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Molecular basis for Golgi maintenance and biogenesis

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The Golgi apparatus contains thousands of different types of integral and peripheral membrane proteins, perhaps more than any other intracellular organelle. To understand these proteins' roles in Golgi function and in broader cellular processes, it is useful to categorize them according to their contribution to Golgi creation and maintenance. This is because all of the Golgi's functions derive from its ability to maintain steady-state pools of particular proteins and lipids, which in turn relies on the Golgi's dynamic character — that is, its ongoing state of transformation and outgrowth from the endoplasmic reticulum. Here, we categorize the expanding list of Golgi-associated proteins on the basis of their role in Golgi reformation after the Golgi has been disassembled. Information gained on how different proteins participate in this process can provide important insights for understanding the Golgi's global functions within cells.

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Abbreviations
ER endoplasmic reticulum
ERGIC ER–Golgi intermediate carrier

Introduction

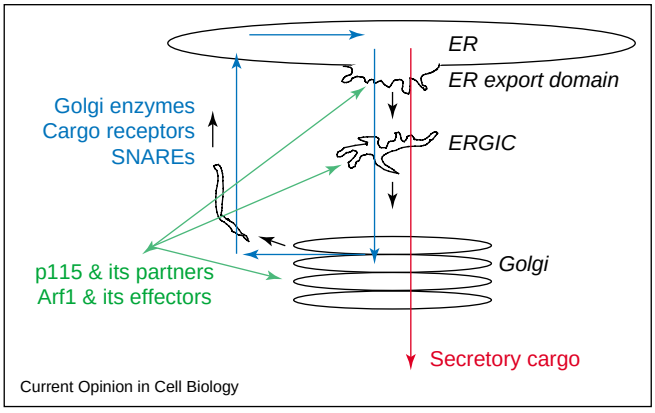
The Golgi apparatus performs three major functions essential for growth, homeostasis and division of eukaryotic cells. First, it operates as a carbohydrate factory for the processing and modification of proteins and lipids moving through the secretory pathway [1]. Second, it serves as a station for protein sorting and transport, receiving membrane from the ER and delivering it to the plasma membrane or other intracellular sites [2]. Finally, it acts as a membrane scaffold onto which diverse signaling, sorting and cytoskeleton proteins adhere [3,4,5••].

These distinct Golgi functions operate within a structure that is unique among subcellular organelles in many ways, including its composition as a stacked array of cisternae and connecting tubules/vesicles, its enormous diversity of protein components (>1000 different types) [6], and its unrivaled capacity to dynamically transform in response to specific stimuli or other cellular changes. Examples of the Golgi's dynamic behavior include its reversible disassembly during mitosis and under experimentally induced conditions (e.g. osmotic stress or treatment with BFA, Exo1 or ilimaquinone [7–10]), and its rebuilding at peripheral ER export sites in response to microtubule disruption or expression of mutated proteins that function in ER-to-Golgi trafficking [11,12].

The Golgi's ability to transform itself fundamentally under different conditions is probably due to the fact that proteins only associate with it transiently as they move through other pathways in the cell. Conditions that alter the entry or return of these proteins to the Golgi, therefore, will disrupt Golgi structure and function. Also, many proteins associated with the Golgi are part of large protein complexes [13]. Altering the association of one protein in the complex may affect the stability and localization of others, with downstream consequences for Golgi organization and structure.

That no class of Golgi protein is stably associated with the Golgi has been demonstrated in GFP-based imaging studies examining the trafficking itineraries and residency times of proteins localized in the Golgi (see Figure 1 for a summary of these results). These studies have shown that integral membrane proteins associated with the Golgi, including processing enzymes (i.e. mannosidase II, galactosyltransferase, etc), SNAREs and secretory cargo receptors (i.e. ERGIC53, p24 proteins and KDEL receptors), are continuously exiting and reentering the Golgi by membrane trafficking pathways leading to and from the ER [12,14,15]. Peripheral membrane proteins associated with the Golgi (including the small GTPase Arf1 and its effectors [phosphotidylinositol kinases, lipases, signaling kinases], as well as coatomer, p115 and GRASPs), by contrast, exchange constantly between membrane and cytosolic pools [5••,12,16]. Newly synthesized cargo proteins passing through the Golgi to other destinations (which include both integral membrane and luminal proteins) also spend relatively short periods of time in the Golgi [17,18••]. The residency times for these different classes of Golgi proteins as measured by photobleaching varies enormously: Golgi processing enzymes stay for ~60 min, cargo proteins ~30 min, cargo receptors ~10 min and peripheral proteins ~1 min [12,16].

Figure 1



Trafficking itineraries of proteins associated with the Golgi apparatus. All proteins that associate with the Golgi do so only transiently before moving to other destinations in the cell. Integral membrane and luminal proteins enter the Golgi via transport intermediates formed from ER export sites. Among those are cargo proteins (red) that pass through the Golgi to the cell surface or other cellular destinations and Golgi resident components (blue)(including Golgi enzymes, cargo receptors and SNAREs specific to the ER-Golgi system) which return to the ER through retrograde transport intermediates. Cytoplasmic proteins associated with the Golgi (green) (including Arf1 and its effectors, p115 and its binding partners) continuously bind to and dissociate from ER export domains, ERGIC or Golgi membranes.

Why are proteins only transiently associated with the Golgi? A possible explanation is that the Golgi apparatus may not be a conventional organelle in the sense of being an autonomous entity comprised of stable components. Rather, it appears to function as a steady-state membrane structure that undergoes continuous outgrowth from and reconsumption by the ER through the formation of anterograde and retrograde transport intermediates. Hence, when ER export is specifically inhibited, Golgi membrane components undergoing normal retrograde transport back to the ER are trapped in the ER or at ER export domains, and no Golgi structure persists [12,19–21]. After the Golgi has been dispersed in this manner, it can readily reform when normal ER export activities resume [7,22••,23•].

This steady-state view of the Golgi means that Golgi maintenance and biogenesis are inextricably linked. Peripheral and integral membrane proteins with roles in Golgi maintenance will also be involved in Golgi biogenesis from the ER. Accordingly, one way of making sense of the vast array of proteins associated with the Golgi apparatus is to look at their role in the reformation of the Golgi after it has disassembled. As discussed below, analyzing the function and activity of proteins associated with the Golgi in this manner provides a useful framework for understanding Golgi organization and its broader role in the life cycle of the cell.

