ELSEVIER

Biophysical Chemistry 84 (2000) 261–268

Biophysical Chemistry

www.elsevier.nl/locate/bpc

Reconstitution of the Golgi reassembly process in semi-intact MDCK cells

Fumi Kano[a,b], Kuniaki Nagayama[b], Masayuki Murata[b,*]

[a] *Department of Biophysics, Graduate School of Science, Kyoto University, Kitashirakawa, Sakyo-Ku, Kyoto 606-8502, Japan*
[b] *Department of Molecular Physiology, National Institute for Physiological Sciences, Okazaki 444-8585, Japan*

Received 9 February 2000; received in revised form 2 March 2000; accepted 2 March 2000

Abstract

The Golgi apparatus, which consists of stacks of cisternae during interphase, is fragmented or dispersed throughout the cytoplasm at the onset of mitosis. A sea sponge metabolite, ilimaquinone (IQ), causes Golgi membranes to vesiculate. And after its removal, the vesiculated membranes reassemble into stacks of cisternae in the perinuclear region. To study the mechanism of Golgi membrane dynamics during mitosis, we have reconstituted the reassembly process of IQ-induced vesiculated Golgi membranes in streptolysin O-permeabilized Mardin–Darby canine kidney (MDCK) cells. Monitoring the dynamics of Golgi membranes labeled with a green fluorescence protein (GFP)-tagged protein, we dissected the process into two elementary components: the reassembly of vesiculated Golgi membranes into punctate structures; and the subsequent reformation of these structures into stacks of cisternae near the nucleus. Using morphometric analysis, we studied the kinetics and biochemical requirements for the process, and revealed that an NEM-sensitive factor, cytoplasmic dynein, and GTP binding protein were involved in the Golgi reassembly.

Keywords: Golgi apparatus; Semi-intact cells; GFP; Reconstitution; Ilimaquinone; Membrane dynamics

1. Introduction

In mammalian cells, the Golgi apparatus shows a dynamic conversion of its structure in response to the cell cycle [1,2]. In interphase, the Golgi apparatus consists of stacks of cisternae and tubular structures, located at the pericentriolar position. At the onset of mitosis, the Golgi components disperse throughout the cell to be partitioned into daughter cells. The dispersed Golgi components in the vesicles begin to appear as large vesicles at telophase [3,4]. After cytokinesis, the stacks of cisternae are reformed near the

* Corresponding author. Tel.: +81-564-55-7815; fax: +81-564-52-7913.
E-mail address: mmurata@nips.ac.jp (M. Murata)

0301-4622/00/$ - see front matter
PII: S0301-4622(00)00133-2

nucleus. Recently, Lippincott-Schwartz and colleagues proposed the Golgi inheritance through retrograde transport, where at the onset of mitosis the Golgi components relocate to the ER via retrograde transport and at telophase budded from the ER to reappear near the nucleus [5]. Additional information would be required to resolve the question how the Golgi complex is inherited. Thus the molecular mechanisms by which the Golgi membranes disperse and reform along with the cell cycle have recently started to be elucidated [6,7].

Malhotra and colleagues reconstituted the reassembly process of Golgi membranes dispersed by the sea sponge metabolite, ilimaquinone (IQ) [8,9]. IQ causes the Golgi membrane to break down into small vesicles of 50–60 nm in diameter and inhibits vesicular transport from the ER [10]. Upon removal of IQ, the vesiculated Golgi membranes fuse, and subsequently reassemble into stacks of cisternae, which are indistinguishable from the intact Golgi morphologically and functionally [9]. They treated NRK (normal rat kidney) cells with IQ to induce the disassembly of the Golgi apparatus, then washed out the reagent and permeabilized the cells by freeze–thaw. When the permeabilized cells were incubated with bovine brain cytosol and ATP-regenerating system, the vesiculated Golgi membranes fused with each other and formed larger punctate structures throughout the cytoplasm. By using the assay, they demonstrated that the vesiculated Golgi membranes fused into large vesicles of 200 nm diameter in an NSF (*N*-ethylmaleimide sensitive fusion protein)-dependent manner and then these vesicles further fused to form the cisternae in a reaction catalyzed by an NSF-like ATPase, p97. Warren and colleagues showed that both NSF and p97 were required for the fusion of mitotic Golgi fragments by using an in vitro reconstitution assay [11]. In their assay, isolated Golgi membranes were fragmented by mitotic cytosol, and the mitotic fragments were further incubated with interphase cytosol to induce the cisternal regrowth. Thus, these findings implied that common machinery is involved in both cases: reassembly of IQ-induced vesiculated Golgi membranes and that of mitotic Golgi fragments.

