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GOLGI ARCHITECTURE AND INHERITANCE

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■ **Abstract** Golgi inheritance proceeds via sequential biogenesis and partitioning phases. Although little is known about Golgi growth and replication (biogenesis), ultrastructural and fluorescence analyses have provided a detailed, though still controversial, perspective of Golgi partitioning during mitosis in mammalian cells. Partitioning requires the fragmentation of the juxtanuclear ribbon of interconnected Golgi stacks into a multitude of tubulovesicular clusters. This process is choreographed by a cohort of mitotic kinases and an inhibition of heterotypic and homotypic Golgi membrane-fusion events. Our model posits that accurate partitioning occurs early in mitosis by the equilibration of Golgi components on either side of the metaphase plate. Disseminated Golgi components then coalesce to regenerate Golgi stacks during telophase. Semi-intact cell and cell-free assays have accurately recreated these processes and allowed their molecular dissection. This review attempts to integrate recent findings to depict a more coherent, synthetic molecular picture of mitotic Golgi fragmentation and reassembly. Of particular importance is the emerging concept of a highly regulated and dynamic Golgi structural matrix or template that interfaces with cargo receptors, Golgi enzymes, Rab-GTPases, and SNAREs to tightly couple biosynthetic transport to Golgi architecture. This structural framework may be instructive for Golgi biogenesis and may encode sufficient information to ensure accurate Golgi inheritance, thereby helping to resolve some of the current discrepancies between different workers.

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INTRODUCTION

The Problem of Organelle Inheritance

Undoubtedly, one of the major evolutionary transitions was the transcendence from prokaryotic to eukaryotic cellular existence. The magnitude of this step is clear from the available fossil record, which indicates this transition took four times longer than the transition from inanimate matter to life (López-García & Moreira 1999). The evolution of eukaryotes involved the complex compartmentalization of the cytoplasm into a series of highly innovative, specialized devices, termed organelles, that exist as discrete membrane-bound structures. This reorganization of the cytoplasm allowed a cooperative division of labor, a recurring facet of the major evolutionary transitions (Szathmáry & Maynard Smith 1995). Compartmentalization conferred evolvability (Kirschner & Gerhart 1998), yielding the creation of highly interdependent, yet diverse and discrete microcosms within the cell, each tailored precisely for a specific set of concentrated, essential biochemical reactions. The specialized microenvironments of organelles are maintained by the controlled flux of ions and small molecules across the delimiting organelle membrane. Intimately coupled to this are vesicle transfers between compartments and signal-dependent import systems that together regulate organellar microenvironments (Blobel 1980, Rothman 1994).

As organelles evolved, their architecture and function increased in complexity to meet the selective pressures imposed by the environment on the cell. Increasing architectural and functional complexity likely came at the expense of the capacity to rapidly synthesize an organelle de novo. By de novo assembly, we mean the assembly of a new organelle in the absence of preexisting structures that comprise that organelle. Therefore, an acute selection pressure arose for accurate organelle inheritance such that progeny would not incur the energetically expensive cost of

de novo organelle assembly (if indeed this was possible at all), which might prove deleterious, especially to a unicellular organism in a competitive environment. Just as with the genetic material, organelle inheritance proceeds via intimately coupled, sequential biogenesis (growth and replication), and partitioning (division) phases. Thus, just as with the DNA, organelles are duplicated and correctly apportioned between nascent daughter cells before completion of cytokinesis (Shima & Warren 1998). Therefore, de novo organelle biogenesis in its purest sense is never required, as in all known cases organelles grow by proliferation and inheritance of preexisting organelles (Nunnari & Walter 1996, Lowe 2002). The templates that may govern their replication are inherited and endow progeny with a complete organelle complement. At most, de novo biogenesis may provide a fail-safe mechanism should organelles, for some reason, not be inherited correctly.

The need for accurate organelle inheritance is most obvious for the plasma membrane, the importance of which predates eukaryotic origination since it is an inescapable necessity for all cellular existence. For some organelles the functional complexity that precluded their de novo biosynthesis was coincident with their endosymbiotic origination, as with mitochondria and plastids (López-García & Moreira 1999). Since the progenitors of these organelles were independent organisms, and modern mitochondria and plastids still harbor their own genetic material to encode proteins and tRNAs essential for their function, it is inconceivable that they be synthesized de novo. Similarly, the evolution of the nuclear envelope and endoplasmic reticulum (ER) was likely a contingent irreversibility. This may have involved a symbiosis of Archaea in Bacteria (Horiike et al. 2001, Hartman & Fedorov 2002) or the complex invagination of an ancestral ribosome and translocon-studded plasma membrane (Blobel 1980, Helenius & Aebi 2001). During evolution the ER became the major site of membrane protein and lipid biosynthesis and as an irreversible consequence must be generated from preexisting ER membranes, as there is no obvious alternative mechanism to synthesize them anew.

The requirement for accurate inheritance of organelles of the endomembrane system is less apparent, since they are partially derived from the ER. Do they need to be inherited or can they be assembled de novo should a daughter fail to receive a copy? To what extent do these organelles depend on preexisting templates or instructive structures for the propagation of their architecture and function (Lewin 1998, Kirschner et al. 2000)?

Once an organelle has doubled in biomass in preparation for normal cell division, it must be accurately partitioned between progeny. Organelles adopt one of two partitioning strategies: stochastic or ordered. These strategies need not be mutually exclusive, and which is used may depend on organelle copy number and geographical locale within the cell. The mechanism followed also varies in a cell type- or organism-dependent manner (Warren & Wickner 1996). Ordered partitioning often involves the mitotic spindle and associated astral microtubules, and it is exemplified by the use of the mitotic spindle to accurately partition chromosomes. Stochastic partitioning relies on the organelle being present in multiple

copies dispersed randomly throughout the cytoplasm. Such a distribution would provide each daughter with an equal share of the organelles, provided sufficient copies of the organelle are present. The more copies the more accurate is partitioning as governed by the binomial theorem (Birky 1983, Warren 1993). Organelles such as mitochondria may adopt such a strategy in certain cell types (Rizzuto et al. 1995). Similarly, the pervasiveness of the ER throughout the cytoplasm of mammalian cells may ensure that it too is partitioned using a stochastic mechanism (Zeligs & Wollman 1979).

Precise control over organelle inheritance may also be extremely important if partitioning has to be asymmetric. For example, expulsion of the polar body during oocyte maturation and the inheritance of P-granules (ribonucleoprotein particles) that function in germline specification in *Caenorhabditis elegans* (Hird et al. 1996) are highly asymmetric processes. Similarly, the formation of argosomes, plasma membrane exovesicles, by donor cells and their horizontal transfer or inheritance to neighboring acceptor cells within the same epithelium may provide a mechanism of morphogen spread through epithelia (Greco et al. 2001, Vincent & Magee 2002). Thus, within the context of a multicellular organism, asymmetric partitioning and even targeted partitioning of cellular components may be critical for development and may have arisen by the manipulation of mechanisms that ensure equal partitioning. At the other extreme, the programmed degradation and elimination of organelles is required for some developmental processes such as the terminal differentiation of erythrocytes, keratinocytes, and fiber cells in the eye lens (van Leyen et al. 1998).

Golgi Architecture and Function

The precise sequence of events and the molecular mechanisms that coordinate Golgi architectural inheritance in mammalian cells are the focus of this review. The Golgi apparatus occupies a central position in the classical secretory pathway, where it receives the entire output of de novo synthesized polypeptides from the ER, and functions to distil, posttranslationally process, and sort cargo to their ultimate destinations (Mellman & Simons 1992). Resident enzyme complexes in the intralumenal milieu of Golgi cisternae function to conjugate secretory cargo with elaborate and highly diverse patterns of glycans. This set of modifications is more complex than the glycosylation events that occur in the ER, which serve more to aid in the correct folding of nascent polypeptides (Helenius & Aebi 2001). The complexity of glycosylation conferred by Golgi glycosyltransferases allows functional diversification of mature proteins suited for a spectrum of novel functions of particular importance for the adaptive and innate immune responses (Varki 1998, Rudd et al. 2001). In essence, the Golgi is a factory for evolvability (Kirschner & Gerhart 1998, Gagneaux & Varki 1999). The Golgi apparatus is also the major site of sphingolipid biosynthesis within the cell and acts as a buffer between the glycerolipid-rich ER and the sterol/sphingolipid-rich plasma membrane (Holthuis et al. 2001). Sterols must be rapidly pumped out of the ER since their accumulation

would alter membrane fluidity and might interfere with the translocation of nascent polypeptides (Bretscher & Munro 1993).

Typically, the Golgi apparatus consists of a series of flattened cisternal membranes that are closely apposed and aligned in parallel to form a stack (Figure 1*A*) (Rambourg & Clermont 1997, Ladinsky et al. 1999). The Golgi stack is bound on either face by extensive tubulovesicular networks: the *cis*-Golgi network (CGN) and *trans*-Golgi network (TGN). At a minimum, the CGN functions to receive the entire biosynthetic output from the ER, whereas the TGN sorts completed post-translationally modified products onto their final destination. This unique architecture is remarkably conserved throughout eukaryotic evolution; it is even apparent in the diplomonad *Giardia lamblia*, one of the earliest branching extant eukaryotes (Gillin et al. 1996). This indicates that the Golgi apparatus arose very early in eukaryotic evolution, possibly as an ER outgrowth or as a complex invagination of the plasma membrane (Blobel 1980, Helenius & Aebi 2001). Comparative genomics of early branching eukaryotes may provide insight into the evolutionary origins of the Golgi apparatus, but it may be that the latest common ancestor of all eukaryotes already possessed a sophisticated secretory pathway (Dacks & Doolittle 2001).

In plants and lower animal cells, the Golgi apparatus exists as many copies of discrete stacks dispersed throughout the cytoplasm. In contrast, the Golgi apparatus of mammalian cells is a juxtanuclear, often pericentriolar reticulum, where the discrete Golgi stacks are stitched together via tubular continuities that link equivalent cisternae, to yield a compact and twisting interconnected ribbon. The precise orientation of the Golgi ribbon can reflect the area of the cell surface that exocytosis is directed toward; for example, toward the leading edge of a migrating cell or cell wound, or toward the offending cell coupled to a cytotoxic T cell (Kupfer et al. 1982, 1983). This long-range ordering of Golgi structure is dependent on the cytoskeleton and is coordinated at least in part by Rho-GTPases (Nobes & Hall 1999).

Just as structure dictates function at the organismal and cellular level, so also do organellar form and structure influence organellar and cellular function. However, as with other organelles, precisely how Golgi architecture facilitates its function remains unclear. Numerous hypotheses have been proposed (Rothman 1981, Farquhar 1985, Mellman & Simons 1992, Bretscher & Munro 1993, Helenius & Aebi 2001), but since it is not yet possible to precisely manipulate Golgi architecture, the evidence supporting such hypotheses tends to be correlative rather than definitive. A detailed molecular knowledge of how Golgi architecture is maintained will facilitate precise manipulation of the components that underpin it and allow examination of functional predictions concerning the significance of architectural phenotypes.

Evolutionary Constraints on the Mechanism of Golgi Inheritance

The mechanism of Golgi inheritance varies dependent on whether the organism in question possesses a cell wall. Organisms with a cell wall have an intrinsic

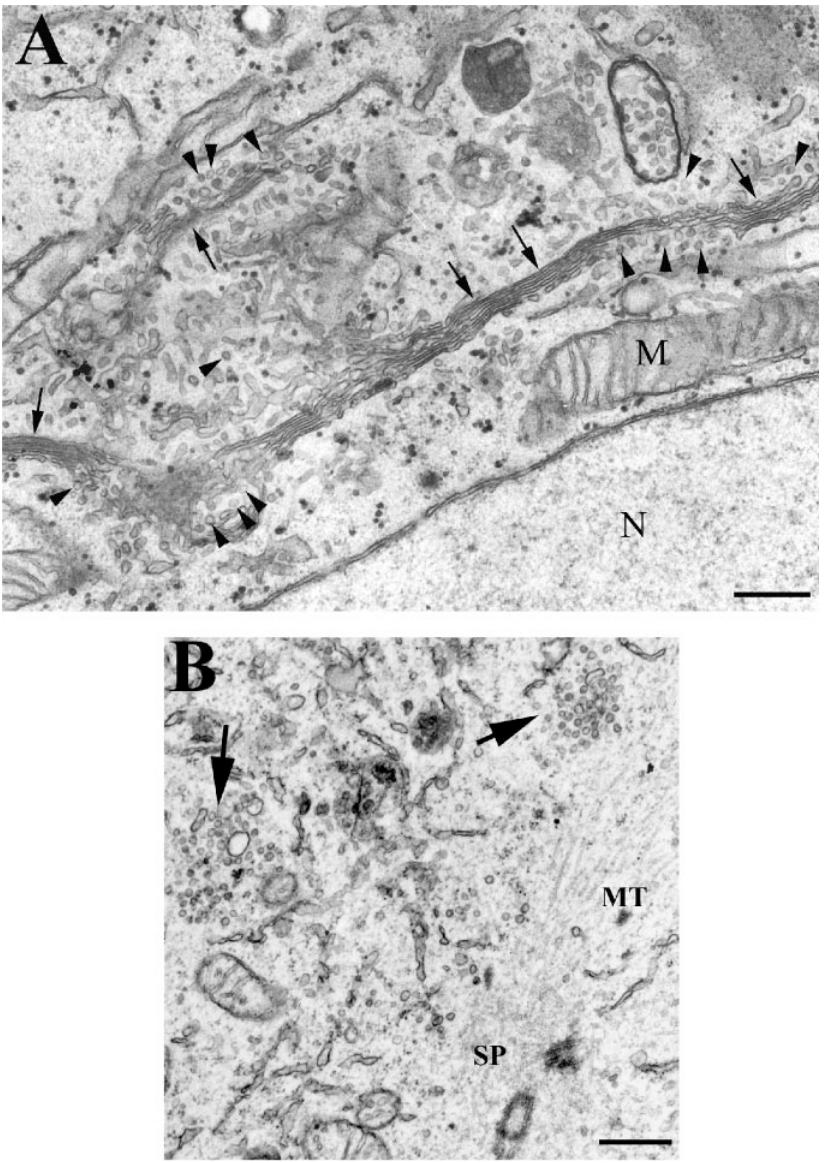

Figure 1 Morphology of the mammalian Golgi apparatus during interphase and mitosis. (*A*) A typical juxtanuclear collection of interconnected Golgi stacks (*arrows*) of an interphase NRK cell. Note the frequent transport vesicles (*arrowheads*). M, mitochondrion; N, nucleus. Bar, 0.5 μm. (*B*) The morphology of the Golgi apparatus of a PtK1 cell during mitosis. Numerous vesicles and tubules constitute the mitotic Golgi cluster (*arrows*) that occurs close to the spindle pole (SP) and microtubules (MT). Bar, 0.3 μm.