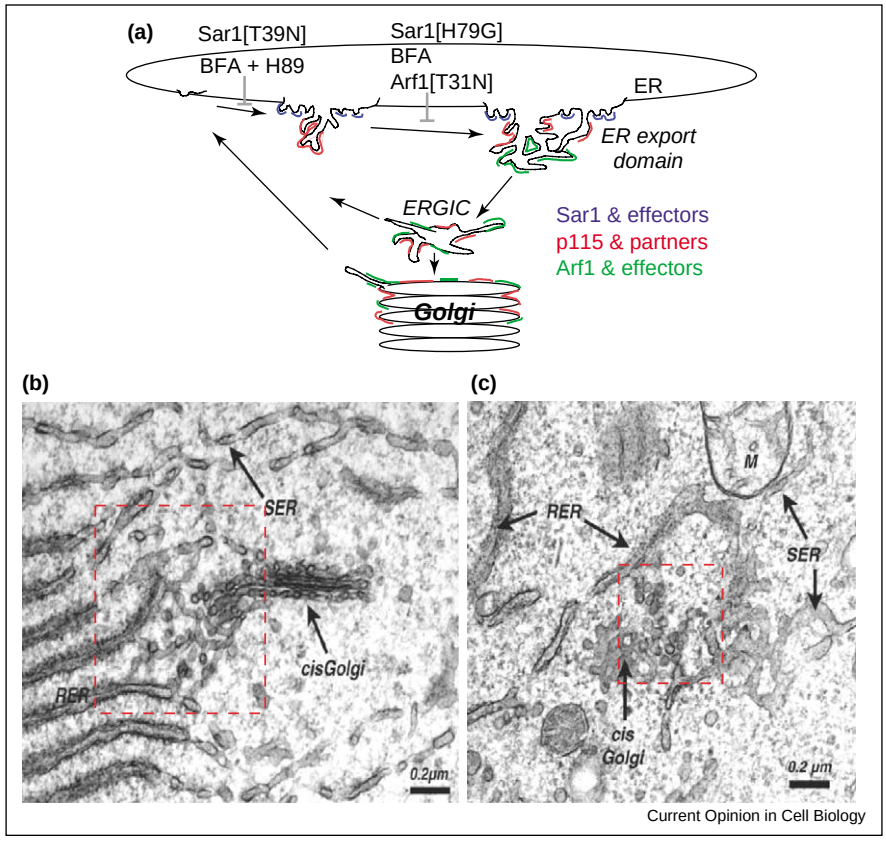
Conditions of Golgi disassembly

To understand the Golgi reformation process, it is helpful to begin with the conditions that cause the Golgi to disassemble and to see how this affects the distribution of steady-state Golgi components (see Figure 2a for these conditions and their effects). The most complete disassembly of the Golgi occurs when the small GTPase known as Sar1 is unable to bind GTP (e.g. in cells expressing the persistently inactive mutant Sar1 [T39N]) [12,19,20]. The steady-state Golgi model explains this effect on the basis of Sar1's role in the generation of ER export sites (micron-sized domains of coated buds and vesicles/tubules that export protein and lipid out of the ER) (Figure 2b,c). Through its dynamic GTP binding and release cycle, Sar1 is able to recruit effector proteins, including COPII coat proteins, to ER membranes and thereby to differentiate and maintain ER export domains [12,24,25]. Because Golgi membrane proteins cycling through the ER travel through these domains to return to the Golgi, interference with normal ER export site distribution or behavior will affect the distribution of the Golgi proteins. Thus, when cells express the GDP-restricted form of Sar1 (Sar1 [T39N]) and ER export sites disappear, Golgi integral membrane proteins are trapped in the ER and Golgi peripheral proteins are retargeted to the ER or cytoplasm [12,20,21]. The net result is the disappearance of any Golgi or Golgi-like structure within cells.

Golgi reassembly as a multi-step process

Reversing conditions in which no Golgi or Golgi-like structures exist within a cell has facilitated the study of Golgi reassembly. Recently, for example, Puri and Linstedt [22••] used a reversible ER export block involving the sequential treatment of cells with BFA and then H89. Treatment of cells with this protocol mimicked the effect of Sar1 [T39N] expression, with Golgi proteins redistributing into either the ER or cytoplasm and no Golgi template or scaffold remaining. Strikingly, when

Figure 2



ER to Golgi transition. **(a)** Effects of mutations in Sar1 and Arf1, as well as the drugs BFA and H89 on assembly of ER export domains. **(b,c)** Ultra-structure of endoplasmic reticulum and Golgi apparatus in COS-7 cells. Note the fenestration of the cis-Golgi in (c) and the continuity of membranes between the smooth ER and the Golgi in both (b) and (c) within these cells. The dashed red box indicates the complex organization of an ER export domain.

the drugs were washed out, the Golgi readily reformed by outgrowth from the ER.

After washout, different Golgi proteins emerged from the ER at different rates, with GM130 being faster than giantin, which was faster than mannosidase II. As this emergence requires that these proteins be recruited first to ER export domains, the sequential emergence may reflect specific roles of these and other proteins in differentiating ER export domains to produce Golgi structures. In this scenario, localized pools of specific lipid and protein species produced at ER export domains by Sar1 activity (e.g. COPII binding and dissociation) would stimulate peripheral proteins to bind to, and Golgi membrane proteins to sort into, these sites. The ensuing differentiation of ER export domains would then lead to recruitment of additional peripheral proteins, causing Golgi enzymes and secretory cargo proteins within the ER to undergo sorting into these domains. The domains would grow larger and inevitably detach from the ER as globular-tubule elements (i.e. ERGIC: endoplasmic reticulum–Golgi intermediate carriers). Clustering and fusion of these elements would then give rise to the Golgi apparatus.

This multi-step process of Golgi reassembly would presumably involve differential regulation at each step. The regulatory machinery involved may intersect and/or act at multiple steps, but each step would be dependent on the success of the prior step. For example, differentiation and functioning of ER export domains would precede the formation of ERGIC that emerge from these sites. Recent work in cells expressing the Sar1 [H79G] mutant, in which Sar1 is able to bind GTP but cannot efficiently hydrolyze it, supports this multi-step view [12,20]. In these cells, ER export domains form in the same way and have a similar overall ultrastructure as in control cells [12], with COPII coat proteins localized to these sites. However no Golgi apparatus is observed and protein secretion is inhibited. This suggests that Sar1 must engage in an ongoing process of GTP binding and hydrolysis in order to orchestrate the protein machinery involved in the loading of cargo into ER export sites and the differentiation of ER

export sites into ERGIC (see Figure 2a). Whether ERGIC formation occurs by fusion of vesicles budding out from ER exit sites (as is commonly thought) or by direct maturation of an ER exit site into an ERGIC is unclear ([18••], see also figure 2b and c), but without Sar1 GTP binding and hydrolysis this process does not occur.