Unfortunately, however, the final products of fused Golgi membranes in Malhotra's reconstitution system remained dispersed throughout the cytoplasm. They supposed that microtubule integrity is essential for complete Golgi reassembly in the pericentriolar region and their failure is due to the disorganization of microtubules (networks) caused by their permeabilization procedure.

In this paper, we demonstrate the complete reconstitution of the reassembly process for IQ-induced vesiculated Golgi membranes (hereafter described as Golgi reassembly) in semi-intact MDCK cells. In our study, semi-intact cells were prepared by a two-step treatment using a bacterial pore-forming toxin, streptolysin O (SLO). By this treatment, almost all of the cytosolic factors are depleted but the structures of intracellular organelles and cytoskeletons are unaffected. In addition, we used a GFP-tagged Golgi-resident membrane protein, mouse galactosyltransferase-GFP (GT-GFP), as a probe to monitor Golgi morphology. Fluorescence microscopic observation revealed that the Golgi-reassembly can be morphologically dissected into two elementary processes; the fusion of dispersed Golgi membranes to form punctate structures throughout the cell, and the subsequent relocation of these structures near the nucleus and return of the characteristic morphology of the Golgi apparatus. We have also used a morphometric analysis to quantify the morphological changes to the Golgi apparatus during the reassembly, and investigated the kinetics and biochemical requirements of the process.

2. Experimental

2.1. Cell culture and reagents

MDCK cells were maintained in DMEM (Nissui, Japan) supplemented with 10% fetal bovine serum (JRH Biosciences, Lenexa, KS), 100 U/ml penicillin G, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone at 37°C in a 5% CO_2 incubator. IQ was a gift from Dr Vivek Malhotra (University of California, San Diego) and was stored as a

25-mM solution in DMSO. Streptolysin O (SLO) was purchased from Dr Sucharit Bhakdi (University of Meintz, Germany). Anti-dynein antibody was obtained from Sigma (St. Louis, MO). All other chemicals were purchased from Sigma or Wako Chemicals (Osaka, Japan).

2.2. DNA construction and stable transfectants

Complementary DNA encoding 1–60 amino acids of mouse galactosyltransferase was placed upstream of EGFP cDNA in EGFP-N1 vector (Clontech, Palo Alto, CA). The MDCK cells were transfected with LipofectAMINE PLUS (GIBCO BRL, Rockville, MD). Stable transfectants, named MDCK-GT, were selected in complete medium containing 0.3 mg/ml GENETICIN (GIBCO BRL).

2.3. Golgi reassembly assay

The MDCK-GT, grown on polycarbonate membranes, were incubated with 5 μg/ml taxol (Sigma) for 30 min at 37°C to stabilize the microtubules. They were then incubated with preactivated SLO (0.5 μg/ml) for 20 min at 4°C. After the excess SLO had been washed out with cold PBS, the cells were incubated with TB [25 mM Hepes-KOH (pH 7.4), 115 mM potassium acetate, 2.5 mM $MgCl_2$, 1 mM DTT, 2 mM EGTA] for 20 min at 37°C to permeabilize the plasma membranes. It was affirmed that $>95\%$ of cells were permeabilized by these treatments. Sixty percent of cytosolic proteins were depleted in semi-intact cells, which is confirmed by measuring the activity of leaked cytosolic lactate dehydrogenase [12]. Permeabilized cells were incubated with 50 μM IQ containing ATP-regenerating system (1 mM ATP, 8 mM creatine kinase, and 50 μg/ml creatine phosphate) for 30 min at 33°C. And then, semi-intact cells having dispersed Golgi membranes were washed with TB for 15 min at 4°C to remove IQ. The cells were incubated with L5178Y cytosol (prepared as described by Pimplikar et al. [13]) and ATP-regenerating system at 33°C for 90 min to reassemble the dispersed Golgi membranes. After appropriate treatments, cells were fixed with 1% formaldehyde in TB and observed by an Axiobert 135 fluorescence microscope (Carl Zeiss Inc., Jena, Germany).