need to continue secreting material during cell division, to maintain the cell wall at all times and avoid osmotic shock (Makarow 1988). In contrast, organisms that lack a cell wall may deactivate exocytosis during cell division, as secretion is not required for their passage through cell division, until cytokinesis, when fusion of secretory vesicles with the plasma membrane may facilitate closure of the intercellular bridge (Robinson & Spudich 2000). The need for secretion during mitosis places constraints on the mechanism of Golgi inheritance, since a functional Golgi apparatus must be maintained throughout the cell cycle. Thus, in such organisms the Golgi apparatus is not vesiculated or returned to the ER during mitosis, but rather it is partitioned between nascent daughter cells as preexisting discrete units (Preuss et al. 1992, Nebenführ et al. 2000). This may require their division by medial fission as in *Toxoplasma*, *Trypanosoma*, and *Trichomonas* (Hager et al. 1999, Field et al. 2000, Benchimol et al. 2001, Pelletier et al. 2002). However, this is not as dramatic as the morphological transformation that occurs in mammalian cells (Lucocq & Warren 1987, Lucocq et al. 1987, 1989). Because the mammalian Golgi apparatus is a single-copy organelle, restricted to a tight juxtanuclear locale, the problem is to evenly distribute it between progeny. This problem is circumvented by a shutdown of exocytosis, a concurrent fragmentation of the Golgi apparatus into thousands of vesicles, and their dispersal throughout the cell that partitions equal amounts of Golgi membrane to the nascent progeny (Figure 1*B*) (Warren 1993). This naturally occurring phenomenon provides a unique opportunity to deconstruct the molecular landscape behind the establishment, biogenesis, and maintenance of Golgi architecture.

GOLGI APPARATUS ARCHITECTURE DURING THE MAMMALIAN CELL CYCLE

Interphase Biogenesis

During interphase, the complex architecture of the Golgi apparatus must duplicate in preparation for appropriate segregation between progeny. How this process occurs and is regulated remains largely mysterious. Many EM and fluorescence images from disparate organisms give the impression that this proceeds by lateral growth followed by medial fission (Troyer & Cameron 1980, Garcia-Herdugo et al. 1988, Hager et al. 1999).

However, it is unclear whether the mammalian Golgi apparatus is constantly increasing in size throughout G1, S, and G2, or whether it doubles in size during S-phase, as with the nuclear DNA and the total cellular phospholipid (Jackowski 1996). In this regard, it is interesting that the number of ER exit sites doubles between G1 and G2 (Hammond & Glick 2000), perhaps in response to a signal for increased secretory output. It may be that a strictly regulated replication event occurs during S-phase that may even require licensing factors to ensure it only happens once every cell cycle. Doubling phospholipid mass at S-phase is uncoupled from DNA replication as inhibitors of DNA replication do not affect phospholipid

accumulation, and inhibition of phospholipid synthesis has no immediate effect on DNA replication (Boggs et al. 1995). Similarly, arresting cells at the G1/S boundary with the DNA synthesis inhibitor aphidocolin does not block Golgi biogenesis. Instead, the Golgi continues to increase in size concomitant with increasing cell size. Upon release from the aphidocolin block, cells proceed normally through mitosis, but the number of mitotic Golgi clusters (MGCs) that facilitate Golgi inheritance increases (relative to cells not treated with aphidocolin) proportionate to the increased Golgi and cell size (Shima et al. 1997). Thus, cell and Golgi size appear to be tightly coupled, yet precisely how cell size is regulated is also obscure, but likely reflects complex interplay between competing extracellular cues and their downstream intracellular transcriptional networks (Conlon & Raff 1999).

The mechanism and regulation of Golgi duplication prior to cytokinesis may be intimately coupled to mechanisms that regulate the amount and composition of the organelle that occur in response to secretory demand. Golgi compartments increase or decrease in surface and volume in precise accordance with the amount of membrane flux through them (Griffiths et al. 1989, Rambourg & Clermont 1997, Morin-Ganet et al. 2000). This is also apparent when comparing different cell types; for example, the surface and volume of Golgi compartments is very large in exocrine pancreatic secretory cells, but scant in reticulocytes where there is very little secretion.

At the simplest level, the biogenesis of any organelle requires that the cell coordinately increase the synthesis of the proteins and lipids that comprise it. For the Golgi apparatus this reflects a combination of input and selective retention of material synthesized in the ER, coupled with Golgi-mediated sphingolipid biosynthesis and the assembly of peripheral membrane protein complexes on the cytosolic face of the Golgi membrane. Golgi components synthesized in the ER arrive in vesiculotubular clusters (VTCs) at the CGN (Presley et al. 1997, Scales et al. 1997, Marra et al. 2001) and are sorted to their correct location in the CGN, stack, or TGN by a combination of retrieval and retention mechanisms that depend on both protein-protein (Nilsson et al. 1993, 1994, 1996; Slusarewicz et al. 1994; Barr et al. 2001) and protein-lipid interactions (Bretscher & Munro 1993, Munro 1998). Golgi sphingolipid biosynthesis and increasing sterol concentration (and so bilayer thickness) towards its *trans*-face may facilitate lipid-based sorting mechanisms that may distinguish transmembrane cargo by the length of their transmembrane domain (Munro 1998). Hence, the biosynthetic sphingolipid output of the Golgi could help establish its biochemical and morphological polarity (Bretscher & Munro 1993, Holthius et al. 2001).

Golgi residents are filtered away from the secretory cargo, which as a consequence is distilled at the exit face or TGN (Orci et al. 1997, 1998, 2000a,b). This anterograde membrane flow is counterbalanced by retrograde membrane flow to maintain the surface area of previous compartments, ensure escaped ER residents return to the ER, and to recycle transport machinery required for anterograde flow (Lippincott-Schwartz et al. 2000). This antagonistic anterograde/retrograde

membrane flux establishes a finely tuned equilibrium between the acquisition and removal of membrane. Despite this dynamic equilibrium, a Golgi structure is generated that exhibits both morphological and biochemical polarity (Farquhar 1985, Nilsson et al. 1993, Orci et al. 1998, Ladinsky et al. 1999). Golgi matrix proteins targeted from the cytoplasm may coordinate or maintain this polarity (Ward et al. 2001, Yoshimura et al. 2001, Pfeffer 2001) and ensure that the Golgi architecture is robust despite this huge membrane flux through it (White et al. 2001). Given the rate of membrane flow, it is likely that such a matrix is highly dynamic to keep pace with these changes (Ward et al. 2001, Marra et al. 2001), and consequently must possess sophisticated self-organizing and/or assembly properties (Kirscher et al. 2000, Misteli 2001).

Golgi identity and function is highly dependent on membrane-fusion events: first, vesicle transfer between different compartments (heterotypic fusion) and second, the maintenance of the delimiting membrane of a compartment by fusion with identical copies (homotypic fusion). Golgi heterotypic fusion is controlled by NSF (Malhotra et al. 1988), and homotypic fusion may be controlled by p97, both highly conserved AAA ATPases (Patel & Latterich 1998). Provided each enveloping membrane has markers that determine specificity and identity, the differential content of compartments naturally follows. Compartmental identity is achieved in part by the inherent specificity of cognate interactions that occur between members of the SNARE [soluble N-ethylmaleimide sensitive factor (NSF) attachment protein (SNAP) receptor] superfamily (Scales et al. 2000), whereby, in the simplest sense, a unitary v-SNARE on a vesicle interacts uniquely with its cognate, three-component t-SNARE on an acceptor membrane (Pelham 2001a). This interaction, termed a *trans*-SNARE complex or SNAREpin, docks a vesicle to its target membrane and either induces spontaneous bilayer mixing (Chen et al. 1999, McNew et al. 2000a) or signals to downstream components that directly catalyze fusion (Ungermann et al. 1998, Peters et al. 2001). However, specificity is further predicated by a preceding layer of regulation, termed vesicle tethering. Vesicle tethering is a SNARE-independent event and requires the activity of peripheral membrane proteins, which are often extended coiled-coil fibrous proteins or large multiprotein complexes, and seems to be coordinated by Rab-GTPases (Zerial & McBride 2001).

The coordinate increase in synthesis of organellar components is achieved by transcriptional networks that sense fluctuations in demand for organelle function, and induce altered expression of genes encoding organellar proteins (Nunnari & Walter 1996). This is perhaps most clear in the case of the unfolded protein response (UPR), where unfolded proteins in the lumen of the ER are sensed by the lumenal domain of the transmembrane kinase Ire1p (Patil & Walter 2001). Ire1p transmits this information to the cytoplasm and directly facilitates the translation of the transcription factor Hac1p, which induces the upregulation of a suite of compensatory proteins (Travers et al. 2000). These include ER chaperones and translocation machinery as well as glycoslyation enzymes, lipid biosynthetic enzymes, and vesicle transport proteins at all levels of the secretory pathway (Travers

et al. 2000). The UPR induces the coordinated biogenesis of ER/Golgi protein and membrane (Cox et al. 1997, Travers et al. 2000). By extension, a process akin to or perhaps even overlapping with the transcriptional network sparked during the UPR probably regulates the biogenesis of the ER and Golgi apparatus in coordination with an increase in cell size and/or stage of the cell cycle. The precise delineation of pathways that control cell size or signal for increased secretion may illuminate how organelle biogenesis is induced and regulated.

Can a Golgi apparatus be generated de novo? When severe depletion of Golgi membranes has been induced experimentally, new Golgi membranes can reform only extremely slowly (Flickinger 1968, Zorn et al. 1979, Maniotis & Schliwa 1991). In contrast, when Golgi membranes are completely depleted in microsurgically derived cytoplasts, there appears to be no method to regenerate them anew from proximal or distal compartments despite their previous connection by continuous membrane traffic (Pelletier et al. 2000, Klumperman 2000). Secretory cargo such as CD8 and VSVG are packaged into COPII vesicles as usual and exit the ER to form VTCs that are mobile on microtubules (Pelletier et al. 2000). These VTCs mature to the COPI/p115 positive stage (Pelletier et al. 2000), the final stage of their maturation before becoming *cis*-cisternae (Scales et al. 1997, Marra et al. 2001). However, the VTCs remain trapped at this stage and do not become a Golgi stack, possibly due to the absence of the *cis*-Golgi matrix components GM130 and GRASP65 that facilitate the late stages of VTC maturation into *cis*-Golgi cisternae (Marra et al. 2001). If Golgi matrix components are dispersed by brefeldin A (BFA) treatment prior to microsurgery, a functional Golgi apparatus can reform in cytoplasts upon BFA washout (Pelletier et al. 2000). BFA is a fungal metabolite that prevents nucleotide exchange onto the ARF1-GTPase and leads to loss of ARF1 and COPI binding to Golgi membranes (Lippincott-Schwartz et al. 2000). This induces Golgi resident enzymes to relocate to the ER and Golgi matrix proteins to disseminate throughout the cytosol (Seemann et al. 2000a). Note that the reformation of a Golgi apparatus after BFA washout does not constitute de novo biogenesis as defined here, since it occurs in the presence of preexisting Golgi matrix structures (Seemann et al. 2000a). The Golgi apparatus then appears to be an autonomous organelle, responsible for its own replication and partitioning.

Conversely, if the perinuclear recycling endosome is excluded from cytoplasts, it is able to reform de novo from a combination of vesicles derived from early endosomes and cytosolic factors (Sheff et al. 2002), in a manner similar to the formation of late endosomes from early endosomes (Mellman 1996). This variability in propensity of organelles of the endomembrane system to assemble de novo may reflect differences in their architectural complexity that is intimately coupled to their function. Whereas the Golgi apparatus performs essential glycosylation and sphingolipid biogenesis as well as sorting, endosomes exclusively sort. Thus, endosomes possess only a simple architecture that can be synthesized de novo, whereas the elaborate Golgi architecture necessitates autonomous replication and division.

Consequently, all the information required to efficiently construct the Golgi apparatus may not reside solely in the genetic material. Rather, within the elaborate structure of the Golgi apparatus resides epigenetic information, or a template, that preexists in the architecture of Golgi cisternae, and which may represent the site for the deposition of newly synthesized material (Lewin 1998). It may even be that components of the Golgi matrix, a proteinaceous scaffold that persists after the extraction of Golgi membranes with detergent (Slusarewicz et al. 1994, Nakamura et al. 1995, Fath et al. 1997), represent such a Golgi template (Seemann et al. 2000a, 2002; Pfeffer 2001; Lowe 2002). This structural matrix or template contains the GRASP family of Golgi stacking proteins (Barr et al. 1997, Shorter et al. 1999), the Golgin family of long, fibrous, extensively coiled-coil peripheral and transmembrane proteins (Seemann et al. 2000a,b; Short et al. 2001) and a spectrin/ankyrin framework (De Matteis & Morrow 2000). Indeed, both GRASP65 and GM130 appear to be deposited directly onto existing Golgi matrix upon their translation by cytosolic ribosomes (Yoshimura et al. 2001). Transmission of such a preformed structure to offspring is achieved by inheritance of the organelle itself. It may even be that the conformation of a protein or set of proteins is the inherited element. Such a structure is likely to possess highly dynamic, self-organizing and flexible properties (Kirschner et al. 2000, Misteli 2001) so that it can accommodate and facilitate maturing cisternae and vesicle transfer while maintaining both Golgi biochemical and morphological polarity (Pelham 2001b, Pfeffer 2001). However, it is fundamentally different from structures required for formation of recycling endosomes since it cannot be formed anew (Pelletier et al. 2000, Sheff et al. 2002). Even if it can be formed anew, but at a very slow rate, its presence or inheritance facilitates the rapid biogenesis of the organelle. Therefore, it defines the autonomous identity of the Golgi apparatus and is instructive for Golgi biogenesis. Analogy may be drawn to the centrioles of *Chlamydomonas*, where templated assembly of centrioles occurs at twice the rate of de novo assembly (Marshall et al. 2001).