Determining what proteins associate with ER export domains in Sar1 [H79G]-expressing cells therefore may provide clues to how these sites become differentiated.

Several types of Golgi proteins are localized to ER export sites, including p115, so-called 'matrix proteins' having long coiled-coil domains (e.g. GM130 and GRASP65) and cargo receptors (e.g. KDELR, ERGIC53 and p24 proteins) [12,20,26]. This led to the initial view that they were persistent elements of the Golgi apparatus unrelated to the ER [26], but their co-localization with ER export site markers (including markers for COPII components) and morphological appearance soon revealed they were ER export domains [12,20]. Within these export domains, the Golgi proteins showed dynamic behavior. Photobleaching experiments revealed that GRASP65 (as well as the COPII protein, Sec13) underwent rapid binding to and dissociation from the export domains, whereas ERGIC53 moved continuously in and out of the domains from surrounding ER membranes [12]. These findings raise the possibility that Golgi matrix proteins and cargo receptors have roles in the biogenesis of ER export sites.

Sar1-based biogenesis of ER export domains and their differentiation by p115 and its interacting partners

The formation of ER export domains is a complex process involving Sar1-mediated COPII coat recruitment onto the ER, coat polymerization into coated buds and the elaboration of tubular elements [25,27,28]. The clustered, COPII-coated ER membrane profiles seen in many electron micrograph images represent only a small part of an ER export domain, which has a diameter of 0.5–1μm and significant overall surface area [29] (Figure 2b,c). Importantly, the COPII-coated budding zones within ER export domains are not the only places where protein export into the secretory pathway occurs [18••,30,31]. Recent studies have shown that cargo (such as procollagen) [18••] are exported by a process of tubule outgrowth from non-coated regions of ER export domains. Given these characteristics of ER export domains it is likely that other proteins in addition to Sar1 and its immediate regulators/effectors (e.g. COPII proteins) are necessary for the formation and functioning of these sites.

As mentioned before, peripheral Golgi proteins and cargo receptors localize to ER export sites, both at steady-state and when Sar1 is in a persistently GTP-bound state; these proteins could play a role in the formation and/or function of ER export domains. The best protein candidate for orchestrating such a role is p115. Comprised of several protein-binding domains, p115 interacts with a variety of proteins with known roles in ER-to-Golgi transport, including other coiled-coil domain proteins (i.e. GM130, giantin and GRASP65), SNARE proteins (i.e. syntaxin 5, Bet1 protein and Sly1 protein), Rab1 GTPase and GBF1 (the exchange factor for Arf1 GTPase) [32–36,37••,38,39•]. Once recruited to membranes at ER export sites, p115 could initiate a cascade of regulatory interactions among these proteins that would lead to Golgi biogenesis and maintenance. The interaction of p115 with SNARE proteins, for example, may allow membranes budding out from ER export domains to fuse homotypically [39•], while its interactions with GM130 and giantin could drive tethering interactions between membranes and the cytoskeleton [40,41]. Interactions of p115 with GRASPs, on the other hand, may facilitate sorting of p24 cargo receptors into forward-directed transport intermediates [38], while its interaction with GBF1 could modulate Arf1 [37••], whose GTPase activity underlies the recruitment of dozens of proteins to membranes [3,5••,42]. Because p115 is thought to be recruited to membranes by Rab1 and/or Rab2 [43–45], which localize not only to ER export domains but also to ERGIC and Golgi membranes [36,46], p115 and its interacting partners could act to differentiate membranes at multiple stages during the process of Golgi biogenesis.

Evidence suggesting that p115 and its interacting partners are important for the functioning of ER export domains has come from studies examining the effects of down-regulating and/or perturbing the activity of these proteins. Down-regulation of p115, GM130 (at high temperatures) or molecules associated with p115 or GM130 (including rab1b, rab2a and golgin 84) blocks all secretory trafficking to the Golgi [39•,47–51]. Moreover, a phenotype emerges that is similar to what is observed when Sar1 is persistently active: Golgi enzymes are redistributed into the ER, and p115 and matrix and/or cargo receptor proteins are located at ER export domains. Thus, unless p115 and its interacting partners are fully active, ER export domains (initiated by Sar1 activity) cannot give rise to ERGICs that translocate through the cytoplasm and fuse with each other to form Golgi elements.

Building a Golgi apparatus: role of Arf1 GTPase

What molecules, or sets of molecules, might be recruited by p115 and its interacting partners to drive Golgi biogenesis? To address this question, it is useful to examine the molecules that are not recruited to ER export domains when the Golgi is absent, such as in Sar1 [H79G]-expressing cells or in cells where p115 or rab proteins are down-regulated. These molecules include the small GTPase called Arf1, Arf1's effectors (including COPI, clathrin, ankyrin, spectrin and others), Golgi enzymes, and secretory cargo proteins [12,20,32,39•,52].

Of this list, Arf1 is the most likely candidate for coordinating the machinery required for globular-tubule transport intermediate (i.e. ERGIC) outgrowth from ER export sites, and for their clustering and fusion to generate Golgi elements. Like Sar1, Arf1 is a GTPase that exchanges its GDP for GTP. In its GTP-bound state, Arf1 is active and associates with membranes. Upon hydrolysis of its GTP to GDP, Arf1 becomes inactive and dissociates from membranes. In its active, membrane-bound form, Arf1 recruits dozens of other cytosolic proteins to ERGIC and Golgi membranes [3,4,5••,42]. Included among these are the following: COPI coat proteins, which bind to and cluster cargo receptor proteins [2,16,53,54]; lipid-modifying enzymes, such as phosphotidylinositol kinases and phospholipases [55,56], which create a lipid environment distinct from ER membranes; and ankyrin and spectrin proteins that form a scaffold onto which many cytoskeletal and signaling molecules adhere [3]. The cytoskeletal proteins recruited onto Golgi membranes by the ankyrin/spectrin meshwork include actin, tubulin, vimentin, dynein, dynamin and myosin isoforms [57–61]. Among these, the dynein/dynamin complex is thought to mediate the microtubule-dependent clustering of ERGIC [16,62], while myosin VI may stabilize Golgi membranes in the centrosomal region or help to mediate post-Golgi budding events [60].