2.4. Morphometric analysis

We classified the Golgi structures morphologically into three types during reassembly as: stage I (dispersed Golgi), dispersed throughout the cell; stage II (punctate Golgi), punctate in structure and distributed in the cytoplasm; and stage III (reformed Golgi), re-fused Golgi membranes localized near the nucleus. Three hundred cells in random fields were counted in each sample. Means and deviations are expressed for three independent experiments.

2.5. NEM treatment

Cytosol was treated with 1 mM *N*-ethylmaleimide (NEM) for 30 min on ice. Then, NEM was quenched by subsequent incubation with 4 mM DTT for 30 min on ice.

2.6. Kinetic modeling of the Golgi reassembly processes

To extract information from the kinetics data, we assumed the single linear first-order kinetics model shown in Fig. 3b. Standard kinetic analysis was applied to the data plots. There are two independent rate constants, $k1$ and $k2$, representing the rate-limiting steps for stage I–II and II–III, respectively. The kinetics data, percent stage I–III, were fitted simultaneously using the least-squares optimization procedure by Sigma Plot scientific graph system (Jandel Corporation, CA).

3. Results

3.1. Reconstitution of IQ-induced Golgi-disassembly and subsequent reassembly of the dispersed Golgi membranes in semi-intact MDCK cells

To study the dynamic morphological changes to the Golgi apparatus in semi-intact cells, we obtained stable transfectants of MDCK cells ex-

pressing GT-GFP continuously. One of these transfectants, MDCK-GT8, grown on polycarbonate membranes, was incubated with taxol to stabilize microtubules and then treated with SLO by a two-step procedure. The semi-intact cells were incubated with 50 μM IQ and ATP-regenerating system (hereafter, described as ATP) at 33°C. As shown in Fig. 1, at the 0-min mark, the Golgi apparatus had a ribbon-like morphology around the nucleus, which is a typical construction of the Golgi apparatus when MDCK cells form an epithelial sheet on polycarbonate membranes. At 20 min after addition of IQ, the Golgi apparatus broke into smaller, punctate structures. These punctate structures subsequently dispersed throughout the cytoplasm after 40 min incubation. Morphological observation of the disassembly revealed it to consist of two elementary processes: the disorganization of the intact Golgi apparatus into large punctate structures; and subsequent fragmentation and the dispersal of these structures throughout the cytoplasm.

After being washed with excess TB to remove IQ, cells containing dispersed Golgi membranes were further incubated with L5178Y cytosol (protein concentration: 1.9–2.4 mg/ml) and ATP at 33°C. After 10 min incubation with cytosol and ATP, the dispersed Golgi membranes fused with each other and formed larger vesicles (punctate structures) throughout the cytoplasm. As the incubation proceeded, the punctate Golgi membranes migrated to the perinuclear region and fused to form the juxtanuclear Golgi. The Golgi reassembly also consists of two processes; the fusion of the dispersed membranes and the relocation of the fused membranes near the nucleus.