Prophase

Ultrastructural and fluorescence analyses on live and fixed cells have provided deep insight into the dynamic morphological changes that occur in Golgi architecture during mammalian M-phase. At the onset of prophase, the compact juxtanuclear Golgi ribbon fragments into its constituent stacks, which migrate to encircle the nucleus (Figure 2, 3) (Lucocq et al. 1987; Misteli & Warren 1995a; Shima et al. 1997, 1998; Zaal et al. 1999; Jokitalo et al. 2001). This migration appears to be coincident with that of the centrosomes to opposite poles of the nucleus and likely reflects the redistribution of microtubules that occurs at this stage. A compact pericentriolar Golgi ribbon is contingent upon an intact microtubule cytoskeleton and is maintained by dynein motors (Corthesy-Theulaz et al. 1992). Disrupting microtubules with nocodazole fragments the Golgi ribbon into its constituent stacks and redistributes them to the cell periphery, where they are

often in proximity to ER exit sites (Rogalski et al. 1984, Cole et al. 1996, Shima et al. 1998, Hammond & Glick 2000). Indeed, the Golgi apparatus itself can nucleate the formation of microtubules, by virtue of a subset of γ-tubulin that resides on its cytoplasmic face, emphasizing the intimate relationship between Golgi and centrosome migration at prophase (Chabin-Brion et al. 2001).

Prometaphase to Anaphase: Mitotic Golgi Clusters as the Unit of Golgi Inheritance

The fate of the perinuclear Golgi stacks at the onset of metaphase has recently been a subject of considerable debate (Figure 2) (Shima et al. 1997, 1998; Jesch & Linstedt 1998; Farmaki et al. 1999; Zaal et al. 1999; Prescott et al. 2001; Jokitalo et al. 2001; Jesch et al. 2001b). At the onset of prometaphase, the perinuclear Golgi stacks fragment into a disseminated array of tubulovesicular clusters, termed MGCs (Lucocq et al. 1987, 1989; Lucocq & Warren 1989; Pypaert et al. 1993, Misteli &Warren 1995a; Shima et al. 1997, 1998; Zaal et al. 1999; Jokitalo et al. 2001). This dramatic morphological transfiguration occurs coincident with high levels of CDK1 kinase activity, which also triggers nuclear envelope disassembly and microtubule rearrangements (Nigg 2001). Fragmentation reflects, at least in part, the mitotic inhibition of heterotypic and homotypic Golgi fusion events, which shift the dynamic Golgi equilibrium from stacked Golgi cisternae to clusters of vesicles and tubular remnants (Warren 1993). The precise morphological composition of MGCs varies in a cell type–dependent manner. In HeLa cells (Lucocq et al. 1987), PtK-1 cells (Schroeter et al. 1985), parotid acinar cells (Tamaki & Yamashina 1991), NRK cells (Burke et al. 1982, Seemann et al. 2002), and thyroid epithelia (Zeligs & Wollman 1979), these clusters are composed of 50- to 70-nm vesicles, whereas in L929 fibroblasts (Moskalewski & Thyberg 1990), melanoma cells (Maul & Brinkley 1970), and chondrocytes (Moskalewski et al. 1977), the clusters are more tubular and even cisternal in composition. MGCs contain all the Golgi resident enzymes and peripheral membrane proteins so far tested, with the exceptions of p115 (Shima et al. 1997, Lowe et al. 2000), βIII spectrin (Beck et al. 1994), and Golgi ankyrin (Beck et al. 1997). This depletion of Golgi-associated p115, spectrin, and ankyrin likely plays a substantial role in the dissolution of Golgi form.

Precisely why the Golgi stacks fragment further into MGCs is not clear, since accurate Golgi inheritance can occur via these intermediates and does so in organisms that maintain a cell wall during mitosis. Further fragmentation may ensure very accurate stochastic inheritance or may be a consequence of the mitotic inhibition of vesicle-mediated transport, allowing ATP conservation solely for the purposes of cell division. However, the dramatic changes in Golgi morphology may have additional functions such as the release of essential mitotic factors that are usually sequestered in the Golgi stack. For example, the RIIα subunit of protein kinase A is associated with the Golgi apparatus during interphase, but is released during mitosis to occupy a distinct subcellular position (Keryer et al. 1998). Thus Golgi

framentation per se may be required for successful passage through mammalian M-phase (Sutterlin et al. 2002).

Just as with the interphase Golgi ribbon, MGCs are embedded in an electron-dense matrix (Mollenhauer & Morré 1978, Cluett & Brown 1992, Lucocq et al. 1987, Pypaert et al. 1993) and are often found in close proximity to ER exit sites (Lucocq et al. 1989, Pypaert et al. 1993, Prescott et al. 2001). What happens to the MGCs is where the controversy arises (Figure 2). The majority of evidence demonstrates that MGCs, which shed vesicles to varying degrees, persist throughout the subsequent phases of mitosis, and serve to nucleate Golgi reassembly that occurs during telophase (Figures 2*A*, 3) (Moskalewski et al. 1977; Burke et al. 1982; Schroeter et al. 1985; Lucocq et al. 1987, 1989; Moskalewski & Thyberg 1990; Pypaert et al. 1993; Souter et al. 1993; Misteli & Warren 1995a; Asada &Yagura 1995; Shima et al. 1997, 1998; Jesch & Linstedt 1998; Farmaki et al. 1999; Lowe et al. 2000; Prescott et al. 2001; Jokitalo et al. 2001; Jesch et al. 2001b). By prometaphase the MGCs have relocated in a centripetal manner and are concentrated in radial arrays surrounding the nascent mitotic spindle asters (Figure 3) (Shima et al. 1998, Whitehead & Rattner 1998, Jokitalo et al. 2001). The radial arrays of MGCs further separate just prior to metaphase. One subpopulation remains in close apposition to the mitotic spindle pole, while another subpopulation is dispersed into the cell periphery by interactions with astral microtubules (Figure 3) (Shima et al. 1998). In addition, MGCs relocalize to either spindle pole to finely balance the amount of Golgi membrane on either side of the metaphase plate (Jokitalo et al. 2001). The mechanisms that sense and adjust such imbalance are obscure, but highly accurate Golgi partitioning is achieved during prometaphase.

The extent to which these MGCs fragment and shed vesicles into the cell periphery via the astral microtubules appears to vary extensively. Initial EM studies revealed that metaphase HeLa cells contain 10–300 Golgi clusters, and those with fewer clusters have larger numbers of free vesicles, suggesting clusters shed vesicles as they proceed through mitosis (Lucocq et al. 1989). This has also been documented by the loss of fluorescence from MGCs that occurs on proceeding from prophase through anaphase (Lowe et al. 2000, Jokitalo et al. 2001, Jesch et al. 2001b). Indeed, it appears that the mitotic Golgi is in dynamic equilibrium between clustered and free vesicles. Disruption of microtubules during M-phase leads to a rapid aggregation of Golgi vesicles with clusters to make larger structures, suggesting that this equilibrium is maintained by microtubule motors (Jesch et al. 2001b).

During anaphase the Golgi remains in equilibrium between MGCs and free Golgi vesicles. MGCs remain associated with the separating spindle poles during anaphase and persist in this orientation until midtelophase (Figure 3) (Shima et al. 1998, Jokitalo et al. 2001, Seemann et al. 2002). MGCs are now static, remaining stably associated with the spindle pole, and no further transfer of material between opposite poles occurs (Jokitalo et al. 2001).

Remarkably, despite this dramatic morphological transformation, the MGC retains the *cis-trans* polarity of resident proteins observed in the interphase Golgi

stack (Shima et al. 1997). That a polarized remnant of the Golgi apparatus persists through mitosis is highly suggestive of an underlying template that organizes the biochemical architecture of Golgi membranes. This template persists independently of stacked, cisternal Golgi architecture and so may even represent a blueprint for the subsequent, rapid reassembly of Golgi stacks at telophase. This underlying matrix persists even when Golgi enzymes are returned to the ER by treatment with BFA (Seemann et al. 2000a). Remarkably, if these cells are then allowed to pass through mitosis, these Golgi structural elements are accurately partitioned between progeny using the mitotic spindle poles and astral microtubules in a manner virtually indistinguishable from normal Golgi inheritance (Figure 3) (Seemann et al. 2002). This is consistent with the ability of the Golgi matrix to move along microtubule tracks even in the absence of membranes (Fath et al. 1997). Like MGCs, matrix structures are found in proximity to, but distinct from, ER exit sites (Lucocq et al. 1989, Prescott et al. 2001, Seemann et al. 2002). This argues that a Golgi structural template is the unit of Golgi inheritance and can specify the rebuilding of a functional Golgi apparatus at any time (Seemann et al. 2000a, 2002; Pelletier et al. 2000).

Intriguingly, one component of the Golgi matrix, GRASP65, may also function as a component of the spindle checkpoint in yeast (Norman et al. 1999). This highly conserved cell-cycle checkpoint arrests cells in metaphase until all pairs of sister chromatids are attached to the mitotic spindle, and it is consistently defective in human tumor cell lines (Nigg 2001). Deletion of GRH1 (the yeast GRASP65 homologue) causes defects in the spindle checkpoint (Norman et al. 1999). Although the precise role played by Grh1p in the spindle checkpoint is unclear, it is intriguing that a Golgi structural protein is required for the successful passage through mitosis, especially since in mammalian cells, GRASP65 only becomes exposed to the cytoplasm during mitosis (Barr et al. 1997). One possibility is that part of the spindle checkpoint also involves successful alignment of all templates to be inherited by daughter cells, and not only alignment of chromosomes and centrosomes. Thus, cells will not proceed to anaphase until all the cellular components (including the Golgi units of inheritance) to be inherited are correctly allocated between nascent progeny.

Prometaphase to Anaphase: A Merged ER/Golgi Compartment as the Unit of Golgi Inheritance

The view of the MGC as the unit of Golgi inheritance has been challenged by Lippincott-Schwartz and colleagues, based on the hypothesis that the Golgi apparatus is in dynamic equilibrium with the ER (Figure 2*B*) (Zaal et al. 1999, Lippincott-Schwartz et al. 2000). During interphase it is possible to perturb this equilibrium with BFA or by blocking ER export with dominant negative forms of Sar1p which cause a redistribution of Golgi enzymes to the ER (Lippincott-Schwartz et al. 1989, Girod et al. 1999, Seemann et al. 2000a, Miles et al. 2001). Since ER exit is blocked at mitosis (Featherstone et al. 1985, Farmaki et al. 1999,

Prescott et al. 2001), it is suggested that Golgi inheritance is achieved by a sudden, bulk retrograde flow of Golgi residents to the ER that occurs during prometaphase (Zaal et al. 1999). The MGC is suggested to be the transport intermediate between Golgi and ER (Zaal et al. 1999, Lippincott-Schwartz et al. 2000). Golgi residents are held in the ER until telophase when the mitotic inhibition of ER exit is relieved (Featherstone et al. 1985, Souter et al. 1993). COPII vesicle formation is blocked at mitosis since COPII coat protomers redistribute from the ER to the cytosol (Farmaki et al. 1999, Hammond & Glick 2000, Prescott et al. 2001). At telophase ER exit resumes, and Golgi residents emerge to reform the Golgi in a manner akin to recovery from BFA. Since the ER is extremely well dispersed throughout the cell during mitosis, such relocation of Golgi residents would ensure accurate Golgi inheritance as a consequence of stochastic ER inheritance (Zeligs & Wollman 1979).

Mitotic Golgi dynamics were observed using predominantly transient expression of the Golgi enzymes GalT and MannII whose lumenal domains were tagged with a triple GFP concatamer (Zaal et al. 1999). This indicated that the Golgi fragmented, as described previously in prophase (Shima et al. 1997, 1998), but then displayed a diffuse staining pattern from metaphase through to telophase that qualitative EM and fluorescence recovery after photobleaching (FRAP) suggested may correspond to the ER. At telophase this diffuse staining pattern reverted to a Golgi ribbon staining (Zaal et al. 1999).

Subsequent studies suggest that many of these results were the consequence of slow or abortive folding of the GalT-GFP constructs used, leading to their mislocalization in the ER (Prescott et al. 2001, Jokitalo et al. 2001, Jesch et al. 2001b, Seemann et al. 2002). Under steady-state interphase conditions, ~30% of this GalT-GFP was found in the ER (Zaal et al. 1999), suggesting that the triple GFP concatamer may be causing misfolding or mislocalization of the enzyme (Jokitalo et al. 2001). All previous studies have found only trace GalT in the ER, unless the enzyme is highly overexpressed (Lucocq et al. 1987; Nilsson et al. 1991, 1993, 1994). One study found that MannII was present in the ER and MGCs of metaphase cells (Thyberg & Moskalewski 1992), but this result could be due to the accumulation of newly synthesized enzyme (Farmaki et al. 1999).

In addition, when Golgi and ER membranes have been covisualized during mitosis by immunofluorescence their patterns are clearly distinct (Jokitalo et al. 2001, Jesch et al. 2001b). MGCs can readily be seen between gaps in the complex ER network of tubules throughout mitosis, even in cells stably expressing the GalT-GFP used by Zaal et al. (Jokitalo et al. 2001). Furthermore, when the MGCs have fragmented further to disseminated Golgi vesicles, as in HeLa cells, the diffuse staining is clearly distinct from an ER stain, with the ER being largely excluded from the spindle pole/midbody region, in contrast to Golgi markers (Jesch et al. 2001b). The diffuse haze reported by Zaal et al. may well be 50–70-nm Golgi vesicles that have been shed from MGCs (Lucocq et al. 1989, Jokitalo et al. 2001, Jesch et al. 2001b). These vesicles may be moving rapidly on microtubules during

metaphase, and this might help explain the rapid FRAP observed by Zaal et al. (Jesch et al. 2001b).