Together, this group of Arf1-dependent proteins and their interacting partners could carry out the following functions: first, to further differentiate the lipid environment of ER export sites so Golgi enzymes and cargo can sort into these sites; second, to facilitate detachment of globular-tubule clusters (i.e. ERGIC) from the now cargo-laden regions of ER export sites; third, to mediate the clustering and fusion of these structures; and fourth, to stimulate retrieval of specific membrane components back to the ER through retrograde transport. The later process would be essential for organizing carbohydrate-processing enzymes, nucleotide–sugar transporters and proton pumps within the Golgi so that they could behave as an interacting system for rapid and efficient conversion of glycoprotein and glycolipid substrates into their products.

Evidence that supports Arf1 having a critical role in these processes comes from the finding that when the GTPase activity of Arf1 is inhibited, for example in cells expressing the persistently inactive form of Arf1, Arf1 [T31N], or in cells treated with BFA (which prevents Arf1 from binding to membranes), none of these processes occurs. Yet ER exit domains are present, together with dynamically localized COPII proteins, p115, matrix proteins and cargo receptors [12,22••,63,64••] (as diagrammed in Figure 2a).

Recruitment of Arf1 to membranes is mediated by the guanosine exchange factor GBF1, which itself undergoes dynamic binding and dissociation from membranes (Jackson and Lippincott-Schwartz, unpublished). Recently, Sztul and colleagues showed that p115 interacts with GBF1 [37••]. Thus, p115 might regulate GBF1's association with membranes and thereby indirectly control Arf1's membrane recruitment. This is consistent with the finding that during treatment of cells with BFA, which is thought to inactivate GBF, p115 continues to associate with membranes [37••]. In BFA-treated cells, p115 and Sar1 activities would allow ER export sites to form, but due to the inhibition of Arf1 the ER export sites would not give rise to cargo-enriched ERGIC (which would be capable of merging to form a Golgi).

The unique protein and lipid environment of the Golgi, maintained by Arf1's activity, is known to serve as a platform for signaling and regulating molecules with diverse functions. These include heterotrimeric G proteins, small G protein Ras, PKA, PI(3) kinase, IQGAP, eNOS, Nir2, PI4Kβ and Cdc42 (see Box 1). Other cytoplasmic proteins associated with the Golgi have roles in the nucleus and cytoplasm, including casein kinase, cyclin B2, tankyrase and Cullin family members and CtBP/BARS. Still other Golgi-associated proteins are kinases and activators of kinases; these include PKC, Myt1, and calmodulin kinases. Finally, a number of polo-like kinases, previously thought to be located on centrosomes, reside on the Golgi including Plk-3 and Sak1 [5••,65]. These proteins have roles in regulating microtubule dynamics.

Many of the activities of these signaling and regulatory molecules depend on the membrane environment of the

Box 1 Golgi-associated signaling and regulatory molecules

Heterotrimeric G proteins [4]

G(i3), Gα, G βγ

Small G proteins [4,36,42,43,53,73,75•]

Arf1, Arf3
Arl family members
Ras, Rac, Cdc42
Rab family members

Protein kinases [3,5••,55,71,76,78,79]

Phosphotidyl-inositol kinases
PKA
PKC isoforms
Casein kinase isoforms
Calmodulin kinase

Protein Kinase D

Myt1 kinase

Polo-like kinases, Plk- 3, Sak1
MEK kinases, MEKK2, MEKK4
Mammalian Ste20 kinases, YSK1 and MST4

Cytoskeletal regulatory proteins [3,4,57]

Dynein, dynamin
Myosin I, II, V, VI
Ankyrin isoforms

Spectrin isoforms
Kinesins
IQGAP, WASP/Arp2/3

Phospholipases [3,5••,55,56]

Phospholipase A_2

Phospholipase D_2

Others [3,4,5••,77•,80]

Cullin2, Cullin3
Endothelial nitric-oxide synthase (eNOS)
Phosphotidylinositol transfer protein, Nir2
Phosphatidylinositol phospholipid phosphatase, PTEN2
Tankyrase1, Tankyrase 2
CtBP/BARS

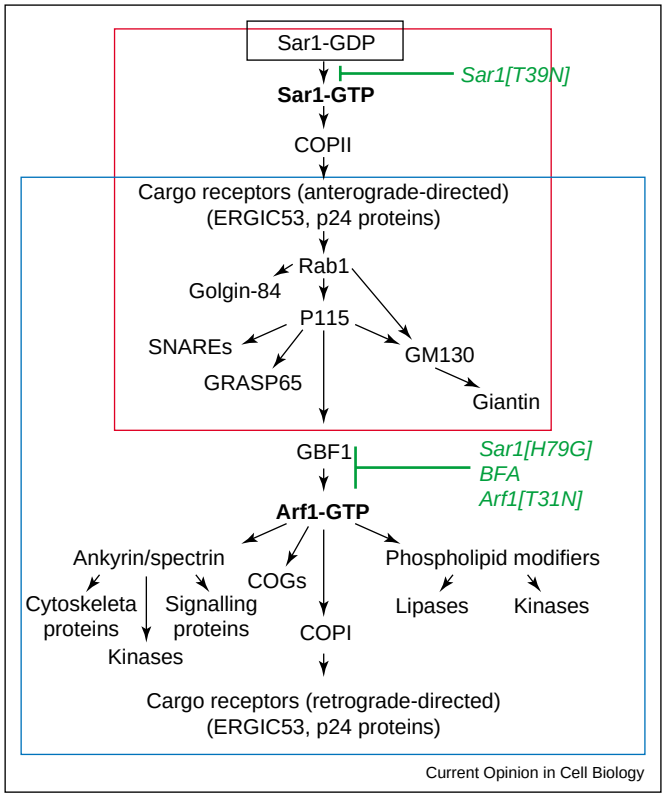
Golgi created specifically by Arf1 and its effectors. As an example, they undergo rapid dissociation from Golgi membranes when Arf1 is acutely inactivated with BFA [3,5••]. Interestingly, Arf1 is inactivated early in mitosis [5••]. The ensuing release of these signaling and regulatory proteins into the cytoplasm could potentially therefore have roles in orchestrating mitotic processes occurring in the cytoplasm. Consistent with this, when mitotic Golgi disassembly is inhibited by treatments that prevent Arf1 from becoming inactive, the Arf1-dependent Golgi peripheral proteins do not release from the Golgi and mitotic defects in chromosome segregation and furrow ingression during cytokinesis arise [5••]. This suggests that Arf1-dependent dispersal of peripheral Golgi proteins in mitosis is important for coordinating the behavior of Golgi membranes, chromosomes and the cytoskeleton during mitosis. The inactivation of Arf1 in mitosis thus provides a means for redistributing these proteins into the cytoplasm so that they can perform their mitotic functions.