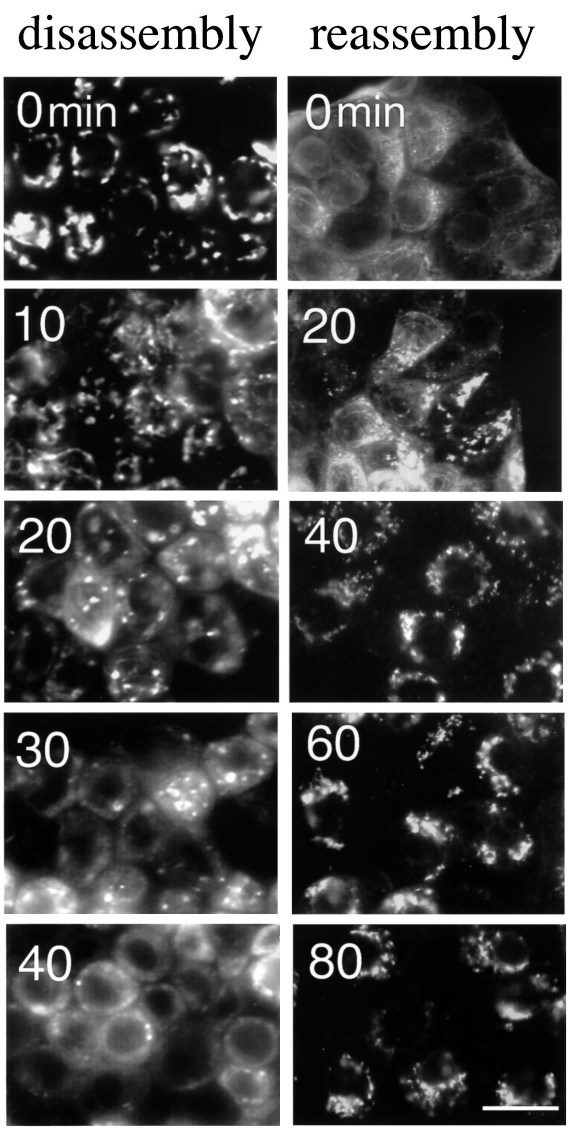

Fig. 1. Morphological changes to GFP-tagged Golgi membranes during Golgi disassembly and reassembly. Semi-intact MDCK-GT cells were incubated with 50 μM IQ and ATP-regenerating system at 33°C for 0, 10, 20, 30, and 40 min. The cells were fixed and viewed with fluorescence microscope (disassembly). After being washed out IQ, the cells were incubated with L5178Y cytosol and ATP-regenerating system at 33°C for 0, 20, 40, 60, and 80 min. Fusion of the vesiculated Golgi membranes and their relocation into the pericentriolar region were observed (reassembly). Bar = 20 μm.

3.2. Kinetics of the Golgi reassembly

Next we developed a morphometric analysis to quantify the morphological changes to the Golgi apparatus during reassembly. As shown in Fig. 2, morphologically, the Golgi apparatus was classified as: stage I (dispersed Golgi), stage II (punctate Golgi), and stage III (reformed Golgi). For morphometric analysis, the cells which showed one of the three typical morphologies of the Golgi apparatus described above were enumerated in each sample and the percentage of cells at each stage was expressed (see Section 2).

Morphometric analysis was used to investigate

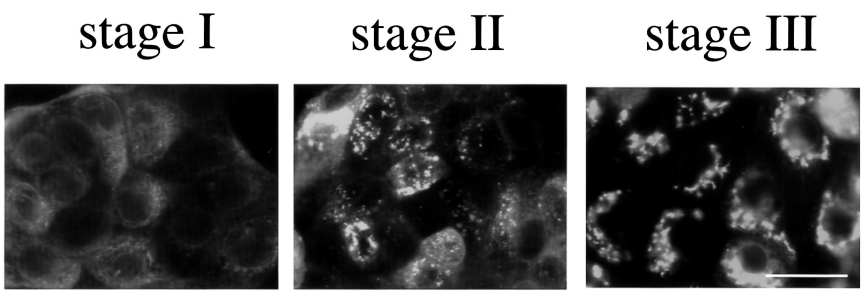

Fig. 2. Morphological dissection of the Golgi membranes during the Golgi reassembly. The process was dissected into three stages in the Golgi reassembly assay: stage I, Golgi membranes dispersed throughout the cytoplasm; stage II, punctate Golgi structures distributed throughout the cell; and stage III, reformed Golgi membranes localized near the nucleus. Bar = 20 μm.

the kinetics of the IQ-induced Golgi reassembly process in semi-intact MDCK-GT cells (Fig. 3a, left panel). The percentage of stage I cells decreased from 90% to 15% although that of stage III cells increased to approximately 60% at 80 min incubation with cytosol and ATP. The percentage of stage II increased to 40% followed by a decrease to 25%. The time-dependent increase followed by the decrease in the percentage of stage II cells support that the punctate structures are intermediates in the Golgi reassembly process.