Biochemical fractionation of mitotic cells shows that the ER and the Golgi apparatus remain entirely independent during mitosis (Jesch & Linstedt 1998, Jesch et al. 2001b, Seemann et al. 2002). Furthermore, immunoisolation of MGCs with anti-GM130 antibodies causes no coprecipitation of multiple ER markers, such as PDI, calnexin, or components of the COPII coat (Seemann et al. 2002). Merger can only be detected by biochemical means if Golgi residents are first redistributed to the ER with BFA prior to entering mitosis (Jesch & Linstedt 1998, Jesch et al. 2001b, Seemann et al. 2002). Even under this condition, components of the Golgi matrix (i.e., Golgins and GRASPs) cannot be detected in the ER by either biochemical, immunofluorescence, or immunogold EM techniques (Jesch & Linstedt 1998, Jesch et al. 2001b; Seemann et al. 2002). This template may then be the essential unit of Golgi inheritance, especially if it is involved in the regulation of the spindle checkpoint (Norman et al. 1999). Furthermore, BFA no longer redistributes Golgi residents to the ER if added during mitosis (Jesch & Linstedt 1998), suggesting that Golgi to ER retrograde transport, like many membrane fusion events, is inhibited during mitosis (Warren 1993). This is the exact opposite of the proposed 20-fold increase in the rate of Golgi to ER retrograde transport necessary to explain the sudden merger of Golgi and ER at prometaphase (Zaal et al. 1999).

The fact that the Golgi matrix can partition in the absence of the enzyme-containing membranes that normally populate this structure (Figure 3) provides at least a partial resolution of the discrepancies in Golgi localization during mitosis reported by many groups. It may not matter whether the enzymes arrive in progeny via the ER or with the Golgi matrix fraction, provided there is accurate inheritance of the matrix (Seemann et al. 2002). It may even be that as long as the Golgi matrix is inherited the Golgi apparatus can be rapidly regenerated by the population of this structure with de novo synthesized Golgi enzymes. The underlying matrix would then be instructive for rapid Golgi biogenesis. This leaves open the question as to the mechanism adopted by the enzymes for their inheritance. It may be that they are mostly carried by the matrix fraction into the daughter cells during normal cell division. There may, however, be occasions when they need to be inherited via the ER, e.g., when the enzymes are redistributed to the ER by BFA in the preceding interphase (Seemann et al. 2002). Such movement might even reflect mitotic processes other than inheritance. More experimentation is required to examine the consequences of the route taken by Golgi enzymes.

Telophase

At telophase, within minutes of a sudden decrease in CDK1 activity, MGCs are transformed into discrete stacks of cisternae in a 10-min period. The tubules and vesicles of MGCs fuse with incoming Golgi vesicles to generate first short single cisternae, which then stack (Lucocq et al. 1989, Souter et al. 1993). Each layer of

the stack then grows laterally, rather than individual cisternae growing and then stacking. Once a certain critical size is reached, these Golgi stacks coalesce in the pericentriolar, juxtanuclear region, utilizing microtubules, and interconnect to reform the Golgi ribbon (Figure 3) (Corthesy-Theulaz et al. 1992; Shima et al. 1997, 1998). This process entails the extension of tubules that link the discrete stacks to recreate a ribbon-like structure (Shima et al. 1997). Inhibition of centrosome migration to the juxtanuclear region by perturbing HsEg5 (a BimC kinesin-like motor) function with microinjected antibodies inhibits the coalescence of the discrete Golgi stacks at this stage (Whitehead & Rattner 1998). Golgi stacks reassemble before the arrival of proteins transported from the ER (Souter et al. 1993), strongly suggesting that Golgi reassembly is independent of the ER. The extreme rapidity of Golgi reassembly makes it intrinsically difficult to study in vivo. However, a cell-free system that mimics many of these events has begun to elucidate the underlying molecular mechanisms (see below).

Cytokinesis

At cytokinesis two pools of Golgi membranes can often be found on opposite sides of the nucleus (Figure 3) (Shima et al. 1998, Seemann et al. 2002). One pool is in close proximity to the intercellular bridge that connects the separating cells. This pool of Golgi membranes may be due to the centrosomal migration to this area of the cell that is essential for daughter cell separation (Piel et al. 2001). In order for cells to divide there must be a membrane-fusion event either between the inner leaflets of the plasma membrane or between vesicles and the plasma membrane to seal the intercellular bridge (Robinson & Spudich 2000). The reorientation of the centrosome/Golgi may reflect the need to direct secretion toward the intercellular bridge and ensure plasma membrane sealing during cell separation. Testing this possibility will require uncoupling centrosome and Golgi migration.

MOLECULAR MECHANISMS OF GOLGI DISASSEMBLY

Cell-Free Assays to Deconstruct Mitotic Regulation of Golgi Architecture

Although the in vivo ultrastructural and fluorescence studies described above provide an initial understanding of mitotic Golgi behavior, a more reductionist approach is required to deconstruct the molecular workings of these processes. Thus, semi-intact cell assays (Acharya et al. 1998, Kano et al. 2000) and a cell-free system have been developed to reconstruct these events (Misteli &Warren 1994; Rabouille et al. 1995a,b; Shorter & Warren 1999). Cells gently permeabilized with digitonin and extracted with high salt can be incubated with mitotic cytosol, and changes in Golgi morphology monitored by immunofluorescence or EM. So far, this approach has only reconstituted the fragmentation phase of the process. In contrast, a cell-free system has reconstituted both the fragmentation and reassembly

phases of the Golgi inheritance cycle. Highly purified rat liver Golgi stacks (RLG) are incubated in mitotic cytosol to generate mitotic Golgi fragments (MGF). These fragments, on incubation with interphase cytosol or purified cytosolic components, will reassemble into Golgi stacks (Figure 4). This provides a readily manipulatable biochemical system within which the sequence of morphological events can be precisely followed by quantitative EM. That the Golgi apparatus can be systematically disassembled and reassembled in vitro demonstrates the innate self-organizing potential of this organelle and its underlying structural matrix (Misteli 2001).

Initial Golgi Ribbon Breakdown

A semi-intact NRK cell assay has indicated a role for Polo-like kinase 1 (Plk1) and MEK1 in the initial fragmentation of the Golgi ribbon into its constituent stacks, which occurs during early prophase (Acharya et al. 1998, Colanzi et al. 2000, Sutterlin et al. 2001). Plk1 phosphorylates GRASP65, and this may facilitate the initial fragmentation of the Golgi ribbon and cisternal unstacking (see below). MEK1 seems to activate a novel Golgi-associated ERK activity, distinct from ERK1 and ERK2, that is essential for Golgi fragmentation (Acharya et al. 1998, Colanzi et al. 2000). However, tyrosine-phosphorylated ERK2 specifically accumulates in the nucleus and on the Golgi in late G2/prophase, and elevation of tyrosine-phosphorylated ERK2 by increased MEK1 expression appeared to disrupt the Golgi apparatus at the light microscope level (Cha & Shapiro 2001). These effects were independent of ERK2 kinase activity, but did require the tyrosine phosphorylation of ERK2. However, since no EM observations were made in this study, it remains unclear to what extent this activity reflects mitotic disassembly (Cha & Shapiro 2001).

It is proposed that the MEK1 activity required for Golgi fragmentation occurs uniquely in mitosis, and does not require ERK1 or ERK2 activation since it is

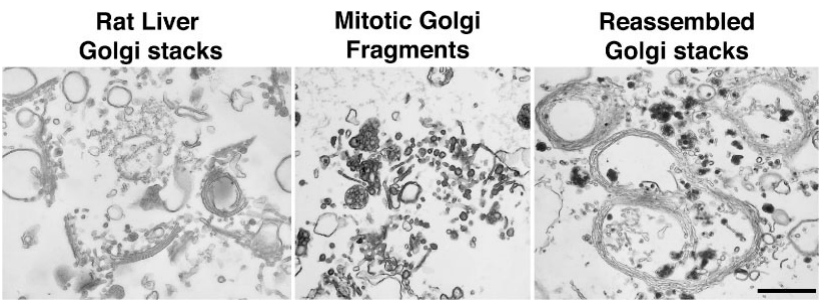

Figure 4 Cell-free mitotic Golgi fragmentation and reassembly. Rat liver Golgi stacks (*left panel*) were incubated with mitotic cytosol to generate a population of mitotic Golgi fragments (*middle panel*). Incubation of mitotic Golgi fragments with interphase cytosol regenerates stacked Golgi architecture (*right panel*). Bar, 0.5 μm.

supported by a form of MEK1 that is proteolytically cleaved by the anthrax toxin (Colanzi et al. 2000). Consistent with this proposal, cells pass through mitosis and fragment their Golgi normally in the presence of anthrax toxin (Lowe et al. 1998). How the MEK1/ERK pathway induces initial mitotic Golgi fragmentation awaits the clarification of downstream effector molecules. Presumably, these effectors should be activated by MEK1 that has been proteolytically cleaved by the anthrax toxin, but not in the presence of PD98059 (Colanzi et al. 2000), and may provide the means to identify them. However, Golgi fragmentation proceeds normally in vivo in the presence of the specific MEK1 inhibitors PD98059 and U0126 (Lowe et al. 1998, Draviam et al. 2001), suggesting that the requirement for MEK1 can be bypassed. In addition, MEK1 activation is not yet considered a general feature of the mammalian cell cycle (Abrieu et al. 2001, Nigg 2001). Thus, MEK1 may facilitate fragmentation of the Golgi ribbon but may not be an obligatory step.

COPI-Dependent Fragmentation

In another semi-intact cell assay, this time using MDCK cells, sequential roles for MEK1 and CDK1 were perceived during mitotic fragmentation (Kano et al. 2000). MEK1 was required for the initial breakdown of the Golgi ribbon into large punctate fragments, which by EM appear to be Golgi stacks. After this phase, CDK1 was required for the subsequent transformation of these stacks into dispersed Golgi vesicles (Kano et al. 2000). In the cell-free system, mitotic disassembly of Golgi stacks proceeds via two distinct, concurrent fragmentation pathways that require CDK1, but not MEK1 (Misteli & Warren 1994, 1995b; Lowe et al. 1998). The COPI-dependent pathway proceeds as COPI vesicles continue to bud from all levels of the Golgi stack (Sönnichsen et al. 1996), but are unable to tether and so fuse with their target membrane (Misteli &Warren 1994, Nakamura et al. 1997). This pathway likely consumes the peripheral rims of cisternae and accounts for up to 65% of the total cisternal membrane (Misteli & Warren 1994, 1995b; Sönnichsen et al. 1996). A COPI-independent pathway converts the flattened cisternal cores into a heterogeneous array of tubulovesicular profiles (Misteli &Warren 1995b).

A possible molecular explanation for the accumulation of COPI vesicles at mitosis is that the binding of p115 to Golgi membranes is significantly inhibited during mitosis (Figure 5) (Levine et al. 1996, Sohda et al. 1998, Lowe et al. 2000). p115 is a highly conserved, homodimeric vesicle-tethering protein, required for intra-Golgi (Waters et al. 1992, Seemann et al. 2000b) and ER-Golgi transport (Allan et al. 2000, Moyer et al. 2001) that juxtaposes membranes during interphase by simultaneously binding two Golgins, GM130 in one membrane and Giantin in the other. GM130 and Giantin are long, rod-like fibrous proteins due to an extensive coiled-coil domain structure typical of Golgins (Linstedt et al. 1993, Nakamura et al. 1995). GM130 is restricted to Golgi cisternae, whereas Giantin is also present in COPI vesicles (Nakamura et al. 1995, Sönnichsen et al. 1998, Martínez-Menárguez et al. 2001). Thus, p115 may tether COPI vesicle to cisterna or cisterna to cisterna, depending upon the topological restriction of Giantin, and so

couple stacked Golgi structure to processive COPI vesicle flow (Shorter & Warren 1999; Linstedt 1999; Orci et al. 1998, 2000b). Thus, COPI vesicles may flow along a Giantin-p115-GM130 axis or vector. At mitosis, the extreme basic N-terminal domain of GM130, comprising the p115 binding site, is directly phosphorylated on serine 25 by cyclin B-CDK1, with the effect of potently inhibiting p115 binding (Nakamura et al. 1997, Lowe et al. 1998). Although p115 can still bind Giantin (Sönnichsen et al. 1998), it is no longer able to cross-link to GM130. As a result, COPI vesicles accumulate, as they are unable to tether, and so fuse, and intra-Golgi transport is inhibited by CDK1 (Figure 5) (Collins & Warren 1992, Stuart et al. 1993, Fernández &Warren 1998).

In vivo, GM130 is phosphorylated during prophase at the onset of Golgi fragmentation, as revealed by an antibody that specifically recognizes GM130 phosphorylated on serine 25 (Lowe et al. 2000). GM130 remains phosphorylated until telophase, when it is dephosphorylated (at least in vitro) by PP2A containing the Bα regulatory subunit (Lowe et al. 2000). GM130 phosphorylation and dephosphorylation is synchronous with p115 dissociation and reassociation with Golgi membranes in addition to Golgi fragmentation and reassembly (Lowe et al. 2000). This strongly suggests that these events are intimately linked. Furthermore, ectopic expression of cyclin B-CDK1 in G0/G1 phase cells induces GM130 phosphorylation and a mitotic-like reorganization of the Golgi apparatus (Draviam et al. 2001).

