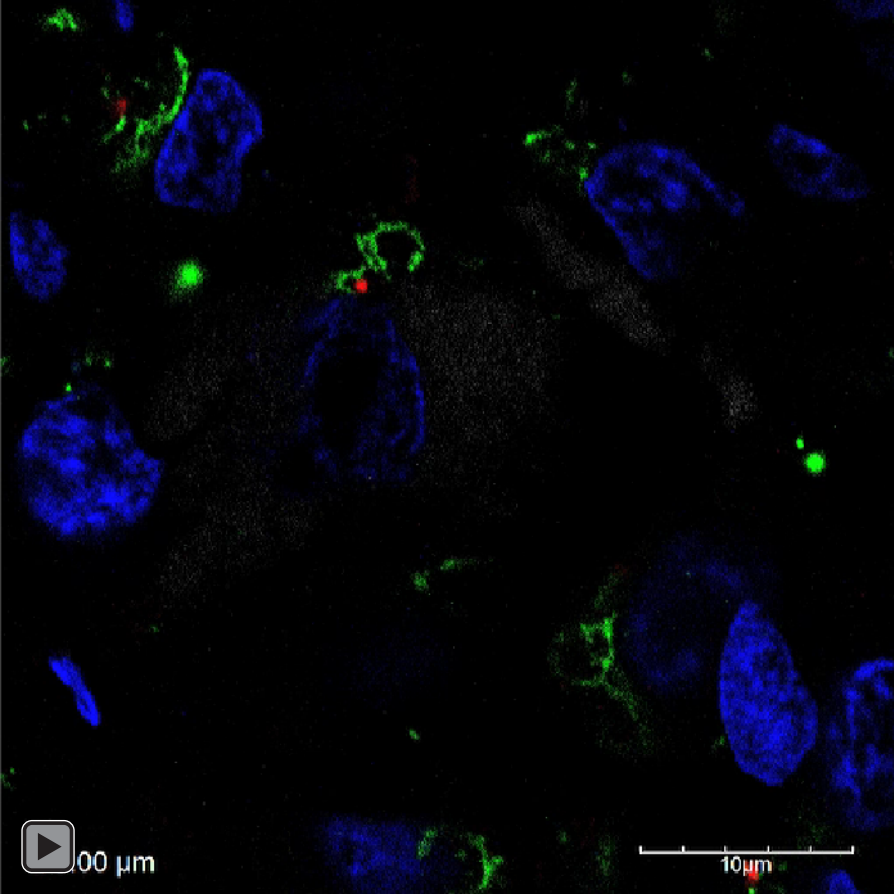
Cup-like shape Golgi
open to the apical membrane

Basal

Apical

Nucleus

Nucleus

Nucleus

A

B

C

exocrine acinus

Nucleus

D
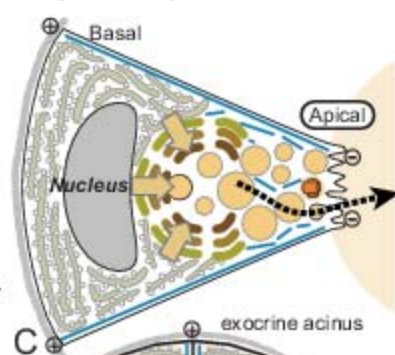
⊕ —— ⊖   microtubule

MTOC / centrosome (γtubulin positive)