We next examined whether the kinetic data plots obtained above could be fit to the model in Fig. 3b. We assumed that the reaction is a simple first-order reaction and the Golgi reassembly consists of three stages arranged in series. To see whether the model could account for the data, and to extract rate parameters, we used least-squares optimization. Experimental data of the percentage of cells at each stage (left panel) and the corresponding model solution (right panel) are represented in Fig. 3a. Simulated data plots fitted the experimental data very well, and the mean rate constants (±S.E.) were obtained as $0.0274 \pm 0.0029\ \mathrm{min}^{-1}$ for $k1$ (from stage I to II) and $0.0285 \pm 0.0041\ \mathrm{min}^{-1}$ for $k2$ (from stage II to III). The optimal fitting strongly indicates that the punctate Golgi structures at stage II are intermediates in the Golgi reassembly process.

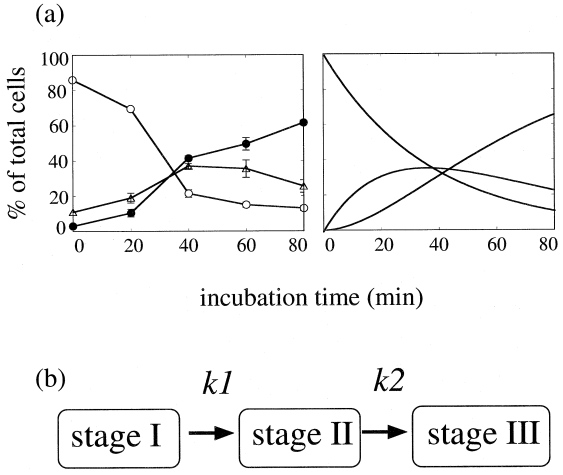

Fig. 3. Kinetics and modeling of the Golgi reassembly. (a) Time-dependent kinetic changes in the percentage of cells at each stage are expressed in the left panel. Open circles represent stage I; open triangles, stage II; and closed circles, stage III. Right panel shows the corresponding simulation curve, assuming the reaction is expressed as simple linear first order kinetics as shown in (b). (b) Simple linear first-order kinetic model for the Golgi reassembly process. In this model, the vesiculated Golgi membranes reassemble from stage I to II and II to III at a rate equal to a rate constant (i.e. $k1$ and $k2$).

3.3. Morphometric analysis of the IQ-induced Golgi reassembly: biochemical requirements

By using the above reassembly assay, we investigated the biochemical requirements for the process. Semi-intact cells, whose Golgi membranes were dispersed by IQ treatment, were incubated with cytosol under various conditions at 33°C for 80 min and then subjected to morphometric analysis. The reassembly process required ATP and its hydrolysis (Fig. 4, ATP and AMP-PNP). Addition of ATP only was enough for the formation of the punctate Golgi (stage II), al-