Golgi formation through sequential activity of Sar1 and Arf1

The multi-step process of Golgi biogenesis described here thus appears to be orchestrated in sequence by the GTPase activities of Sar1 and Arf1 and their effectors (Figure 3). In this scheme, Sar1 GTPase activity initiates the process of Golgi biogenesis by COPII-mediated sorting of specific integral membrane proteins (e.g. ERGIC53, p24 proteins and KDEL receptor) to ER sub-domains. Clustering of these proteins results in changes in bilayer thickness and composition at these sites, leading to the recruitment and activation of molecules like Rab1, which in turn recruits p115 to these sites. The ability of P115 to interact with SNAREs and matrix proteins then causes the nascent ER export sites to differentiate into ERGIC (i.e. immature Golgi) by stimulating membrane transformation and fusion events in this local region. (Once the Golgi is formed, Rab1- and Rab2-mediated recruitment of p115 and its partners continues to stimulate membrane transformation, tethering and fusion events in Golgi membranes.)

The activity of Arf1 at ER export domains is what allows these domains to transform into dynamic transport intermediates capable of packaging diverse types of secretory cargo, sorting selected molecules back to the ER and translocating through the cytoplasm. When fused together, these intermediates comprise the Golgi. Arf1 activation may be a result of the recruitment of GBF1 by

Figure 3



Schematic organization of Golgi biogenesis. **(a)** Recruitment of proteins involved in the biogenesis of the Golgi as a steady-state system is based on the sequential GTPase activities of Sar1 and Arf1. Note that different sets of interacting protein pathways associate with different compartments :ER (black box), ER export domain (red box), ERGIC and Golgi membranes (blue box).

p115, as GBF1 is the likely guanosine exchange factor for Arf1 [37••,64••]. Once Arf1 is active on the ER export site membranes, it recruits a large number of effector proteins (including ankyrin, spectrin, COPI, signaling proteins and phospholipid-modifying proteins) that allow the ER export domain to transform first into ERGIC and then into Golgi membranes.

This process involves a functional as well as a morphological transformation of membranes since the structures comprising ER export sites and ERGIC (i.e. clusters of tubules and vesicles) are different in appearance from those comprising the Golgi (i.e. compact flattened stacks of cisternae). Determining what Golgi-associated proteins are responsible for converting Golgi membranes into flattened stacks of cisternae and how this is mediated is an active area of investigation [66,67]. An important clue comes from recent work observing the behavior of ER membranes. These membranes are capable of dramatically remodeling from a reticular network into a tight stack of cisternae (reminiscent of the Golgi and other stacked organelles) upon overexpression of ER proteins with cytoplasmic domains capable of undergoing low affinity interactions [68•]. A similar mechanism, therefore, might explain how the Golgi obtains its specific morphological shape.

While the focus of this review has been the mechanisms underlying Golgi biogenesis in interphase, Arf1 and Sar1 have been shown to be sequentially inactivated and then re-activated during mitosis [5••,69]. This raises the possibility that the activities of these GTPases are relevant to the multi-step process of mitotic Golgi reassembly [5••]. Further research is needed to address this possibility given the complexity and varying viewpoints of mitotic Golgi breakdown and reassembly [5••,70–72].

Conclusions

The Golgi apparatus is a steady-state organelle that can be assembled from the ER *de novo* in the absence of any pre-existing structure. This *de novo* assembly depends on the sequential activities of Sar1 and Arf1. Sar1 activity leads to the initial recruitment of numerous peripheral proteins to ER export domains (including Rab1, p115, SNAREs and matrix proteins) that permit these domains to differentiate into ERGIC. These structures are then further differentiated by the activity of Arf1 and its numerous effectors, producing Golgi membranes (enriched in secretory cargo and processing enzymes) that are morphologically and geographically distinct from ER. Of the many Golgi-associated proteins whose functions remain to be unraveled, several are likely to be involved in modulating the activities of Sar1, Arf1 and its effectors. Others may not be involved in Golgi biogenesis or maintenance at all, but function as a consequence of the Golgi's steady-state structure. These include the following: glycosylating enzymes; molecules with roles in exporting cargo out of the Golgi to the plasma membrane or endosomal system (e.g. Arl family [73]); proteins that integrate Golgi structure with the cytoskeleton (e.g. COGs [74]); and molecules that use Golgi membranes as a platform for regulating signaling events [55,75•,76]. A challenge for the future is to understand how all of the Golgi-associated proteins interact with each other to maintain the Golgi's complex structure and diverse cellular functions.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Farquhar MG, Palade GE: **The Golgi apparatus: 100 years of progress and controversy**. *Trends Cell Biol* 1998, **8**:2-10.

2. Rothman JE: **Mechanisms of intracellular protein transport**. *Nature* 1994, **372**:55-63.

3. De Matteis MA, Morrow JS: **Spectrin tethers and mesh in the biosynthetic pathway**. *J Cell Sci* 2000, **113**:2331-2343.

4. Donaldson JG, Lippincott-Schwartz J: **Sorting and signaling at the Golgi complex**. *Cell* 2000, **101**:693-696.

5. •• Altan-Bonnet N, Polishchuk R, Phair RD, Weigert R, Lippincott-Schwartz J: **A role for Arf1 in mitotic Golgi disassembly, chromosome segregation and cytokinesis**. *Proc Natl Acad Sci USA* 2003, **100**:13314-13319.
A connection between Golgi disassembly in mitosis and chromosome segregation and cytokinesis is revealed. Arf1 is found to be inactivated early in mitosis. This inactivation is shown to be necessary for Golgi membrane disassembly as well as for the dispersal of numerous signaling and regulatory Golgi-peripheral proteins.

