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Modular organization of the mammalian Golgi apparatus

Nobuhiro Nakamura², Jen-Hsuan Wei¹, and Joachim Seemann¹

¹Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

²Department of Molecular Biosciences, Faculty of Life Sciences, Kyoto Sangyo University, Motoyama, Kamigamo, Kita, Kyoto 603-8555, Japan

Abstract

The Golgi apparatus is essential for post-translational modifications and sorting of proteins in the secretory pathway. In addition, it further performs a broad range of specialized functions. This functional diversity is achieved by combining basic morphological modules of cisternae into higher ordered structures. Linking cisternae into stacks that are further connected through tubules into a continuous Golgi ribbon greatly increases its efficiency and expands its repertoire of functions. During cell division, the different modules of the Golgi are inherited by different mechanisms to maintain its functional and morphological composition.

To cope with the increasing size and complexity during evolution, eukaryotic cells organize their cytoplasm into multiple compartments surrounded by membranes. Localizing distinct sets of biological activities to these membrane-bound organelles increases the efficiency and specificity in processing substrates. This approach is best exemplified in the case of the Golgi apparatus. The Golgi accommodates a variety of enzymes with diverse activities including glycosyltransferases, kinases and proteases, and thus acts as a reaction chamber for post-translational modifications. Furthermore, the Golgi also serves as a central sorting station in the secretory pathway, where it receives the output from the endoplasmic reticulum (ER) and allocates the modified proteins and lipids to their final destinations such as lysosomes, secretory granules and the plasma membrane [1]. The Golgi further participates in many other biological processes that are not directly related to post-translational modifications or secretion. These activities include a broad spectrum of cellular events such as intracellular signaling, cytoskeletal organization, cell polarization, cell migration, mitosis, apoptosis, and autophagy [2–5]. So how can the Golgi regulate such a variety of cellular processes? To understand the basis behind it, much attention has been focused on the morphological organization of the Golgi.

Modular composition of the mammalian Golgi apparatus

Early electron microscopy studies described the Golgi as a well-organized structure with similar appearance in different cell types. Three morphologically distinct components were identified: a series of stacked cisternae, groups of vesicles that associate with the cisternae,

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Corresponding authors: Nakamura, Nobuhiro (osaru3@cc.kyoto-su.ac.jp) and Seemann, Joachim (joachim.seemann@utsouthwestern.edu) Tel: 214-648-0317, Fax: 214-648-8694 Tel/Fax: +81 75 705 3018.

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and an electron-dense ground substance surrounding the cisternae [6,7]. Later studies established that the Golgi is not only structurally but also functionally subdivided [8,9], which raised the question whether the Golgi morphology reflects its function. Indeed, the rising complexity of the Golgi organization correlates with its expanded functionality along the evolutionary path, suggesting that Golgi architecture adapts and evolves to support advanced cellular functions. The diversity and versatility of Golgi organization and function is enabled through the combination of different basic entities, which we refer to hereafter as “modules”.

A functional module in cell biology has been described as a discrete unit that functions independently from other components [10]. The advantage of a modular assembly of organelles or biochemical pathways is that the individual components with specific purposes can be separated or combined to achieve different levels of complexity. An important feature of modular composition is that the modules retain their basic activity while obtaining additional function upon assembly. This increases the robustness of the system under conditions where the connectivity or the individual function of modules are challenged [11]. Modular organization is important for processes involving metabolic networking [12], protein synthesis, DNA replication, and mitotic spindle assembly [10]. Other than functional assembly, modular organization can also be applied to structural components. The nucleus, for instance, is compartmentalized into the nucleoplasm and different nuclear bodies that carry out distinct functions [13]. Likewise, membrane-bound organelles, in particular the Golgi, show modular components delineated by lipid membranes.