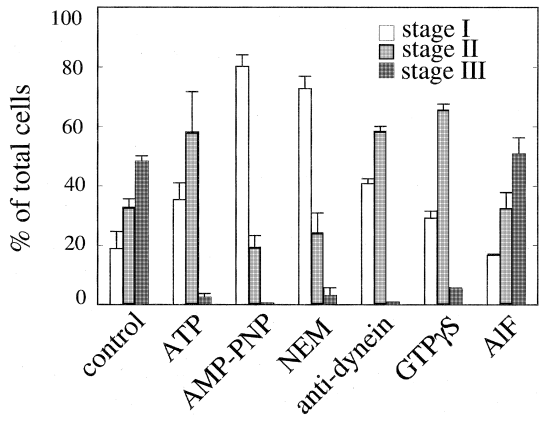

Fig. 4. Biochemical requirements for the Golgi reassembly process. IQ-treated semi-intact MDCK-GT cells were incubated with cytosol/ATP (control), ATP (ATP), cytosol/ATP/1 mM AMP-PNP (AMP-PNP), NEM-treated cytosol/ATP (NEM-cyto), cytosol/ATP/0.4 μg anti-dynein antibody (anti-dynein), cytosol/ATP/1 mM GTPγS (GTPγS), or cytosol/ATP/AlF (AlF, 30 mM NaF and 50 μM $AlCl_3$) at 33°C for 80 min, respectively. After the incubation, the cells were subjected to morphometric analysis. Mean of the percentage of cells at stages I–III was expressed. Vertical bars represent deviations. Protein concentration of cytosol is 2.4 mg/ml.

though the effect of cytosol in semi-intact cells cannot be ignored. NEM-treated cytosol inhibited almost completely the formation of punctate Golgi (Fig. 4, NEM). This indicated that a general fusion protein, NSF, in exogeneously added cytosol, is essential for the formation of stage II Golgi. Addition of anti-dynein antibody especially inhibited the reassembly process at stage II (Fig. 4, anti-dynein), while anti-kinesin antibody did not (data not shown). The result suggested that the minus-end directed microtubule-associated motor protein, cytoplasmic dynein, is involved in the later process (from stage II to III). Indeed, without preincubation of the cells with taxol, the reassembly process did not proceed beyond stage II (punctate Golgi), suggesting that the migration of punctate Golgi to the pericentriolar region is dependent on microtubule integrity and microtubule-associated motor protein(s).

We next examined the involvement of GTP binding proteins in the reassembly process. Addition of GTPγS, a non-hydrolyzable analogue of GTP, inhibited the process from stage II to III, but the aluminum fluoride (AlF) did not (Fig. 4, GTPγS and AlF). G protein(s), which might have a different sensitivity for the reagents, is involved later in the process. However, at this stage, we could not characterize the type of G protein, because both GTPγS and AlF activate trimeric and small G proteins [14–17].

4. Discussion

Malhotra's group reconstituted the reassembly process of the IQ-mediated vesiculated Golgi membranes in semi-permeabilized NRK cells [8,9]. The results they obtained were consistent with our results, especially the ATP-, cytosol- and NSF-requirements for the fusion of vesiculated Golgi membrane.

However, our reconstitution systems revealed several new findings as below. First, we reconstituted not only the fusion of the vesiculated Golgi membranes but also the relocation of the fused membranes to the pericentriolar region in semi-intact MDCK cells. SLO-mediated permeabilization has little effect on the integrity of microtubule networks in MDCK cells, as was supported by indirect immunofluorescence method using anti-α-tubulin antibody (data not shown). The good preservation of microtubule integrity would make it possible to reconstitute the whole process of the Golgi reassembly. Indeed, without taxol treatment, reassembly arrested at stage II (punctate), suggesting that microtubule integrity is important for the relocation process (data not shown).

Second, we dissected the Golgi reassembly into two elementary processes: the fusion of dispersed Golgi membranes (stage I) to form punctate structures (stage II); and the subsequent relocation of these structures near the nucleus to reform the stacks of cisternae (stage III). By morphometric analysis, we analyzed the kinetics of Golgi reassembly and evaluated the rate constant of each process. We found that a simple model comprised of a series of linear rate laws connecting three stages (stage I–III) was sufficient to fit the data plots (Fig. 3a). In mammalian cells, the mitotic Golgi fragments first fuse into large vesi-

cles at telophase, and stacks of cisternae are reconstructed after cytokinesis [18,19]. Punctate Golgi at stage II in our reconstitution system might correspond to the large vesicles at telophase and fused Golgi at stage III to stacks of cisternae after cytokinesis.