6. Wu CC, Yates JR III, Neville MC, Howell KE: **Proteomic analysis of two functional states of the Golgi complex in mammary epithelial cells**. *Traffic* 2000, **1**:769-782.

7. Lippincott-Schwartz J, Yuan LC, Bonifacino JS, Klausner RD: **Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER**. *Cell* 1989, **56**:801-813.

8. Takizawa PA, Yucel JK, Veit B, Faulkner DJ, Deerinck T, Soto G, Ellisman M, Malhotra V: **Complete vesiculation of Golgi membranes and inhibition of protein transport by a novel sea sponge metabolite, ilimaquinone**. *Cell* 1993, **73**:1079-1090.

9. Lee TH, Linstedt AD: **Osmotically induced cell volume changes alter anterograde and retrograde transport, Golgi structure, and COPI dissociation**. *Mol Biol Cell* 1999, **10**:1445-1462.

10. Feng Y, Yu S, Lasell TK, Jadhav AP, Macia E, Chardin P, Melancon P, Roth M, Mitchison T, Kirchhausen T: **Exo1: a new chemical inhibitor of the exocytic pathway**. *Proc Natl Acad Sci USA* 2003, **100**:6469-6474.

11. Cole NB, Sciaky N, Marotta A, Song J, Lippincott-Schwartz J: **Golgi dispersal during microtubule disruption: regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites**. *Mol Biol Cell* 1996, **7**:631-650.

12. Ward TH, Polishchuk RS, Caplan S, Hirschberg K, Lippincott-Schwartz J: **Maintenance of Golgi structure and function depends on the integrity of ER export**. *J Cell Biol* 2001, **155**:557-570.

13. Whyte JR, Munro S: **Vesicle tethering complexes in membrane traffic**. *J Cell Sci* 2002, **115**:2627-2637.

14. Cole NB, Smith CL, Sciaky N, Terasaki M, Edidin M, Lippincott-Schwartz J: **Diffusional mobility of Golgi proteins in membranes of living cells**. *Science* 1996, **273**:797-801.

15. Presley JF, Cole NB, Schroer TA, Hirschberg K, Zaal KJ, Lippincott-Schwartz J: **ER-to-Golgi transport visualized in living cells**. *Nature* 1997, **389**:81-85.

16. Presley JF, Ward TH, Pfeifer AC, Siggia ED, Phair RD, Lippincott-Schwartz J: **Dissection of COPI and Arf1 dynamics *in vivo* and role in Golgi membrane transport**. *Nature* 2002, **417**:187-193.

17. Hirschberg K, Miller CM, Ellenberg J, Presley JF, Siggia ED, Phair RD, Lippincott-Schwartz J: **Kinetic analysis of secretory protein traffic and characterization of Golgi-to-plasma-membrane transport intermediates in living cells**. *J Cell Biol* 1998, **143**:1485-1503.

18. •• Mironov AA, Mironov AA Jr, Beznoussenko GV, Trucco A, Lupetti P, Smith JD, Geerts WJ, Koster AJ, Burger KN, Martone ME *et al.*: **ER-to-Golgi carriers arise through direct en bloc protrusion and multistage maturation of specialized ER exit domains**. *Dev Cell* 2003, **5**:583-594.
The authors investigate the trafficking from the ER to the Golgi of procollagen and VSVG cargo using correlative video/light electron microscopy and tomography. They show that both cargo proteins are extruded in large membrane structures out of COPII coated ER exit sites but the structures are not a product of the budding and fusion of COPII dependent vesicles.

19. Kuge O, Dascher C, Orci L, Rowe T, Amherdt M, Plutner H, Ravazzola M, Tanigawa G, Rothman JE, Balch WE: **Sar1 promotes vesicle budding from the endoplasmic reticulum but not Golgi compartments**. *J Cell Biol* 1994, **125**:51-65.

20. Miles S, McManus H, Forsten KE, Storrie B: **Evidence that the entire Golgi apparatus cycles in interphase HeLa cells: sensitivity of Golgi matrix proteins to an ER exit block**. *J Cell Biol* 2001, **155**:543-555.

21. Stroud WJ, Jiang S, Jack G, Storrie B: **Persistence of Golgi matrix distribution exhibits the same dependence on Sar1p activity as a Golgi glycosyltransferase**. *Traffic* 2003, **4**:631-641.

22. •• Puri S, Linstedt AD: **Capacity of the Golgi apparatus for biogenesis from the endoplasmic reticulum**. *Mol Biol Cell* 2003, **14**:5011-5018.
This study uses a reversible ER export block to manipulate the activities of Sar1 and Arf1 and addresses the relative contributions of each GTPase in the biogenesis of Golgi membranes from the ER.

23. • Stroud WJ, Jiang S, Jack G, Storrie B: **Persistence of Golgi matrix distribution exhibits the same dependence on Sar1p activity as a Golgi glycosyltransferase**. *Traffic* 2003, **4**:631-641.
Using confocal video microscopy this study investigates the dynamics of transitional ER exit sites and Golgi structures and shows their tight spatio-temporal coupling in the budding yeast.

24. Aridor M, Fish KN, Bannykh S, Weissman J, Roberts TH, Lippincott-Schwartz J, Balch WE: **The Sar1 GTPase coordinates biosynthetic cargo selection with endoplasmic reticulum export site assembly**. *J Cell Biol* 2001, **152**:213-229.

25. Barlowe C, d'Enfert C, Schekman R: **Purification and characterization of SAR1p, a small GTP-binding protein required for transport vesicle formation from the endoplasmic reticulum**. *J Biol Chem* 1993, **268**:873-879.

26. Seemann J, Jokitalo E, Pypaert M, Warren G: **Matrix proteins can generate the higher order architecture of the Golgi apparatus**. *Nature* 2000, **407**:1022-1026.

27. Stephens DJ: ***De novo* formation, fusion and fission of mammalian COPII-coated endoplasmic reticulum exit sites**. *EMBO Rep* 2003, **4**:210-217.

28. Antonny B, Schekman R: **ER export: public transportation by the COPII coach**. *Curr Opin Cell Biol* 2001, **13**:438-443.

29. Bannykh SI, Rowe T, Balch WE: **The organization of endoplasmic reticulum export complexes**. *J Cell Biol* 1996, **135**:19-35.

30. Muniz M, Morsomme P, Riezman H: **Protein sorting upon exit from the endoplasmic reticulum**. *Cell* 2001, **104**:313-320.