The most striking morphological feature of the Golgi is the parallel alignment of flattened, disk-shaped cisternae into stacks. Each stack of cisternae is flanked on two faces by fenestrated tubular-reticular networks, the *cis*-Golgi network (CGN) and the *trans*-Golgi network (TGN) [14]. The role of the CGN is to exchange proteins and lipids between the ER and the Golgi, whereas cargo is sorted from the TGN in distinct carriers to different cellular destinations. A typical mammalian cell generally contains four to six cisternae per stack, but the number varies among organisms and cell types [15,16]. Although morphologically indistinguishable, the cisternae within a stack differ greatly in their biochemical compositions as well as the reactions they carry out. Each cisterna fulfills certain biosynthetic tasks by forming a separate reaction compartment that hosts a unique mixture of glycosylation enzymes [17,18]. While moving through the different Golgi cisternae, cargo molecules become exposed and gradually modified by the sequential actions of the glycosylation enzymes.

The modular assembly of cisternae into stacks is thought to increase the fidelity of the Golgi in biosynthetic pathways. Distributing different enzymes into distinct cisternae instead of combining them in a single compartment may facilitate correct and efficient post-translational modifications. Unlike the ER, the Golgi does not possess a rigorous quality control mechanism either to retain immature reaction intermediates or to eliminate incorrectly processed end products. Therefore, even when Golgi resident glycosyltransferases are mutated or inhibited, underglycosylated proteins are still secreted [19]. The localization and concentration of a particular set of enzymes in specific cisternae raises the ratio of enzyme to its substrate. This effectively increases the yield and fidelity of the glycosylation products even in the absence of a direct quality control. Moreover, since transport vesicles can only form at the rims of stacked cisternae, the surface area available for vesicle budding is much reduced upon stacking [20]. As a consequence, cisternal stacking prolongs the duration of cargo exposure to the enzymes and correspondingly increases the quality of glycan products.

On the other hand, stacking further enhances the efficiency of secretion. By packing cisternae close together, transport vesicles do not need to travel over long distances. Tethering complexes present on cisternae, such as the COG complex [21,22], CASP [23] and p115 [24], further improve the rate of transport by physically linking budding vesicles to the target cisternae. The proximity of cisternal membranes within a stack might be of particular importance for plant cells, where polarized Golgi stacks are highly motile and move on actin filaments along the cortical ER network to collect secretory cargo [25,26]. Assembling cisternae into a single stack ensures efficient cargo processing and intercisternal trafficking in plant cells while the stack is on the move. In addition, the proximity of cisternae allows the formation of tubular connections between different cisternae within a stack. In response to the increase in secretion load, tubules form across cisternae to allow fast transfer of cargo [27,28*,29]. The tubules between heterotypic cisternae are only observed in mammalian cells, exemplifying that not all advanced features made available through stacking are implemented universally in all systems.

Despite the gain of function by combining Golgi membranes into stacks, individual cisternae are fully capable of supporting basic biosynthetic functions. Stacks are the prevailing structure in nearly all eukaryotic cells. One of the few exceptions is the yeast *Saccharomyces cerevisiae* where cisternae are not arranged into stacks [30]. Instead, single cisternae with an enzyme composition corresponding to *cis*, *medial*, *trans* or TGN modules are dispersed throughout the cell [31]. Secretory cargo becomes sequentially exposed to different enzymes as the cisterna gradually changes its composition [32,33]. Since cisternae are functional no matter isolated or closely apposed, they can be regarded as the basic morphological module of the Golgi.

As the basic morphological unit, the cisternae can be further deconstructed based on the molecular properties into two different modules, including Golgi-resident enzymes and structural matrix proteins. Under physiological conditions the Golgi enzymes continuously cycle between the Golgi and the ER [34]. Although normally localized and functioning in Golgi cisternae, the enzymes remain fully active upon redistribution into the ER. For instance, S1P and S2P are two Golgi-resident proteases responsible for processing membrane-bound precursors of transcription factors. Upon stimulation, the cleaved, mature transcription factors such as SREBP, ATF6 and CREB3L1 are liberated from the membrane and then enter the nucleus to switch on genes required for cholesterol homeostasis, unfolded protein response and antiviral signaling [35]. However, activation of these transcription factors can also be achieved without activation signals through relocating S1P and S2P into the ER with the fungal metabolite Brefeldin A (BFA), indicating that the activity of enzymes is independent of their localization [36,37]. Similarly, Golgi resident oligosaccharide-modifying enzymes also function in the ER where they remain active and rapidly process glycoproteins [38,39]. Therefore, enzymes represent a functional module of the Golgi cisternae.