Third, based on the analysis, we also evaluated the biochemical requirements for each process quantitatively and found that GTP binding protein regulates the latter process of the reassembly. Various kinds of GTP binding proteins were demonstrated to regulate the Golgi function and organization. One of the most characterized GTPases, involved in Golgi function, is Ras-like GTPase, the Rab family [20,21]. Rab 1, 2, and 6 are reported to be involved in vesicular transport to or through the Golgi apparatus [22–26]. It was also reported that trimeric G proteins regulate the Golgi structure [27–29]. For example, $G_{\beta\gamma}$, a subunit of trimeric G protein, mediates the signal of IQ-induced Golgi fragmentation [28,29]. In our reconstitution system, IQ-induced Golgi disassembly was inhibited by GTPγS or AlF (Kano et al., unpublished data). It is interesting that AlF inhibited the Golgi disassembly but not reassembly. The different sensitivities of G proteins to the reagents during disassembly and reassembly would provide a clue to the identity of the G protein involved in each process.

Our reconstitution system allows one to dissect complex intracellular processes morphologically and biochemically, and to screen and evaluate the biochemical requirements and kinetics for each process as described above. This system would also be of use for the study of the dynamic organization of other organelles, such as mitotic nuclear membrane dynamics.

Acknowledgements

We would like to thank Dr M. Furuse at Kyoto University for the mouse cDNA library and helpful discussions. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (B) from the Ministry of Education, Science, Sports and Culture of Japan (M.M) and by a JSPS Research Fellowship for Young Scientists (F.K.).

References

[1] N. Cabrera-Poch, R. Pepperkok, D.T. Shima, Inheritance of the mammalian Golgi apparatus during the cell cycle, Biochim. Biophys. Acta 1404 (1998) 139–151.
[2] M. Lowe, N. Nakamura, G. Warren, Golgi division and membrane traffic, Trends Cell Biol. 8 (1998) 40–44.
[3] T. Misteli, G. Warren, COP-coated vesicles are involved in the mitotic fragmentation of Golgi stacks in a cell-free system, J. Cell Biol. 125 (1994) 269–282.
[4] S.A. Jesch, A.D. Linstedt, The Golgi and endoplasmic reticulum remain independent during mitosis in HeLa cells, Mol. Biol. Cell 9 (1998) 623–635.
[5] K.J.M. Zaal, C.L. Smith, R.S. Polishchuk, N. Altan, N.B. Cole, J. Ellenberg, K. Hirschberg, J.F. Presley, T.H. Roberts, E. Siggia, R.D. Phair, J. Lippincott-Schwartz, Golgi membranes are absorbed into and reemerged from the ER during mitosis, Cell 99 (1999) 589–601.
[6] U. Acharya, A. Mallabiabarrena, J.K. Acharya, V. Malhotra, Signaling via mitogen-activated protein kinase (MEK1) is required for Golgi fragmentation during mitosis, Cell 92 (1998) 183–192.
[7] M. Lowe, C. Rabouille, N. Nakamura, R. Watson, M. Jackman, E. Jamsa, D. Rahman, D.J. Pappin, G. Warren, Cdc2 kinase directly phosphorylates the *cis*-Golgi matrix protein GM130 and is required for Golgi fragmentation in mitosis, Cell 94 (1998) 783–793.
[8] U. Acharya, R. Jacobs, J.M. Peters, N. Watson, M.G. Farquhar, V. Malhotra, The formation of Golgi stacks from vesiculated Golgi membranes requires two distinct fusion events, Cell 82 (1995) 895–904.
[9] U. Acharya, J.M. McCaffery, R. Jacobs, V. Malhotra, Reconstitution of vesiculated Golgi membranes into stacks of cisternae: requirement of NSF in stack formation, J. Cell Biol. 129 (1995) 577–589.
[10] P.A. Takizawa, J.K. Yucel, B. Veit, D.J. Faulkner, T. Deerinck, G. Soto, M. Ellisman, V. Malhotra, Complete vesiculation of Golgi membranes and inhibition of protein transport by a novel sea sponge metabolite, ilimaquinone, Cell 73 (1993) 1079–1090.
[11] C. Rabouille, T.P. Levine, J.M. Peters, G. Warren, An NSF-like ATPase, p97, and NSF mediate cisternal regrowth from mitotic Golgi fragments, Cell 82 (1995) 905–914.
[12] R.L. Schnaar, P.H. Weigel, M.S. Kuhlenschmidt, Y.C. Lee, S. Roseman, Adhesion of chicken hepatocytes to polyacrylamide gels derivatized with *N*-acetylglucosamine, J. Biol. Chem. 253 (1978) 7940–7951.
[13] S.W. Pimplikar, E. Ikonen, K. Simons, Basolateral protein transport in streptolysin O-permeabilized MDCK cells, J. Cell Biol. 125 (1994) 1025–1035.
[14] A.G. Gilman, G proteins: transducers of receptor-generated signals, Annu. Rev. Biochem. 56 (1987) 615–649.