31. Morsomme P, Prescianotto-Baschong C, Riezman H: **The ER v-SNAREs are required for GPI-anchored protein sorting from other secretory proteins upon exit from the ER**. *J Cell Biol* 2003, **162**:403-412.

32. Alvarez C, Garcia-Mata R, Hauri HP, Sztul E: **The p115-interactive proteins GM130 and giantin participate in endoplasmic reticulum–Golgi traffic**. *J Biol Chem* 2001, **276**:2693-2700.

33. Kondylis V, Rabouille C: **A novel role for dp115 in the organization of tER sites in *Drosophila***. *J Cell Biol* 2003, **162**:185-198.

34. Nakamura N, Lowe M, Levine TP, Rabouille C, Warren G: **The vesicle docking protein p115 binds GM130, a cis-Golgi matrix protein, in a mitotically regulated manner**. *Cell* 1997, **89**:445-455.

35. Sonnichsen B, Lowe M, Levine T, Jamsa E, Dirac-Svejstrup B, Warren G: **A role for giantin in docking COPI vesicles to Golgi membranes**. *J Cell Biol* 1998, **140**:1013-1021.

36. Allan BB, Moyer BD, Balch WE: **Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion**. *Science* 2000, **289**:444-448.

37. •• Garcia-Mata R, Sztul E: **The membrane-tethering protein p115 interacts with GBF1, an ARF guanine-nucleotide-exchange factor**. *EMBO Rep* 2003, **4**:320-325.
Using a two-hybrid assay the authors report an interaction between p115 and GBF1, the guanosine exchange factor of Arf1. The interaction is then demonstrated *in vitro* as well as *in vivo*. Mutations in GBF1 that lead to loss of binding to p115 result in the disruption of Golgi membranes.

38. Shorter J, Watson R, Giannakou ME, Clarke M, Warren G, Barr FA: **GRASP55, a second mammalian GRASP protein involved in the stacking of Golgi cisternae in a cell-free system**. *EMBO J* 1999, **18**:4949-4960.

39. • Puthenveedu MA, Linstedt AD: **Gene replacement reveals that p115/SNARE interactions are essential for Golgi biogenesis**. *Proc Natl Acad Sci USA* 2004, **101**:1253-1256.
Using RNAi technology the authors knock out p115 and show it to be necessary for Golgi biogenesis. They also report that only the SNARE binding domain and not the phosphorylation sites or the golgin-binding domains are necessary for Golgi biogenesis.

40. Linstedt AD, Jesch SA, Mehta A, Lee TH, Garcia-Mata R, Nelson DS, Sztul E: **Binding relationships of membrane tethering components. The giantin N terminus and the GM130 N terminus compete for binding to the p115 C terminus**. *J Biol Chem* 2000, **275**:10196-10201.

41. Lesa GM, Seemann J, Shorter J, Vandekerckhove J, Warren G: **The amino-terminal domain of the Golgi protein giantin interacts directly with the vesicle-tethering protein p115**. *J Biol Chem* 2000, **275**:2831-2836.

42. Nie Z, Hirsch DS, Randazzo PA: **Arf and its many interactors**. *Curr Opin Cell Biol* 2003, **15**:396-404.

43. Short B, Preisinger C, Korner R, Kopajtich R, Byron O, Barr FA: **A GRASP55-rab2 effector complex linking Golgi structure to membrane traffic**. *J Cell Biol* 2001, **155**:877-883.

44. Moyer BD, Allan BB, Balch WE: **Rab1 interaction with a GM130 effector complex regulates COPII vesicle cis–Golgi tethering**. *Traffic* 2001, **2**:268-276.

45. Weide T, Bayer M, Koster M, Siebrasse JP, Peters R, Barnekow A: **The Golgi matrix protein GM130: a specific interacting partner of the small GTPase rab1**. *EMBO Rep* 2001, **2**:336-341.

46. Tisdale EJ: **Rab2 interacts directly with atypical protein kinase C (aPKC) ι/λ and inhibits aPKCι/λ-dependent glyceraldehyde-3-phosphate dehydrogenase phosphorylation**. *J Biol Chem* 2003, **278**:52524-52530.

47. Vasile E, Perez T, Nakamura N, Krieger M: **Structural integrity of the Golgi is temperature-sensitive in conditional-lethal mutants with no detectable GM130**. *Traffic* 2003, **4**:254-272.

48. Alvarez C, Garcia-Mata R, Brandon E, Sztul E: **COPI recruitment is modulated by a Rab1b-dependent mechanism**. *Mol Biol Cell* 2003, **14**:2116-2127.

49. Diao A, Rahman D, Pappin DJ, Lucocq J, Lowe M: **The coiled-coil membrane protein golgin-84 is a novel rab effector required for Golgi ribbon formation**. *J Cell Biol* 2003, **160**:201-212.

50. Satoh A, Wang Y, Malsam J, Beard MB, Warren G: **Golgin-84 is a rab1 binding partner involved in Golgi structure**. *Traffic* 2003, **4**:153-161.

51. Tisdale EJ, Balch WE: **Rab2 is essential for the maturation of pre-Golgi intermediates**. *J Biol Chem* 1996, **271**:29372-29379.

52. Brandon E, Gao Y, Garcia-Mata R, Alvarez C, Sztul E: **Membrane targeting of p115 phosphorylation mutants and their effects on Golgi integrity and secretory traffic**. *Eur J Cell Biol* 2003, **82**:411-420.

53. Bonifacino JS, Lippincott-Schwartz J: **Coat proteins: shaping membrane transport**. *Nat Rev Mol Cell Biol* 2003, **4**:409-414.

54. Bigay J, Gounon P, Robineau S, Antonny B: **Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature**. *Nature* 2003, **426**:563-566.

55. De Matteis M, Godi A, Corda D: **Phosphoinositides and the Golgi complex**. *Curr Opin Cell Biol* 2002, **14**:434-447.

56. Brown WJ, Chambers K, Doody A: **Phospholipase A2 (PLA2) enzymes in membrane trafficking: mediators of membrane shape and function**. *Traffic* 2003, **4**:214-221.

57. Allan VJ, Thompson HM, McNiven MA: **Motoring around the Golgi**. *Nat Cell Biol* 2002, **4**:E236-E242.

58. Stow JL, Fath KR, Burgess DR: **Budding roles for myosin II on the Golgi**. *Trends Cell Biol* 1998, **8**:138-141.

59. Zakrzewska E, Perron M, Laroche A, Pallotta D: **A role for GEA1 and GEA2 in the organization of the actin cytoskeleton in *Saccharomyces cerevisiae***. *Genetics* 2003, **165**:985-995.