In contrast to enzymes, Golgi structural proteins do not cycle through the ER [40]. These proteins form a dynamic proteinous matrix that contains members of the golgin and GRASP (Golgi Re-Assembly Stacking Protein) families [41,42]. The matrix was first described in early EM studies as a ground substance in which the Golgi membranes are embedded [7]. It is sufficient to form a ribbon-like structure resembling the typical Golgi in the perinuclear region, even in the absence of enzymes or proper organization of cisternae [40]. The matrix also recruits glycosylation enzymes *in vitro* [43], which may play an important role in Golgi biogenesis. When the Golgi is removed from the cell by laser microsurgery, a functional Golgi reforms after 12 hours. Interestingly, matrix proteins are detected prior to the reappearance of enzymes and knock-down of the matrix protein GM130 significantly delays the restoration of a functional Golgi [44**]. These findings indicate that the matrix lays the

ground for recruiting enzymes to the Golgi, and hence can be viewed as the basic module that confers Golgi its identity.

Matrix modules can also function when located outside the normal Golgi. In the follicular epithelium of *Drosophila melanogaster*, for example, a pool of the Golgi protein GRASP is localized to the plasma membrane where it participates in unconventional secretion [45]. During the remodeling of the epithelium, integrin alpha subunits are made in the ER and reach the plasma membrane even when transport through the Golgi is impaired. Strikingly, this bypass of the canonical Golgi route depends on GRASP, as the integrin fails to be delivered to the correct location after GRASP-knockdown. GRASP has also been implicated in unconventional secretion of other cargo such as acyl-CoA binding protein in *Dictyostelium discoideum* [46] and *Pichia pastoris* [47], as well as the delivery of cystic fibrosis transmembrane conductance regulator mutant protein ($\Delta F508$ -CFTR) to the cell surface [48*]. Mechanistically, the role of GRASP in the bypass of the Golgi remains to be determined. However, unconventional secretion of the cargo still requires the Golgi component GRASP. It is possible that other Golgi modules dislocated from the Golgi also participate. In this scenario Golgi elements form a functionally coupled unit without the assembly of morphologically detectable cisternae or stacks.

Formation of the Golgi ribbon

Combining the modules of stacks into a higher-organized continuous ribbon further broadens the repertoire of Golgi functions in vertebrates. While several simple unicellular eukaryotes carry only a single stack, most organisms have multiple copies of stacks that are dispersed throughout the cell [49]. Unique to vertebrates, the stacks are interconnected by tubular reticular structures into a twisted ribbon-like structure in the perinuclear area next to the centrosomes [14,50]. The restricted juxtanuclear localization plays an important role in the establishment of cell polarity in vertebrates. The polarized localization of the ribbon facilitates the directional delivery of vesicles to a restricted area of the plasma membrane, which is important for the outgrowth of dendrites in neurons [51,52], fibroblast migration during wound healing [53–55], and the immune response [56].

Ribbon biogenesis depends on microtubules to gather stacks (Figure 1). This initial step is then followed by tethering and homotypic fusion of the stacks into a continuum in the perinuclear region of the cell. Essential for the peri-centriolar positioning and maintenance of the Golgi ribbon is the polarized array of microtubule filaments emanating from the centrosomes, the major microtubule-organizing center (MTOC). The Golgi stack itself also forms an MTOC that locally polymerizes and organizes microtubules [57]. Recent studies showed that microtubule arrays derived from both MTOCs coordinately position and organize the ribbon. Depolymerization of microtubules with nocodazole breaks the ribbon into individual stacks that are distributed throughout the cell. Upon washout, Golgi-derived microtubules gather stacks locally in the cell periphery, which are then transported along centrosome-organized microtubules towards the peri-centrosomal area [58,59]. Interfering with either stage causes defects in ribbon assembly, which is concomitant with altered polarized secretion and directional cell migration [60**].