A number of other mechanisms likely ensure the cessation of ER-Golgi and intra-Golgi transport during mitosis that may facilitate Golgi fragmentation and accurate Golgi inheritance. ER-Golgi transport is retarded since COPII vesicle formation is inhibited during mitosis owing to the release of COPII coat protomers from the ER to the cytosol (Famarki et al. 1999, Prescott et al. 2001). The mechanism behind this is obscure. The lack of input of membrane into the Golgi apparatus from the ER may contribute to Golgi fragmentation were the cisternae to mature themselves to extinction (Pelham 2001b). However, this explanation is unsatisfactory as intra-Golgi transport (and so vesicle transfer and cisternal maturation) also ceases at mitosis at both the *cis*-medial (Collins et al. 1992, Stuart et al. 1993) and medial-*trans* levels (Fernández & Warren 1998), and TGN to plasma membrane transport is also retarded (Hesketh et al. 1984, Oliver et al. 1985, Kreiner & Moore 1990, Kanki & Newport 1991). A block on ER exit could induce a redistribution of Golgi enzymes to the ER, as occurs when dominant negative Sar1p mutants are expressed (Seemann et al. 2000a, Ward et al. 2001). However, since redistribution of enzymes to the ER does not appear to occur during mitosis (Jokitalo et al. 2001, Jesch et al. 2001b, Seemann et al. 2002), possibly owing to a block in Golgi-ER retrograde transport (Jesch & Linstedt 1998, Farmaki et al. 1999), such a mechanism would not explain Golgi fragmentation.

Therefore, it is likely that mechanisms acting at the level of the intra-Golgi transport machinery induce Golgi fragmentation. Both heterotypic and homotypic fusion events may be inhibited within the Golgi apparatus. The best-defined is the inhibition of p115/GM130 binding that occurs at mitosis, which blocks COPI vesicle transfer (heterotypic fusion). However, p115 phosphorylation is also inhibited

during mitosis (Sohda et al. 1998). p115 phosphorylation by a CKII-like kinase native to Golgi membranes is an essential event for postmitotic Golgi reassembly, and may elicit a mechanical transition from COPI vesicle tethering (SNARE independent) to vesicle docking (SNARE dependent) (Dirac-Svejstrup et al. 2000, Shorter et al. 2002). Inhibition of this kinase activity during mitosis might reinforce the inhibition of p115/GM130 binding such that if any binding did occur, even then the COPI vesicle would not fuse with its acceptor compartment. The exact identity of the kinase is unknown.

Other hints as to the mechanisms of Golgi fragmentation have come from the cell-free system. For instance, the AAA ATPases NSF and p97 are depleted on MGF relative to starting RLG (Shorter & Warren 1999). NSF mediates COPI vesicle fusion with Golgi membranes (Malhotra et al. 1988), and although its adaptor α-SNAP is present on MGF, NSF is not (Shorter & Warren 1999). That p97 no longer binds suggests that Golgi homotypic fusion is also blocked. How these events are regulated is unclear, but one hint comes from the fact that the p97 cofactor p47 (Kondo et al. 1997) is phosphorylated by CDK1 during mitosis (Mayr et al. 1999). How this affects p97/p47 activity is unclear, but may preclude p47 binding to the Golgi t-SNARE syntaxin-5, which is essential for p97/p47 function in Golgi homotypic fusion (Rabouille et al. 1998).

Rab1 is also phosphorylated by CDK1 during mitosis, and this induces increased Rab1 membrane association (Bailly et al. 1991). Rab1 phosphorylation may alter interactions between Rab1 and its effectors GM130 and p115, and so affect COPI vesicle-tethering reactions (Allan et al. 2000, Moyer et al. 2001, Weide et al. 2001). This may also contribute to COPI-dependent fragmentation.

Finally, MGF contains only unengaged or disassembled SNAREs, i.e., there are no fully assembled (SDS-resistant) SNARE complexes and cognate SNAREs no longer coimmunoprecipitate from MGF, in contrast to RLG (Müller et al. 2002). The mechanism behind this inhibition of SNARE complex assembly is unclear, but may be a simple consequence of the inhibition of upstream vesicle-tethering events that are essential for SNARE assembly to occur (Shorter et al. 2002). p115 also catalyzes SNAREpin formation directly, so its release from the Golgi membrane at mitosis may ensure that neither vesicle tethering nor docking can occur (Shorter et al. 2002). However, it may also be that the SNAREs themselves are subject to direct mitotic regulation.

COPI-Independent Fragmentation

COPI-independent fragmentation converts the cisternal cores into a series of tubular networks, tubules, and heterogeneously sized vesicles, and is most clearly discerned when mitotic cytosol is depleted of coatomer (Misteli & Warren 1995b). This pathway consumes 40–50% of Golgi cisternae (Misteli & Warren 1994, 1995b), and the hallmark tubular network formation that indicates COPI-independent fragmentation is readily identifiable in vivo (Misteli & Warren 1995a). This fragmentation may result from a mitotic inhibition of p97-mediated homotypic

Golgi membrane fusion that constitutively antagonizes a background membrane-fission activity. In the absence of homotypic fusion, membrane fission could proceed unchecked. Membrane fission entails the inner lumenal leaflets of the membrane bilayer coming into apposition and fusing (Rothman & Warren 1994, Warren 1995, Misteli 1996). When this activity is coupled to ARF/coatomer a COPI vesicle forms, whereas in their absence a tubule or fenestration results. Relaxation of the Golgi scaffolding matrix at mitosis may allow more random collisions between the inner leaflets of cisternae, and may generate fenestrae in the midregions of the cisternae, and tubules/vesicles at the periphery (Warren 1995, Misteli 1996). The Golgi membrane fission activity of BARS-50 may be important in this process (Weigert et al. 1999). Alternatively, Golgi-specific endophilins may promote this activity (Farsad et al. 2001). Both BARS-50 and endophilin possess acyltransferase activity and may directly modify the lipid bilayer (converting lysophosphatidic acid to phosphatidic acid) in a manner conducive in certain orientations (e.g., at the constricted neck of a nascent vesicle) for membrane fission (Barr and Shorter 2000). Tubule formation may also require in some way cytosolic phospholipase A_2 isoforms (de Figueiredo et al. 1998). However, the precise molecular mechanisms involved in tubule formation are unclear. To what extent these factors contribute to COPI-independent Golgi fragmentation is also unclear.

Tubules and tubular networks are striking morphological features of the Golgi apparatus (Sciaky et al. 1997, Ladinsky et al. 1999). This has raised speculation that they may be involved in transport processes (Sciaky et al. 1997). Cell-free intra-Golgi transport can still occur under certain conditions where COPI vesicle formation is blocked, suggesting that tubules may substitute as transport vehicles (Elazar et al. 1994, Happe et al. 1998). In addition, Golgi enzymes return to the ER via a COPI-independent retrograde transport pathway that may utilize tubules as the transport vector (Girod et al. 1999). COPI-independent mitotic fragmentation may be a result of uncoupling these pathways from membrane fusion.

Unstacking Golgi Cisternae

Concomitant with the initial COPI-coupled and COPI-uncoupled fission of Golgi cisternae, the cisternae also unstack. The mean number of cisternae per stack decreases as fragmentation proceeds, both in vivo and in vitro (Misteli & Warren 1994, 1995a). Unstacking is rapid and is completed prior to the consumption of Golgi cisternae by the COPI-dependent and -independent pathways, suggesting that it may function to make more membrane surface available to cytosolic factors that catalyze these fragmentation reactions. The precise mechanism by which cisternae unstack is unclear, but must involve a relaxation or reconfiguration of the Golgi matrix that usually holds the cisternae together. The mitotic inhibition of GM130/p115 binding may contribute to unstacking, as p115 can link cisternae via bridging Giantin and GM130 in adjacent cisternae (Shorter & Warren 1999). However, this is unlikely to be the major stacking mechanism, as p115 can be removed from Golgi membranes with high salt without unstacking Golgi cisternae (Waters et al. 1992, Cluett & Brown 1992). Stacking does require protein-protein

interactions since it can be disrupted by mild proteolysis (Cluett & Brown 1992). Addition of certain proteases liberates single cisternae, which maintain their shape, suggesting that cisternal form is maintained by an intralumenal matrix inaccessible to proteases, perhaps composed of Golgi enzyme oligomers (Cluett & Brown 1992; Nilsson et al. 1994, 1996).

Another family of Golgi matrix proteins, the GRASPs, may provide this salt-resistant stacking activity. Two members of this family have so far been characterized: GRASP65 (Barr et al. 1997, 1998) and GRASP55 (Shorter et al. 1999, Short et al. 2001). Both GRASPs are required for the stacking of Golgi cisternae during reassembly and are highly conserved, oligomeric proteins that are N-terminally myristoylated (Barr et al. 1997, 1998). Each GRASP anchors a Golgin to the membrane, GM130 in the case of GRASP65 (Barr et al. 1998), and Golgin-45 in the case of GRASP55 (Short et al. 2001). Both GRASPs contain CDK1 phosphorylation sites and are phosphorylated during mitosis. Indeed, GRASP65 appears to be the major mitotic Golgi phosphoprotein (Barr et al. 1997). The mitotic phosphorylation of GRASPs may somehow reconfigure the interactions that hold cisternae together. GRASP55 is phosphorylated by ERK2 during mitosis, but what effect this has on GRASP55 activity is unknown (Jesch et al. 2001a).

Plk1 is implicated in mitotic Golgi fragmentation as it phosphorylates GRASP65 at multiple residues during mitosis (Lin et al. 2000). Substrate inhibition of Plk1 and a dominant negative form of the kinase inhibit mitotic Golgi fragmentation in semi-intact NRK cells (Sutterlin et al. 2001). Whether GRASP65 is the target required for Plk1 to exert its effects is unclear, as the functional and biochemical consequences of GRASP65 phosphorylation by Plk1 remain undetermined. It may be that a combination of Plk1/CDK1 phosphorylation disrupts GRASP65 interactions and induce cisternal unstacking. Whether other Golgi substrates of Plk1 are important for mitotic fragmentation remains unclear.

Most of the phosphorylation sites in GRASP65 reside in its S/P rich C-terminal domain. Intriguingly, during apoptosis GRASP65 is specifically cleaved by caspase-3 at three sites in this region (Lane et al. 2002). During apoptosis the Golgi apparatus is also disassembled (Mancini et al. 2000), and mutation of the three caspase-3 cleavage sites in GRASP65 retards cisternal unstacking and Golgi fragmentation in apoptotic cells (Lane et al. 2002). Apoptotic cleavage of GRASP65 may ensure that cells cannot traverse the spindle checkpoint during mitosis (Norman et al. 1999). Caspase-2 mediated cleavage of Golgin-160 also contributes to the apoptotic Golgi disassembly process (Mancini et al. 2000). Regulation of the C-terminal domain of GRASP65 during mitosis and apoptosis may drive unstacking and fragmentation. The function of this domain is not clear, but it is not the GM130-binding domain (Barr et al. 1998).

MOLECULAR MECHANISMS OF GOLGI REASSEMBLY

Incubation of isolated MGF with interphase cytosol induces their reassembly into Golgi stacks, which mimics the morphological events that occur during the initial assembly of Golgi stacks at telophase in vivo (Souter et al. 1993, Rabouille

et al. 1995b). This similarity strongly implies that this process has been faithfully reproduced in vitro. Golgi reassembly requires interplay between soluble factors and SNAREs via two intersecting pathways controlled by the AAA proteins NSF and p97 (Rabouille et al. 1995a, 1998). These two pathways can sustain maximal cisternal regrowth individually (i.e., their effects are not additive with respect to membrane fusion). However, if studied in isolation the two pathways generate Golgi membranes of distinct morphology. Only when added in combination is the reassembly of MGF in interphase cytosol reproduced (Shorter & Warren 1999).

NSF-Dependent Cisternal Regrowth

The requirement for interphase cytosol for the reassembly of Golgi cisternae from MGF can be replaced with the purified components NSF, α-SNAP, γ-SNAP, and p115 (Rabouille et al. 1995a). Incubation of MGF with these purified proteins generates stacks of Golgi cisternae whose individual cisternae are shorter than those generated by interphase cytosol, suggesting they have not been able to fuse homotypically and grow laterally (Shorter & Warren 1999).

NSF is a barrel-shaped hexamer (Fleming et al. 1998). Each monomer contributes one stave of the barrel and consists of a N-terminal domain, followed by two ATPase cassettes: the D1 and D2 domains (Tagaya et al. 1993). NSF functions via interactions with SNAREs, integral membrane proteins that recruit NSF to membranes via SNAPs (Clary et al. 1990, Söllner et al. 1993). As a consequence of bilayer mixing, assembled v-/t-SNARE complexes accumulate in the fused membranes and must be disassembled to allow their recycling for future membrane-fusion events (Mayer et al. 1996). The N-terminal domain of NSF binds *cis*-SNARE complexes via α-SNAP, and this binding is enhanced by γ-SNAP (Clary et al. 1990). Upon ATP hydrolysis by the D1 domain of NSF, the *cis*-SNARE complex is disassembled (Söllner et al. 1993, Mayer et al. 1996). SNARE disassembly ensures a conformational switch between the ATP/ADP states of the NSF hexamer, which may generate a rotational force that is transmitted via SNAP proteins and may help unwind the helical SNARE bundle (Hanson et al. 1997, Owen & Schiavo 1999).

The SNARE disassembly function of NSF is not required during Golgi reassembly (Müller et al. 1999, 2002). Rather, NSF-catalyzed SNARE disassembly occurs during the mitotic fragmentation of Golgi stacks, and SNARE complexes do not reform, possibly due to the mitotic inhibition of upstream vesicle-tethering events (Nakamura et al. 1997, Müller et al. 2002). Despite containing an abundant source of disassembled SNAREs, MGF must still be incubated with NSF and SNAPs for Golgi membrane fusion to occur. This second NSF activity is distinct from ATPase-dependent SNARE disassembly in that it can be accomplished by D1 ATPase mutants (G274E and E329Q) as well as in the presence of ATPγS, AMP-PNP, and AMP-PCP (Müller et al. 1999, 2002). This activity is not supported by ADP or in the presence of ATP-depletion systems, suggesting that NSF-ATP is required (Müller et al. 2002). However, NSF (G274E) was unable to catalyze Golgi

reassembly unless the SNAREs had previously been disassembled by wild-type NSF during mitotic fragmentation. Thus, disassembled SNAREs are a prerequisite for this second NSF function. Kinetic analysis revealed that NSF/α-SNAP complete their function very early in Golgi reassembly, within the first 5 to 15 min (Müller et al. 2002), consistent with findings for NSF in homotypic vacuole fusion (Mayer et al. 1996).