60. Warner CL, Stewart A, Luzio JP, Steel KP, Libby RT, Kendrick-Jones J, Buss F: **Loss of myosin VI reduces secretion and the size of the Golgi in fibroblasts from Snell's waltzer mice**. *EMBO J* 2003, **22**:569-579.

61. Togo T, Steinhardt RA: **Nonmuscle myosin IIA and IIB have distinct functions in the exocytosis-dependent process of cell membrane repair**. *Mol Biol Cell* 2004, **15**:688-695.

62. Percival JM, Hughes JA, Brown DL, Schevzov G, Heimann K, Vrhovski B, Bryce N, Stow JL, Gunning PW: **Targeting of a tropomyosin isoform to short microfilaments associated with the Golgi complex**. *Mol Biol Cell* 2004, **15**:268-280.

63. Scales SJ, Pepperkok R, Kreis TE: **Visualization of ER-to-Golgi transport in living cells reveals a sequential mode of action for COPII and COPI**. *Cell* 1997, **90**:1137-1148.

64. •• Garcia-Mata R, Szul T, Alvarez C, Sztul E: **ADP-ribosylation factor/COPI-dependent events at the endoplasmic reticulum-Golgi interface are regulated by the guanine nucleotide exchange factor GBF1**. *Mol Biol Cell* 2003, **14**:2250-2261.

The study provides evidence for GBF1 being the primary guanosine exchange factor for Arf1. Using various mutants of GBF1 they demonstrate that GBF1 is necessary for the recruitment of Arf1 and COP1 and the formation of a stable membrane domain that can mature and differentiate into Golgi membranes.

65. Ruan Q, Wang Q, Xie S, Fang Y, Darzynkiewicz Z, Guan K, Jhanwar-Uniyal M, Dai W: **Polo-like kinase 3 is Golgi localized and involved in regulating Golgi fragmentation during the cell cycle**. *Exp Cell Res* 2004, **294**:51-59.

66. Wang Y, Seemann J, Pypaert M, Shorter J, Warren G: **A direct role for GRASP65 as a mitotically regulated Golgi stacking factor**. *EMBO J* 2003, **22**:3279-3290.

67. Cluett EB, Brown WJ: **Adhesion of Golgi cisternae by proteinaceous interactions: intercisternal bridges as putative adhesive structures**. *J Cell Sci* 1992, **103**:773-784.

68. • Snapp EL, Hegde RS, Francolini M, Lombardo F, Colombo S, Pedrazzini E, Borgese N, Lippincott-Schwartz J: **Formation of stacked ER cisternae by low affinity protein interactions**. *J Cell Biol* 2003, **163**:257-269.

The authors demonstrate that the ER can be transformed into a stacked membrane system through the weak interactions of the cytoplasmic tails of over-expressed ER proteins.

69. Farmaki T, Ponnambalam S, Prescott AR, Clausen H, Tang BL, Hong W, Lucocq JM: **Forward and retrograde trafficking in mitotic animal cells. ER–Golgi transport arrest restricts protein export from the ER into COPII-coated structures**. *J Cell Sci* 1999, **112**:589-600.

70. Shorter J, Warren G: **Golgi architecture and inheritance**. *Annu Rev Cell Dev Biol* 2002, **18**:379-420.

71. Colanzi A, Suetterlin C, Malhotra V: **Cell-cycle-specific Golgi fragmentation: how and why?** *Curr Opin Cell Biol* 2003, **15**:462-467.

72. Barr FA: **Golgi inheritance: shaken but not stirred**. *J Cell Biol* 2004, **164**:955-958.

73. Panic B, Whyte JR, Munro S: **The ARF-like GTPases Arl1p and Arl3p act in a pathway that interacts with vesicle-tethering factors at the Golgi apparatus**. *Curr Biol* 2003, **13**:405-410.

74. Farkas RM, Giansanti MG, Gatti M, Fuller MT: **The *Drosophila* Cog5 homologue is required for cytokinesis, cell elongation, and assembly of specialized Golgi architecture during spermatogenesis**. *Mol Biol Cell* 2003, **14**:190-200.

75. • Bivona TG, Perez De Castro I, Ahearn IM, Grana TM, Chiu VK, Lockyer PJ, Cullen PJ, Pellicer A, Cox AD, Philips MR: **Phospholipase Cγ activates Ras on the Golgi apparatus by means of RasGRP1a**. *Nature* 2003, **424**:694-698.

This study reveals the Golgi apparatus as a novel location for Ras activity and its activity at the Golgi has important biological consequences such as cellular differentiation and mediation of radioresistance.

76. Preisinger C, Short B, De Corte V, Bruyneel E, Haas A, Kopajtich R, Gettemans J, Barr FA: **YSK1 is activated by the Golgi matrix protein GM130 and plays a role in cell migration through its substrate 14-3-3ζ**. *J Cell Biol* 2004, **164**:1009-1020.

77. • Nardini M, Spano S, Cericola C, Pesce A, Massaro A, Millo E, Luini A, Corda D, Bolognesi M: **CtBP/BARS: a dual-function protein involved in transcription co-repression and Golgi membrane fission**. *EMBO J* 2003, **22**:3122-3130.

A crystallography study that provides information on how CtBP/BARS can function as a lipid modifying enzyme as well as a transcriptional repressor.

78. Liu F, Stanton JJ, Wu Z, Piwnica-Worms H: **The human Myt1 kinase preferentially phosphorylates Cdc2 on threonine 14 and localizes to the endoplasmic reticulum and Golgi complex**. *Mol Cell Biol* 1997, **17**:571-583.

79. Yeaman C, Ayala MI, Wright JR, Bard F, Bossard C, Ang A, Maeda Y, Seufferlein T, Mellman I, Nelson WJ, Malhotra V: **Protein kinase D regulates basolateral membrane protein exit from trans-Golgi network**. *Nat Cell Biol* 2004, **6**:106-112.

80. Litvak V, Tian D, Carmon S, Lev S: **Nir2, a human homolog of *Drosophila* melanogaster retinal degeneration B protein, is essential for cytokinesis**. *Mol Cell Biol* 2002, **22**:5064-5075.