After the stacks are gathered around the centrosomes, they are laterally tethered together. The tethering of stacks depends on the peripheral Golgi proteins GRASP65 and GRASP55. GRASP65 and GRASP55 were first shown to be required for stacking of heterotypic cisternae [61,62], as double knockdown results in a complete loss of stacks [63**]. In addition, individual depletion of GRASP65 or GRASP55 reduces the lateral connections between stacks, suggesting that both proteins provide an initial link in ribbon formation and

maintenance [64,65]. GM130 appears to cooperate with GRASP65 and/or GRASP55 because RNAi of GM130 induces a similar Golgi ribbon fragmentation phenotype [65,66].

Tubules interconnect stacks into the ribbon

Once the stacks are positioned in close proximity, homotypic cisternae of neighboring stacks are laterally fused by tubuloreticular structures into a continuous ribbon [14,50] (Figure 1). The mechanisms leading to this lateral fusion remain largely uncharacterized, but recent analyses indicate that tubules emanating from the Golgi play a key part. Lysophospholipid phatidic acid generated by phospholipase A2 (PLA2) activates tubulation at the Golgi [67]. It is postulated that lysophospholipid induces membrane protrusion causing local negative membrane curvature [68]. PLA2 activity in tubulation is antagonized by lysophospholipid acyltransferases (LPATs) including the lysophosphatidic acid acyltransferase 3 (LPAAT3) [69]. Tubules emanating from the Golgi appear to have two opposing roles. Production of tubules by inhibiting LPATs fragments the Golgi, probably through shedding and tearing of Golgi material by the tubules [70,71]. On the other hand, inhibition of tubulation by reducing PLA2 activity also disrupts the ribbon [72**]. This suggests that under physiological condition tubulation is finely controlled to sustain the ribbon.

The mechanisms that generate tubules are less understood. BFA triggers the formation of tubules from the Golgi that are extended on microtubules toward the cell periphery [73]. The primary targets for BFA are ARF GEFs including GBF1 and BIGs and their inhibition dissociates ARF1 and the COPI coat from the Golgi [74]. COPI dissociation could expose otherwise hidden components required for tubulation and fusion [75], which however remain to be identified. On the other hand, the COPI coat also induces tubules from the Golgi in a PLA2-dependent manner [76]. As BFA tubulation does not rely on COPI, COPI-dependent and COPI-independent mechanisms coexist to regulate tubulation at the Golgi.

How Golgi membranes are pulled into tubules also remains obscure, but molecular motors including kinesin and myosin are good candidates. Indeed, BFA-dependent tubulation is inhibited by overexpression of an inactive mutant of the kinesin motor KIF1C [77]. However, knockout of KIF1C in mice does not affect the Golgi ribbon or BFA-triggered tubulation, suggesting that perhaps other kinesin motors are also involved [78]. In accordance, the Golgi nucleates microtubules that locally gather stacks [57,59]. It is therefore likely that kinesin motors extend tubules along Golgi-derived microtubules to fuse stacks into a ribbon.

Actin filaments may also contribute to tubulation. Golgi phosphoprotein 3 (GOLPH3) binds to PtdIns(4)P-rich *trans*-Golgi membranes and the myosin MYO18A, thus providing pulling force for efficient tubule formation [79**]. Furthermore, the Golgi protein WHAMM binds to microtubules and promotes tubule elongation through stimulation of Arp2/3-mediated actin polymerization [80*]. Lysophosphatidic acid activates the formin mDia through Rho GTPase, thereby inducing actin polymerization around the Golgi. This, together with myosin II activity, unlinks the ribbon [81*]. As lysophospholipid itself induces tubules [67], the activation of the Rho pathway might link membrane buds formed by the lysophospholipid action to myosin and the buds are pulled to form tubules. Tubules clearly play a key role in bridging stacks together, but it remains unknown how the tubules fuse between homotypic cisternae of linked stacks to generate a continuous ribbon.