In vacuole fusion, NSF-catalyzed SNARE disassembly is tightly coupled to the transfer of LMA1 (a thioredoxin/proteinase B inhibitor 2 heterodimer) from NSF to the t-SNARE Vam3p. LMA1 may preserve Vam3p in an activated state conducive for future *trans*-SNARE pairing events (Xu et al. 1998). A similar activity is suggested for GATE-16, a component of the intra-Golgi transport machinery, which binds NSF and GOS-28, a Golgi v-SNARE (Nagahama et al. 1996, Sagiv et al. 2000). GATE-16 is an essential component of NSF-driven Golgi reassembly, as specific anti-GATE-16 antibodies abolish cisternal regrowth, and purified GATE-16 enhances cisternal regrowth by ~40% (Müller at al. 2002). Furthermore, NSF/α-SNAP stimulates the recruitment of GATE-16 to unpaired GOS-28 on MGF in an ATPase-independent manner. Because this NSF activity is NEM-sensitive, NSF-mediated GATE-16/GOS-28 binding likely represents part of the ATPase-independent NSF activity required for Golgi membrane fusion (Müller et al. 2002).

GATE-16 preferentially binds to unpaired GOS-28 and inhibits GOS-28 binding to its cognate t-SNARE syntaxin-5 in detergent solution (Müller et al. 2002). These effects are highly reminiscent of the inhibition of syntaxin/VAMP binding by Munc18 (Pevsner et al. 1994), and of Sed5p/Bet1p binding by Sly1p (Lupashin & Waters 1997), suggesting that GATE-16 may be a v-SNARE protector (Pfeffer 1999). Thus, GATE-16 may prevent GOS-28 from assembling into unproductive *cis*-SNARE complexes. Conversely, GATE-16 may promote highly regulated SNAREpin assembly at appropriate times. GATE-16, by analogy with LMA1 (Xu et al. 1998), may preserve GOS-28 in a fusion-competent state. GATE-16 is required at a terminal stage of membrane fusion coincident with GOS-28 (Müller et al. 2002). Presumably, GATE-16 must be displaced from GOS-28 for SNAREpin formation to occur. Munc18 has been implicated in a terminal phase of exocytosis coincident with SNARE function, and may induce fusion pore opening (Fisher et al. 2001). Interestingly, the yeast homologue of GATE-16, Apg8p/Aut7p, undergoes a reversible cycle of phosphatidylethanolamine conjugation that is essential for membrane fusion during autophagy (Ichimura et al. 2000). Thus, GATE-16 might be a transient proteolipid that promotes fusion pore opening once it has been displaced from GOS-28 during SNAREpin formation and bilayer mixing.

Kinetic analysis of NSF-driven Golgi reassembly has revealed the sequence of events that occur downstream of NSF/α-SNAP (Figure 5) (Shorter et al. 2002). Following NSF/α-SNAP activity, Giantin-p115-GM130 tethers link COPI vesicles to tubular remnants as a prelude to vesicle docking and fusion (Sönnichsen et al. 1998, Shorter et al. 2002). This event is coordinated by a Rab GTPase, since it is GDI sensitive (Shorter et al. 2002). This may be Rab1 that binds both p115 and

GM130 (Allan et al. 2000, Moyer et al. 2001, Weide et al. 2001) or Rab2, Rab6, or Rab33b, which bind GM130 (Short et al. 2001, Valsdottir et al. 2001). Second, p115 phosphorylation is required (Shorter et al. 2002). p115 is phosphorylated by a CKII-like kinase at serine 942 in its acidic tail region, the GM130/Giantin binding site. This phosphorylation potentiates the interactions between p115/GM130 and p115/Giantin, and so may fasten the tether in place (Dirac-Svejstrup et al. 2000, Shorter et al. 2002). This strengthening of the linkage seems to be required for the transition for the next stage of reaction, vesicle docking, where the v- and t-SNAREs engage.

Microinjection of anti-p115 or anti-Giantin antibodies into cells appears to induce the proteasome-dependent degradation of p115 or Giantin (Puthenveedu & Linstedt 2001). These cells then proceed through mitosis and appear to generate a Golgi ribbon at telophase in the absence of Giantin, but not in the absence of p115 (Puthenveedu & Linstedt 2001). Although the lack of ultrastructural analysis precludes a definite conclusion, since vesiculated Golgi can still appear as a juxtanuclear ribbon by immunofluorescence (Seemann et al. 2000a,b), at a minimum it appears that Giantin is not required for the juxtanuclear clustering of Golgi membranes. It also suggests that p115 perfoms additional functions to linking GM130 to Giantin that are essential for Golgi membrane fusion. This may be in the assembly of cognate SNAREpins (Shorter et al. 2002).

Upon tethering of a COPI vesicle to its acceptor compartment by Giantin-p115-GM130 and after p115 phosphorylation, the v- and t-SNAREs engage. This event is catalyzed by p115 (Shorter et al. 2002, Söllner 2002). The first coiled-coil domain of p115 (CC1) bears weak homology to the SNARE motif, the membrane proximal coiled-coil domain that defines the SNAREs as a protein superfamily (Weimbs et al. 1997, 1998). SNAREpins consist of an internal core of four SNARE motifs, one contributed by the v-SNARE and three by the t-SNARE, that are aligned in parallel to form an exceptionally stable helical bundle (Jahn & Südhof 1999). Transduction of energy from this helical bundle via flexible linker regions to the transmembrane domains of SNAREs may forcibly drive bilayer mixing (McNew et al. 2000b). Furthermore, the precise topological restriction of individual components within an assembled SNAREpin may provide a universal syntax or code that ultimately governs the specificity of membrane fusion events (McNew et al. 2000a, Parlati et al. 2000, Paumet et al. 2001). The SNARE motif-like domain in p115 links the Golgi v-SNARE GOS-28 on the COPI vesicle to its cognate t-SNARE syntaxin-5 on the acceptor membrane (Shorter et al. 2002). This initial linkage catalyzes the specific and tight assembly of a GOS-28/syntaxin-5 complex that once formed no longer requires p115 to maintain it (Shorter et al. 2002).

In this manner, p115 assembles SNAREpins. p115 interacts, via CC1, specifically with only a subset of cellular SNAREs, namely the ER-Golgi SNAREs: syntaxin-5 (Hay et al. 1998), GOS-28 (Nagahama et al. 1996), Bet1p (Hay et al. 1998), membrin (Lowe et al. 1997), rSec22p (Hay et al. 1998), Ykt6p (Zhang & Hong 2001), and GS15 (Xu et al. 1997, Shorter et al. 2002). Furthermore, p115 stimulates the formation of at least three different SNAREpins comprised of syntaxin-5/GOS-28/Ykt6p/Bet1p, syntaxin-5/GOS-28/Ykt6p/GS15, and

syntaxin-5/membrin/ rSec22p/Bet1p (Shorter et al. 2002). Antibody inhibition experiments implicate all these SNAREs in Golgi reassembly (Shorter et al. 2002). This may help explain the requirement for p115 in multiple transport steps between the ER and medial Golgi (Waters et al. 1992, Seemann et al. 2000b, Allan et al. 2000, Marra et al 2001).

When viewed in this light, SNAREs may be seen as short tethers that once assembled into SNAREpins catalyze or signal for membrane fusion. Conversely, one may view the Golgins as extended SNAREs that evolved for the specialized function of long-range vesicle capture. p115 plays a pivotal role in membrane docking by gradually bringing the COPI vesicle closer to its target via these successive interactions. That p115, GM130, and Giantin coimmunoprecipitate with both GOS-28 and syntaxin-5 suggests they may be components of a large tethering complex (Shorter et al. 2002).

p115 also contributes to the specificity of vesicle transfer, since it does not promote noncognate SNARE interactions (Shorter et al. 2002). Furthermore, p115 does not link GOS-28 or syntaxin-5 to themselves. p115 bound to GOS-28 may be restricted to a conformation that is only able to bind syntaxin-5, and not another GOS-28 molecule. Therefore, the p115/SNARE tether must be asymmetric in nature, and a similar situation may exist for Giantin/p115/GM130 interactions. Thus, binding of one SNARE to p115 transmits or encodes specificity to any subsequent p115/SNARE interaction. In this way, p115 may form part of the syntax that ensures that only cognate, topologically correct SNAREpins will assemble, and so enhances vesicle transfer specificity.

Once SNAREpin assembly is completed Golgi membrane fusion occurs rapidly (Shorter et al. 2002), consistent with results obtained with the fusion of dense core granules with the plasma membrane (Chen et al. 1999, Scales et al. 2000) and SNARE-dependent liposome fusion (Weber et al. 1998, McNew et al. 2000a,b). Agents that interfere with SNARE activity blocked NSF-driven cisternal regrowth at a terminal stage of the process (Shorter et al. 2002). Only GATE-16 inhibitors gave a similar kinetic profile (Müller et al. 2002). Therefore, it will be difficult to determine whether other factors are required downstream of SNAREs for Golgi membrane fusion as required for vacuole homotypic fusion (Ungermann et al. 1998, Wickner & Haas 2000, Peters et al. 2001).

p97-Dependent Cisternal Regrowth

By comparison with NSF-dependent cisternal regrowth, the mechanisms that underpin p97-catalyzed cisternal regrowth are much less clear. The requirement for interphase cytosol in reassembly can also be replaced by addition of purified p97 and p47 (Kondo et al. 1997, Rabouille et al. 1998). Incubation of MGF with p97/p47 generates long, single cisternae that only form stacks if supplemented with p115 (Shorter & Warren 1999). The formation of long, single cisternae suggests that p97 may induce homotypic fusion events that fuse like cisternae with like. Thus, it may be that p97 acts to regenerate the cisternal cores from the COPI-independent fragmentation products, and the NSF pathway regenerates the cisternal rims from COPI

vesicles and cisternal remnants. The equilibrium between the p97/NSF pathways may be modulated by cells to generate different cisternal architectures according to cellular needs (Rabouille et al. 1995a, 1998; Shorter & Warren 1999).

Like NSF, p97 is an NEM-sensitive barrel-shaped hexamer. Each monomer constitutes one stave of the barrel and contains a N-terminal domain plus the D1 and D2 ATPase cassettes. Structural analysis suggests that the D1 and D2 domains cooperatively hydrolyze their ATP to generate a large ratchet-like motion and narrowing of the central pore of the hexamer (Zhang et al. 2000, Rouiller et al. 2000). This motion is hypothesized to drive a general unfolding activity of p97 that is unleashed via various adaptor proteins onto targeted substrates (Patel & Latterich 1998, Meyer et al. 2000, Zhang et al. 2000). p47 represents such an adaptor and is a trimer that binds to the N-terminal domain of p97 (Kondo et al. 1997, Yuan et al. 2001). p47 directs p97 activity to Golgi (Kondo et al. 1997, Rabouille et al. 1998) and ER/nuclear envelope membrane-fusion events (Roy et al. 2000, Lavoie et al. 2000, Hetzer et al. 2001). Membrane fusion is somehow mediated by p47 binding to the Golgi t-SNARE syntaxin-5 (Rabouille et al. 1998, Roy et al. 2000). In yeast, p97 exerts its effects on ER homotypic fusion via the t-SNARE Ufe1p (Patel et al. 1998), but in mammalian cells seems to require syntaxin-5 (Roy et al. 2000). ER homotypic fusion seems to be modulated by the tyrosine phosphorylation state of p97 (Lavoie et al. 2000). JAK2-mediated phosphorylation of p97 induces its release from ER membranes, and inhibition of JAK2 promotes ER assembly (Lavoie et al. 2000). This kinase activity is antagonized by PTPH1 phosphatase activity, which if inhibited blocks ER assembly (Lavoie et al. 2000). Another p97 adaptor Ufd1p/Npl4p targets p97-unfoldase activity to postmitotic nuclear reassembly and ER-associated degradation events, but is not required for Golgi reassembly (Meyer et al. 2000, Hetzer et al. 2001, Ye et al. 2001).

Competition for syntaxin-5 binding between α-SNAP and p47 may explain why the NSF and p97 pathways of Golgi reassembly contribute nonadditively to cisternal regrowth (Rabouille et al. 1998). p47 binding to p97 also regulates p97 ATPase activity, but in contrast to α-SNAP and NSF, acts to inhibit the ATPase activity (Meyer et al. 1998), which possibly suggests a different mode of action of p97 relative to NSF.

How p97/SNARE interactions induce membrane fusion is obscure. The mode of action of p97 is thought by analogy to be very similar to that of NSF, except the substrate for p97 is a t-/t-SNARE complex instead of a v-/t-SNARE complex. p97/p47 would bind to assembled *cis*-t-/t-SNARE complexes via p47 and upon ATP hydrolysis by p97 would disassemble them. This activity has not been reconstituted. However, it is supported by evidence from ER homotypic fusion in yeast, which requires only the t-SNARE, Ufe1p, and none of the other known ER-Golgi v- or t-SNAREs (Patel et al. 1998). Furthermore, in a temperature-sensitive Cdc48 (the yeast p97 homologue) mutant at the restrictive temperature, Ufe1p accumulates in SDS-resistant SNARE complexes (Patel et al. 1998). p97-driven Golgi reassembly requires syntaxin-5, but not GOS-28 (Rabouille et al. 1998), although other Golgi v-SNAREs have not been excluded. However, t-/t-SNARE complexes seem structurally unable to form parallel four-helical bundles required

for membrane fusion (Misura et al. 2001) and do not catalyze liposome fusion (Weber et al. 1998). Further, syntaxin-5 is not present in SDS-resistant complexes on MGF (Müller et al. 2002). Thus, it seems unlikely that the assembly or disassembly of t-/t-SNARE complexes contributes to homotypic fusion. Indeed, yeast homotypic vacuole fusion (Wickner & Haas 2000) and TGN homotypic fusion (Paumet et al. 2001, Brickner et al. 2001) is mediated by conventional v-/t-SNAREs. In contrast, homotypic fusion of immature secretory granules requires syntaxin-6 on both membranes (Wendler et al. 2001). Much further experimentation is needed to clarify the molecular mechanisms of p97-driven Golgi reassembly.