[15] G.R. Hoffman, N. Nassar, R.E. Oswald, R.A. Cerione, Fluoride activation of the rho family GTP-binding protein Cdc43Hs, J. Biol. Chem. 273 (1998) 4392–4399.
[16] R. Mittal, M.R. Ahmadian, R.S. Goody, A. Wittinghofer, Formation of a transition-state analog of the Ras GTPase reaction by Ras-GDP, tetrafluoroaluminate, and GTPase-activating proteins, Science (1996) 115–117.
[17] M. Reza, R. Mittal, A. Hall, A. Wittinghofer, Aluminum fluoride associates with the small guanine nucleotide binding proteins, FEBS Lett. 408 (1997) 315–318.
[18] D.T. Shima, K. Haldar, R. Pepperkok, R. Watson, G. Warren, Partitioning of the Golgi apparatus during mitosis in living HeLa cells, J. Cell Biol. 137 (1997) 1211–1228.
[19] D.T. Shima, N. Cabrera-Poch, R. Pepperkok, G. Warren, An ordered inheritance strategy for the Golgi apparatus: visualization of mitotic disassembly reveals a role for the mitotic spindle, J. Cell Biol. 141 (1998) 955–966.
[20] P. Novick, M. Zerial, The diversity of Rab proteins in vesicle transport, Curr. Opin. Cell Biol. 9 (1997) 496–504.
[21] S.R. Pfeffer, Rab GTPases: master regulators of membrane trafficking, Curr. Opin. Cell Biol. 6 (1994) 522–526.
[22] C. Nuoffer, H.W. Davidson, J. Matteson, J. Meinkoth, W.E. Balch, A GDP-bound of rab1 inhibits protein export from the endoplasmic reticulum and transport between Golgi compartments, J. Cell Biol. 125 (1994) 225–237.
[23] F. Peter, C. Nuoffer, S.N. Pind, W.E. Balch, Guanine nucleotide dissociation inhibitor is essential for Rab1 function in budding from the endoplasmic reticulum and transport through the Golgi stack, J. Cell Biol. 126 (1994) 1393–1406.
[24] E.J. Tisdale, W.E. Balch, Rab2 is essential for the maturation of pre-Golgi intermediates, J. Biol. Chem. 271 (1996) 29372–29379.
[25] E.J. Tisdale, A Rab2 mutant with impaired GTPase activity stimulates vesicle formation from pre-Golgi intermediates, Mol. Biol. Cell. 10 (1999) 1837–1849.
[26] O. Martinez, C. Antony, G. Pehau-Arnaudet, E.G. Berger, J. Salamero, B. Goud, GTP-bound forms of rab6 induce the redistribution of Golgi proteins into the endoplasmic reticulum, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 1828–1833.
[27] J.G. Donaldson, R.A. Kahn, J. Lippincott-Schwartz, R.D. Klausner, Binding of ARF and beta-COP to Golgi membranes: possible regulation by a trimeric G protein, Science 254 (1991) 1197–1199.
[28] C. Jamora, P.A. Takizawa, R.F. Zaarour, C. Denesvre, D.J. Faulkner, V. Malhotra, Regulation of Golgi structure through heterotrimeric G proteins, Cell 91 (1997) 617–626.
[29] C. Jamaro, N. Yamanouye, J. Van Lint, J. Laudenslager, J.R. Vandenheede, D.J. Faulkner, V. Malhotra, $G_{\beta\gamma}$-mediated regulation of Golgi organization is through the direct activation of protein kinase D, Cell 98 (1999) 59–68.