Mitotic division of Golgi modules

When cells go through mitosis, the ribbon, the most complex assembly of Golgi modules, needs to be segregated into the daughter cells. Recent evidence indicates that the Golgi is actively passed on to the progeny. In principle, such an inheritance mechanism may not be

absolutely necessary, as the Golgi has the capacity to form *de novo* [82,83]. However, the process of *de novo* Golgi reformation is considerably slow [44**] and cannot meet the vital needs for secretion. Immediately following mitosis, secretion is of critical importance for the completion of cytokinesis [84,85], which requires rapid reformation of a functional Golgi. Conceivably, once the Golgi is present, active inheritance takes the predominant role over other biogenesis strategies by offering great advantages of increased reassembly efficiency. As a comparable case, centrosomes can also form *de novo*, but in reality are inherited by the spindle [86,87]. Centrosomes are not essential for cell division, which has been demonstrated in the centrosome-depleted cells where microtubules together with other cellular components can self-organize into a bipolar spindle [88]. Nevertheless, undoubtedly the emergence of centrosomes maximizes the fidelity and speed of spindle assembly. In developing mutant flies that lost their centrosomes, spindles do form, but division is slow and error-prone [89]. In analogy, an active division mechanism is the most efficient way to rapidly re-establish a functional Golgi after mitosis.

To achieve division, the mammalian Golgi ribbon is disassembled in early mitosis into a collection of vesicular and tubular structures. The mitotic Golgi membranes are then partitioned into the daughter cells where a ribbon is reformed in telophase. As the first step in late G2 phase, the lateral tubular connections between the stacks are severed. This unlinking of the ribbon is critical for the entry into mitosis and requires the Golgi stacking proteins GRASP65 and GRASP55 [90–93]. Although the underlying mechanisms remain largely unclear, it suggests that the proteins that maintain the ribbon during interphase may also be in control for the initiation of the disassembly process.

The modular composition of the Golgi is also reflected by its modular partitioning. Once disassembled, the mitotic Golgi membranes are present in at least two different pools. Parts of the membranes are evenly dispersed as individual vesicular structures throughout the cytoplasm [94,95]. Although these dispersed membranes are sufficient to reform functional stacks in the daughter cells, they lack the factors required for ribbon formation [96]. The second pool of mitotic Golgi membranes concentrates around the spindle poles, indicating a role for the spindle in Golgi partitioning [97]. Indeed, the spindle transmits the Golgi factor(s) required for the reassembly of an interconnected ribbon into the daughter cells. Intriguingly, the clustering of Golgi at the spindle poles is only prominent in organisms that also assemble a ribbon during interphase [49], further supporting the conclusion that the factors to assemble a ribbon are actively inherited. More importantly, it exemplifies that during mitosis different modules of the Golgi are inherited by distinct mechanisms. The current challenge is to uncover the various mechanisms that partition the different modules of the Golgi in order to maintain the higher ordered functionality achieved through its modular organization. Finally, it will be of importance to determine the role of Golgi modules in the regulation of mitosis itself.

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Figure 1.

Steps of the Golgi ribbon assembly. **(a)** Microtubules (red) derived from the Golgi (blue) gather stacks in the cell periphery, which are then moved along centrosome-organized microtubules to the perinuclear area of the cell. **(b)** Tethering proteins further bridge the clustered stacks. **(c)** and **(d)** In the final steps, homotypic cisternae of neighboring stacks are fused by tubular structures elongating on microtubules (red) and/or actin filaments (green) into a continuous ribbon.