Stacking Golgi Cisternae

As single cisternae begin to form they align and tether to form stacks. This stacking process is stimulated by GTPγS and inhibited by microcystin, but the targets of these molecules are unknown (Rabouille et al. 1995b). Stacking of single cisternae requires the formation of Giantin-p115-GM130 tethers at an incipient phase of the reaction; at later time points, stacks are insensitive to disruption of these tethers (Shorter & Warren 1999). Since Giantin-p115-GM130 tether formation is regulated by a Rab-GTPase (Shorter et al. 2002), GTPγS may enhance stacking by enhancing Rab-stimulated tether formation. The second phase of the stacking reaction requires GRASP65 and GRASP55 (Barr et al. 1997, Shorter & Warren 1999, Shorter et al. 1999).

Treatment of MGF with NEM precludes cisternal stacking but not cisternal regrowth during reassembly (Rabouille et al. 1995b, Barr et al. 1997). Using a biotinylated analogue of NEM, it was found that only three proteins are specifically modified by NEM on MGF. Two factors were identified by utilizing this alkylation by the biotinylated NEM analogue as a marker for their chromatographic behavior, and were found to be GM130 and GRASP65 (Barr et al. 1997, 1998). GRASP65 binds to the extreme C-terminal domain of GM130 via a PDZ-like domain (Barr et al. 1998). GRASP65 is found mostly on *cis*-Golgi cisternae and the CGN (Shorter et al. 1999).

In contrast, GRASP55 is localized to medial Golgi cisternae (Shorter et al. 1999). The N-terminal 212 amino acids are highly conserved between the two proteins, after which their primary sequence diverges. GRASP55 lacks many putative serine phosphorylation sites in its C-terminal domain, perhaps explaining why it does not appear to be such a major phosphoprotein at mitosis, unlike GRASP65. Northern blot analysis suggests that both GRASPs are alternatively spliced, raising the possibility of GRASP variants. As well as being myristoylated, GRASP55 is also palmitoylated (Kuo et al. 2000). Soluble GRASPs and antibodies against either GRASP inhibit the stacking process without affecting membrane fusion per se (Barr et al. 1997, Shorter & Warren 1999, Shorter et al. 1999). The distinct localization of GRASP55 suggests it is more responsible for the stacking of medial cisternae (Pfeffer 2001). GRASP55 does not interact with GM130 but instead binds Golgin-45 via a PDZ-like domain (Short et al. 2001). Golgin-45 interacts specifically with Rab2 and is essential for Golgi structure and function (Short et al.

2001). Depletion of Golgin-45 by RNA interference induces Golgi fragmentation and inhibits the transport of VSVG protein to the plasma membrane (Short et al. 2001).

Precisely how the GRASPs function to stack cisternae remains unclear. One function may be to divert vesicle tethers away from the fusion machinery, perhaps acting as an assembly point for the Golgi matrix that may provide a fusion clamp between adjacent cisternae (Rothman & Warren 1994, Shorter et al. 1999, Short et al. 2001). Since the GRASPs are oligomeric in nature, by inserting their lipid groups into opposite membranes and/or by interacting with Golgins or the cytoplasmic tails of Golgi lumenal proteins on opposite membranes they might link cisternae together. Both GRASPs interact with members of the p24 family of cargo receptors as well as certain anterograde transmembrane cargo, such as TGFα (Kuo et al. 2000, Barr et al. 2001). This might provide a means to link adjacent, maturing cisternae together and tightly couple stacking to transport. Similarly, the cytoplasmic tails of the Golgi enzymes MannII and NAGTI also interact directly with the Golgi matrix (Slusarewicz et al. 1994). Although the identity of the receptor in the Golgi matrix remains unidentified, this also provides a potential stacking mechanism since MannII and NAGTI are often found in adjacent cisternae (Nilsson et al. 1993, 1994).

Both GRASPs are reliable markers of Golgi polarity. If the stack of cisternae is composed of cisternae that are maturing along a *cis-trans* vector, the GRASPs will, like the Golgi enzymes, need to be recycled to preceding compartments. It is suggested that Golgi enzymes achieve this by differential entry into retrograde COPI vesicles (Pelham 2001b). *Cis*-Golgi enzymes would have greater access to COPI vesicles than medial or *trans* enzymes. Although there are conflicting reports in the literature (Sönnichsen et al. 1996; Orci et al. 1997, 2000a; Volchuk et al. 2000), there are data to support this notion and that of cisternal maturation (Lanoix et al. 1999, Martinez-Marguez et al. 2001, Bonfanti et al. 1998, Mironov et al. 2001). By contrast, the GRASPs do not seem to enter COPI vesicles. However, one recent FRAP study revealed that GRASP65 may be rapidly shuttling on and off Golgi membranes similar to COPI proteins (Ward et al. 2001). This raises the intriguing possibility that the GRASPs rapidly recycle between cisternae as they mature via a soluble pool, perhaps determined by which GRASP receptors a Golgi cisterna contains. This rapid recycling would keep the cisternae stacked as they matured to allow efficient vesicle transfer between compartments. However, the GFP-GRASP65 used in this study was not the full-length protein (Ward et al. 2001, Barr et al. 1998). Consequently, it was largely soluble (up to 60%; Ward et al. 2001), which may not accurately reflect the endogenous GRASP65 population, which is at most 5% soluble (Barr et al. 1997, Marra et al. 2001, Seemann et al. 2002). Therefore, care must be taken in interpreting these results. Another recent study suggested that a small subpopulation of GRASP65 and GM130 was present on highly dynamic Golgi tubules that emanate from the CGN, and appears to capture incoming cargo from VTCs, as though to initiate the first stages of *cis*-cisterna formation (Marra et al. 2001). These observations give the image of a highly

dynamic Golgi matrix that changes precisely in accordance with the changes in Golgi structure as vesicles transfer between maturing cisternae. The Golgi matrix may then be more akin to the dynamic instability of the cytoskeleton in nature, rather than a static structure (Misteli 2001). This self-organizing matrix encodes sufficient information for accurate Golgi inheritance (Seemann et al. 2002).

FUTURE PERSPECTIVES

The major challenge for the future is to verify the findings from the cell-free system and semi-intact cells in vivo. Observing molecular interactions in real time, by exploiting the photophysical properties of fluorescence emitted by GFP-tagged reporters during the cell cycle, will provide much information concerning cell cycle changes in Golgi molecular architecture (Bastiaens & Pepperkok 2000). The study of Golgi inheritance in genetically tractable organisms also holds much promise for unraveling important aspects of these processes (Rossanese et al. 2001). Similarly, study of Golgi inheritance in protists such as *Trypanosoma* and *Toxoplasma* may ease the study of Golgi biogenesis, since the Golgi stack is a single-copy organelle in these organisms and can be readily monitored using GFP technology (Pelletier et al. 2002). It will also be important to establish the evolutionary conservation of such features as the Golgi matrix and its role in Golgi inheritance in these organisms. Finally, the precise architecture and dynamics of the Golgi matrix itself are extremely important questions. For example, how does the Golgi matrix change to allow a process such as cisternal maturation or mitotic fragmentation? How is Golgi polarity maintained? What are the regulatory mechanisms that dictate Golgi biogenesis or accurate partitioning at prometaphase? The number of questions that remain unanswered make clear that the field is still, in a certain sense, wide open.

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LITERATURE CITED

Abrieu A, Doree M, Fisher D. 2001. The interplay between cyclin-B-CDK1 kinase (MPF) and MAP kinase during maturation of oocytes. *J. Cell Sci.* 114:257–67

Acharya U, Mallabiabarrena A, Acharya JK, Malhotra V. 1998. Signaling via mitogen-activated protein kinase kinase (MEK1) is required for Golgi fragmentation during mitosis. *Cell* 92183–92

Allan BB, Moyer BD, Balch WE. 2000. Rab1

recruitment of p115 into a *cis*-SNARE complex: programming budding COPII vesicles for fusion. *Science* 289:444–48

Asada S, Yagura T. 1995. Golgi membrane vesicles in HeLa mitotic cells are identified with monoclonal antibody made against Golgi cisternal membrane protein p138. *Cell Struct. Funct.* 20:445–53

Bailly E, McCaffrey M, Touchot N, Zahraoui A, Goud B, Bornens M. 1991. Phosphorylation of two small GTP-binding proteins of the Rab family by p34CDK1. *Nature* 350:715–18

Barr FA, Nakamura N, Warren G. 1998. Mapping the interaction between GRASP65 and GM130, components of a protein complex involved in the stacking of Golgi cisternae. *EMBO J.* 17:3258–68

Barr FA, Preisinger C, Kopajtich R, Korner R. 2001. Golgi matrix proteins interact with p24 cargo receptors and aid their efficient retention in the Golgi apparatus. *J. Cell Biol.* 155:885–91

Barr FA, Puype M, Vandekerckhove J, Warren G. 1997. GRASP65, a protein involved in the stacking of Golgi cisternae. *Cell* 91:253–62

Barr FA, Shorter J. 2000. Membrane traffic: Do cones mark sites of fission? *Curr. Biol.* 10:R141–44

Bastiaens PI, Pepperkok R. 2000. Observing proteins in their natural habitat: the living cell. *Trends Biochem. Sci.* 25:631–37

Beck KA, Buchanan JA, Malhotra V, Nelson WJ. 1994. Golgi spectrin: identification of an erythroid beta-spectrin homolog associated with the Golgi complex. *J. Cell Biol.* 127:707–23

Beck KA, Buchanan JA, Nelson WJ. 1997. Golgi membrane skeleton: identification, localization and oligomerization of a 195 kDa ankyrin isoform associated with the Golgi complex. *J. Cell Sci.* 110:1239–49

Benchimol M, Ribeiro KC, Mariante RM, Alderete JF. 2001. Structure and division of the Golgi complex in *Trichomonas vaginalis* and *Tritrichomonas foetus*. *Eur. J. Cell Biol.* 80:593–607

Birky CW Jr. 1983. The partitioning of cytoplasmic organelles at cell division. *Int. Rev. Cytol. Suppl.* 15:49–89

Blobel G. 1980. Intracellular protein topogenesis. *Proc. Natl. Acad. Sci. USA* 77:1496–500

Boggs KP, Rock CO, Jackowski S. 1995. Lysophosphatidylcholine and 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine inhibit the CDP-choline pathway of phosphatidylcholine synthesis at the CTP:phosphocholine cytidylyltransferase step. *J. Biol. Chem.* 270:7757–64

Bonfanti L, Mironov AA Jr, Martinez-Menarguez JA, Martella O, Fusella A, et al. 1998. Procollagen traverses the Golgi stack without leaving the lumen of cisternae: evidence for cisternal maturation. *Cell* 95:993–1003

Bretscher MS, Munro S. 1993. Cholesterol and the Golgi apparatus. *Science* 261:1280–81

Brickner JH, Blanchette JM, Sipos G, Fuller RS. 2001. The Tlg SNARE complex is required for TGN homotypic fusion. *J. Cell Biol.* 155:969–78

Burke B, Griffiths G, Reggio H, Louvard D, Warren G. 1982. A monoclonal antibody against a 135-K Golgi membrane protein. *EMBO J.* 1:1621–28

Cao X, Ballew N, Barlowe C. 1998. Initial docking of ER-derived vesicles requires Uso1p and Ypt1p but is independent of SNARE proteins. *EMBO J.* 17:2156–65

Cha H, Shapiro P. 2001. Tyrosine-phosphorylated extracellular signal-regulated kinase associates with the Golgi complex during G2/M phase of the cell cycle: evidence for regulation of Golgi structure. *J. Cell Biol.* 153:1355–67

Chabin-Brion K, Marceiller J, Perez F, Settegrana C, Drechou A, et al. 2001. The Golgi complex is a microtubule-organizing organelle. *Mol. Biol. Cell* 12:2047–60

Chen YA, Scales SJ, Patel SM, Doung YC, Scheller RH. 1999. SNARE complex formation is triggered by Ca^{2+} and drives membrane fusion. *Cell* 97:165–74

Clary DO, Griff IC, Rothman JE. 1990. SNAPs, a family of NSF attachment proteins involved

in intracellular membrane fusion in animals and yeast. *Cell* 61:709–21

Cluett EB, Brown WJ. 1992. Adhesion of Golgi cisternae by proteinaceous interactions: intercisternal bridges as putative adhesive structures. *J. Cell Sci.* 103:773–84

Colanzi A, Deerinck TJ, Ellisman MH, Malhotra V. 2000. A specific activation of the mitogen-activated protein kinase kinase 1 (MEK1) is required for Golgi fragmentation during mitosis. *J. Cell Biol.* 149:331–39

Cole NB, Sciaky N, Marotta A, Song J, Lippincott-Schwartz J. 1996. Golgi dispersal during microtubule disruption: regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. *Mol. Biol. Cell* 7:631–50

Collins RN, Warren G. 1992. Sphingolipid transport in mitotic HeLa cells. *J. Biol. Chem.* 267:24906–11

Conlon I, Raff M. 1999. Size control in animal development. *Cell* 96:235–44

Corthesy-Theulaz I, Pauloin P, Pfeffer SR. 1992. Cytoplasmic dynein participates in the centrosomal localization of the Golgi complex. *J. Cell Biol.* 118:1333–45

Cox JS, Chapman RE, Walter P. 1997. The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. *Mol. Biol. Cell* 8:1805–14

Dacks JB, Doolittle WF. 2001. Reconstructing/deconstructing the earliest eukaryotes: How comparative genomics can help. *Cell* 107:419–25

de Figueiredo P, Drecktrah D, Katzenellenbogen JA, Strang M, Brown WJ. 1998. Evidence that phospholipase A2 activity is required for Golgi complex and trans Golgi network membrane tubulation. *Proc. Natl. Acad. Sci. USA* 95:8642–47

De Matteis MA, Morrow JS. 2000. Spectrin tethers and mesh in the biosynthetic pathway. *J. Cell Sci.* 113:2331–43

Dirac-Svejstrup AB, Shorter J, Waters MG, Warren G. 2000. Phosphorylation of the vesicle-tethering protein p115 by a casein kinase II-like enzyme is required for Golgi reassembly from isolated mitotic fragments. *J. Cell Biol.* 150:475–88