rER: rough endoplasmic reticulum

cis   trans   Golgi apparatus

secretory granule

flow of secretory proteins from the rER

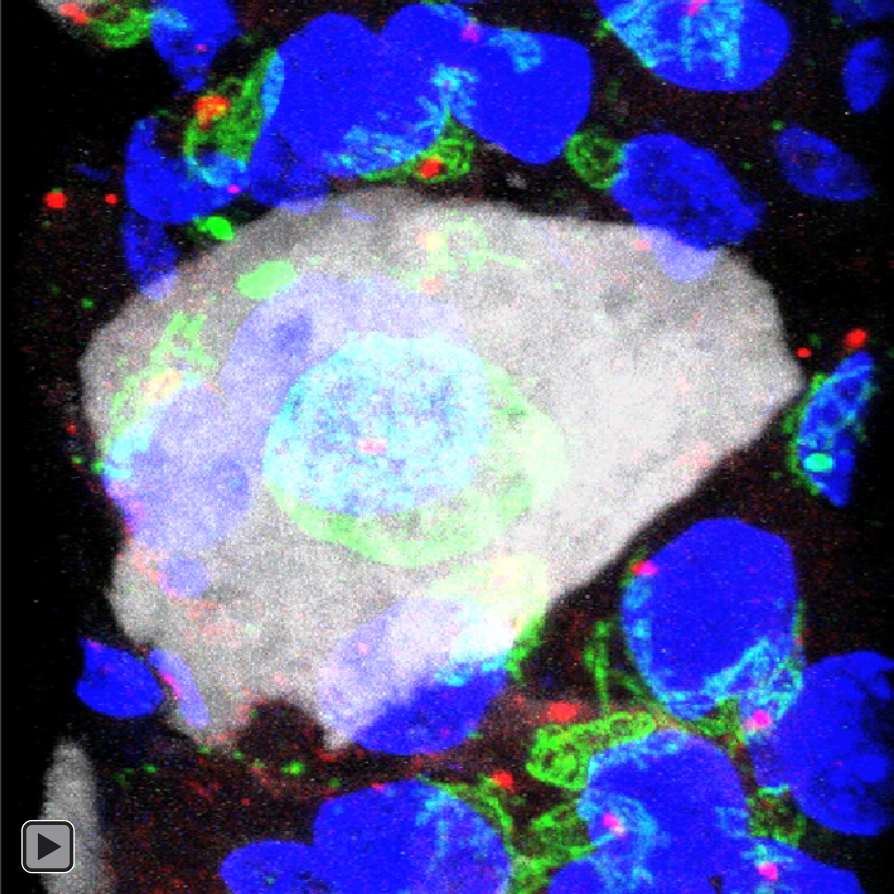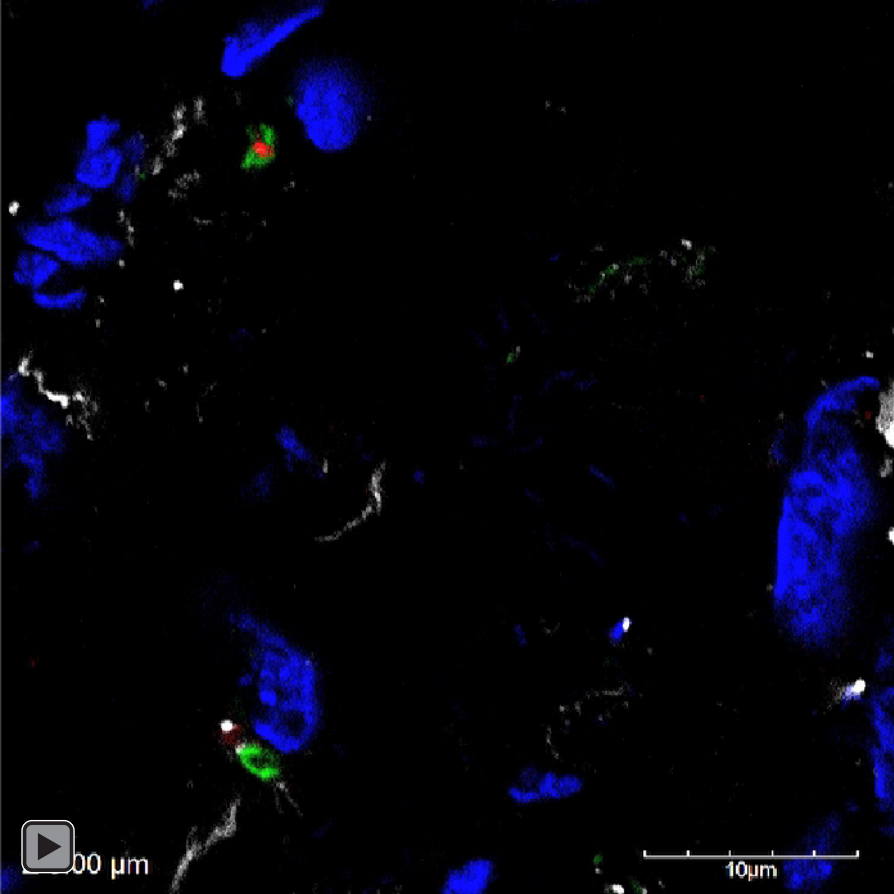flow of secretory proteins from the trans-Golgi network

basal lamina

100 µm

10µm