Draviam VM, Orrechia S, Lowe M, Pardi R, Pines J. 2001. The localization of human cyclins B1 and B2 determines CDK1 substrate specificity and neither enzyme requires MEK to disassemble the Golgi apparatus. *J. Cell Biol.* 152:945–58

Elazar Z, Orci L, Ostermann J, Amherdt M, Tanigawa G, Rothman JE. 1994. ADP-ribosylation factor and coatomer couple fusion to vesicle budding. *J. Cell Biol.* 124:415–24

Farmaki T, Ponnambalam S, Prescott AR, Clausen H, Tang BL, et al. 1999. 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.* 112:589–600

Farquhar MG. 1985. Progress in unraveling pathways of Golgi traffic. *Annu. Rev. Cell Biol.* 1:447–88

Farsad K, Ringstad N, Takei K, Floyd SR, Rose K, De Camilli P. 2001. Generation of high curvature membranes mediated by direct endophilin bilayer interactions. *J. Cell Biol.* 155:193–200

Fath KR, Trimbur GM, Burgess DR. 1997. Molecular motors and a spectrin matrix associate with Golgi membranes in vitro. *J. Cell Biol.* 139:1169–81

Featherstone C, Griffiths G, Warren G. 1985. Newly synthesized G protein of vesicular stomatitis virus is not transported to the Golgi complex in mitotic cells. *J. Cell Biol.* 101:2036–46

Fernández CJ, Warren G. 1998. In vitro synthesis of sulfated glycosaminoglycans coupled to inter-compartmental Golgi transport. *J. Biol. Chem.* 273:19030–39

Field H, Sherwin T, Smith AC, Gull K, Field MC. 2000. Cell cycle and developmental regulation of TbRAB31 localization, a GTP-locked Rab protein from *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 106:21–35

Fisher RJ, Pevsner J, Burgoyne RD. 2001. Control of fusion pore dynamics during exocytosis by Munc18. *Science* 291:875–78

Fleming KG, Hohl TM, Yu RC, Müller SA, Wolpensinger B, et al. 1998. A revised model for the oligomeric state of the N-ethylmaleimide-sensitive fusion protein, NSF. *J. Biol. Chem.* 273:15675–81

Flickinger CJ. 1968. The effects of enucleation on the cytoplasmic membranes of *Amoeba proteus*. *J. Cell Biol.* 37:300–15

Gagneux P, Varki A. 1999. Evolutionary considerations in relating oligosaccharide diversity to biological function. *Glycobiology* 9:747–55

Garcia-Herdugo G, Gonzalez-Reyes JA, Garcua-Navarro F, Navas P. 1988. Growth kinetics of the Golgi apparatus during the cell cycle in onion root meristems. *Planta* 175: 305–12

Gillin FD, Reiner DS, McCaffery JM. 1996. Cell biology of the primitive eukaryote *Giardia lamblia*. *Annu. Rev. Microbiol.* 50:679–705

Girod A, Storrie B, Simpson JC, Johannes L, Goud B, et al. 1999. Evidence for a COP-I-independent transport route from the Golgi complex to the endoplasmic reticulum. *Nat. Cell Biol.* 1:423–30

Greco V, Hannus M, Eaton S. 2001. Argosomes: a potential vehicle for the spread of morphogens through epithelia. *Cell* 106:633–45

Griffiths G, Fuller SD, Back R, Hollinshead M, Pfeiffer S, Simons K. 1989. The dynamic nature of the Golgi complex. *J. Cell Biol.* 108:277–97

Hager KM, Striepen B, Tilney LG, Roos DS. 1999. The nuclear envelope serves as an intermediary between the ER and Golgi complex in the intracellular parasite *Toxoplasma gondii*. *J. Cell Sci.* 112:2631–38

Hammond AT, Glick BS. 2000. Dynamics of transitional endoplasmic reticulum sites in vertebrate cells. *Mol. Biol. Cell* 11:3013–30

Hanson PI, Roth R, Morisaki H, Jahn R, Heuser JE. 1997. Structure and conformational changes in NSF and its membrane receptor complexes visualized by quick-freeze/deep-etch electron microscopy. *Cell* 90:523–35

Happe S, Weidman P. 1998. Cell-free transport to distinct Golgi cisternae is compartment specific and ARF independent. *J. Cell Biol.* 140:511–23

Hartman H, Fedorov A. 2002. The origin of the eukaryotic cell: a genomic investigation. *Proc. Natl. Acad. Sci. USA* 99:1420–25

Hay JC, Klumperman J, Oorschot V, Steegmaier M, Kuo CS, Scheller RH. 1998. Localization, dynamics, and protein interactions reveal distinct roles for ER and Golgi SNAREs. *J. Cell Biol.* 141:1489–502

Helenius A, Aebi M. 2001. Intracellular functions of N-linked glycans. *Science* 291:2364–69

Hesketh TR, Beaven MA, Rogers J, Burke B, Warren GB. 1984. Stimulated release of histamine by a rat mast cell line is inhibited during mitosis. *J. Cell Biol.* 98:2250–54

Hetzer M, Meyer HH, Walther TC, Bilbao-Cortes D, Warren G, Mattaj IW. 2001. Distinct AAA-ATPase p97 complexes function in discrete steps of nuclear assembly. *Nat. Cell Biol.* 3:1086–91

Hird SN, Paulsen JE, Strome S. 1996. Segregation of germ granules in living *Caenorhabditis elegans* embryos: cell-type-specific mechanisms for cytoplasmic localization. *Development* 122:1303–12

Holthuis JC, Pomorski T, Raggers RJ, Sprong H, Van Meer G. 2001. The organizing potential of sphingolipids in intracellular membrane transport. *Physiol. Rev.* 81:1689–723

Horiike T, Hamada K, Kanaya S, Shinozawa T. 2001. Origin of eukaryotic cell nuclei by symbiosis of Archaea in Bacteria is revealed by homology-hit analysis. *Nat. Cell Biol.* 3:210–14

Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, et al. 2000. A ubiquitin-like system mediates protein lipidation. *Nature* 408:488–92

Jackowski S. 1996. Cell cycle regulation of membrane phospholipid metabolism. *J. Biol. Chem.* 271:20219–22

Jahn R, Südhof TC. 1999. Membrane fusion and exocytosis. *Annu. Rev. Biochem.* 68:863–911

Jesch SA, Lewis TS, Ahn NG, Linstedt AD. 2001a. Mitotic phosphorylation of Golgi

reassembly stacking protein 55 by mitogen-activated protein kinase ERK2. *Mol. Biol. Cell* 12:1811–17

Jesch SA, Linstedt AD. 1998. The Golgi and endoplasmic reticulum remain independent during mitosis in HeLa cells. *Mol. Biol. Cell* 9:623–35

Jesch SA, Mehta AJ, Velliste M, Murphy RF, Linstedt AD. 2001b. Mitotic Golgi is in a dynamic equilibrium between clustered and free vesicles independent of the ER. *Traffic* 2:873–84

Jokitalo E, Cabrera-Poch N, Warren G, Shima DT. 2001. Golgi clusters and vesicles mediate mitotic inheritance independently of the endoplasmic reticulum. *J. Cell Biol.* 154:317–30

Kanki JP, Newport JW. 1991. The cell cycle dependence of the secretory pathway in developing *Xenopus laevis*. *Dev. Biol.* 146:214–27

Kano F, Takenaka K, Yamamoto A, Nagayama K, Nishida E, Murata M. 2000. MEK and CDK1 kinase are sequentially required for Golgi disassembly in MDCK cells by the mitotic Xenopus extracts. *J. Cell Biol.* 149:357–68

Keryer G, Yassenko M, Labbe JC, Castro A, Lohmann SM, et al. 1998. Mitosis-specific phosphorylation and subcellular redistribution of the RIIalpha regulatory subunit of cAMP-dependent protein kinase. *J. Biol. Chem.* 273:34594–602

Kirschner M, Gerhart J. 1998. Evolvability. *Proc. Natl. Acad. Sci. USA* 95:8420–27

Kirschner M, Gerhart J, Mitchison T. 2000. Molecular "vitalism." *Cell* 100:79–88

Klumperman J. 2000. The growing Golgi: in search of its independence. *Nat. Cell Biol.* 2:E217–19

Kondo H, Rabouille C, Newman R, Levine TP, Pappin D, et al. 1997. p47 is a cofactor for p97-mediated membrane fusion. *Nature* 388:75–78

Kreiner T, Moore HP. 1990. Membrane traffic between secretory compartments is differentially affected during mitosis. *Cell Regul.* 1:415–24

Kuo A, Zhong C, Lane WS, Derynck R. 2000. Transmembrane transforming growth factor-alpha tethers to the PDZ domain-containing, Golgi membrane-associated protein p59/GRASP55. *EMBO J.* 19:6427–39

Kupfer A, Dennert G, Singer SJ. 1983. Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets. *Proc. Natl. Acad. Sci. USA* 80:7224–28

Kupfer A, Louvard D, Singer SJ. 1982. Polarization of the Golgi apparatus and the microtubule-organizing center in cultured fibroblasts at the edge of an experimental wound. *Proc. Natl. Acad. Sci. USA* 79:2603–7

Ladinsky MS, Mastronarde DN, McIntosh JR, Howell KE, Staehelin LA. 1999. Golgi structure in three dimensions: functional insights from the normal rat kidney cell. *J. Cell Biol.* 144:1135–49

Lane JD, Lucocq J, Pryde JG, Barr FA, Woodman PG, et al. 2002. Caspase-mediated cleavage of the stacking protein GRASP65 is required for Golgi fragmentation during apoptosis. *J. Cell Biol.* 156:495–509

Lanoix J, Ouwendijk J, Lin CC, Stark A, Love HD, et al. 1999. GTP hydrolysis by arf-1 mediates sorting and concentration of Golgi resident enzymes into functional COP I vesicles. *EMBO J.* 18:4935–48

Lavoie C, Chevet E, Roy L, Tonks NK, Fazel A, et al. 2000. Tyrosine phosphorylation of p97 regulates transitional endoplasmic reticulum assembly in vitro. *Proc. Natl. Acad. Sci. USA* 97:13637–42

Levine TP, Rabouille C, Kieckbusch RH, Warren G. 1996. Binding of the vesicle docking protein p115 to Golgi membranes is inhibited under mitotic conditions. *J. Biol. Chem.* 271:17304–11

Lewin B. 1998. The mystique of epigenetics. *Cell* 93:301–3

Lin CY, Madsen ML, Yarm FR, Jang YJ, Liu X, Erikson RL. 2000. Peripheral Golgi protein GRASP65 is a target of mitotic polo-like kinase (Plk) and CDK1. *Proc. Natl. Acad. Sci. USA* 97:12589–94

Linstedt AD. 1999. Stacking the cisternae. *Curr. Biol*. 9:R893–96

Linstedt AD, Hauri HP. 1993. Giantin, a novel conserved Golgi membrane protein containing a cytoplasmic domain of at least 350 kDa. *Mol. Biol. Cell* 4:679–93

Lippincott-Schwartz J, Roberts TH, Hirschberg K. 2000. Secretory protein trafficking and organelle dynamics in living cells. *Annu. Rev. Cell Dev. Biol*. 16:557–89

Lippincott-Schwartz J, Yuan LC, Bonifacino JS, Klausner RD. 1989. Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. *Cell* 56:801–13

Lopez-Garcia P, Moreira D. 1999. Metabolic symbiosis at the origin of eukaryotes. *Trends Biochem. Sci*. 24:88–93

Lowe M. 2002. Golgi complex: biogenesis de novo? *Curr. Biol*. 12:R166–67

Lowe M, Gonatas NK, Warren G. 2000. The mitotic phosphorylation cycle of the *cis*-Golgi matrix protein GM130. *J. Cell Biol*. 149:341–56

Lowe M, Rabouille C, Nakamura N, Watson R, Jackman M, et al. 1998. CDK1 kinase directly phosphorylates the cis-Golgi matrix protein GM130 and is required for Golgi fragmentation in mitosis. *Cell* 94:783–93

Lowe SL, Peter F, Subramaniam VN, Wong SH, Hong W. 1997. A SNARE involved in protein transport through the Golgi apparatus. *Nature* 389:881–84

Lucocq JM, Berger EG, Warren G. 1989. Mitotic Golgi fragments in HeLa cells and their role in the reassembly pathway. *J. Cell Biol*. 109:463–74

Lucocq JM, Pryde JG, Berger EG, Warren G. 1987. A mitotic form of the Golgi apparatus in HeLa cells. *J. Cell Biol*. 104:865–74

Lucocq JM, Warren G. 1987. Fragmentation and partitioning of the Golgi apparatus during mitosis in HeLa cells. *EMBO J*. 6:3239–46

Lupashin VV, Waters MG. 1997. t-SNARE activation through transient interaction with a rab-like guanosine triphosphatase. *Science* 276:1255–58

Makarow M. 1988. Secretion of invertase in mitotic yeast cells. *EMBO J*. 7:1475–82

Malhotra V, Orci L, Glick BS, Block MR, Rothman JE. 1988. Role of an N-ethylmaleimide-sensitive transport component in promoting fusion of transport vesicles with cisternae of the Golgi stack. *Cell* 54:221–27

Mancini, M, Machamer CE, Roy S, Nicholson DW, Thornberry NA, et al. 2000. Caspase-2 is localized at the Golgi complex and cleaves Golgin-160 during apoptosis. *J. Cell Biol*. 149:603–12

Maniotis A, Schliwa M. 1991. Microsurgical removal of centrosomes blocks cell reproduction and centriole generation in BSC-1 cells. *Cell* 67:495–504

Marra P, Maffucci T, Daniele T, Tullio GD, Ikehara Y, et al. 2001. The GM130 and GRASP65 Golgi proteins cycle through and define a subdomain of the intermediate compartment. *Nat. Cell Biol*. 3:1101–13

Marshall WF, Vucica Y, Rosenbaum JL. 2001. Kinetics and regulation of de novo centriole assembly. Implications for the mechanism of centriole duplication. *Curr. Biol*. 11:308–17

Martinez-Menarguez JA, Prekeris R, Oorschot VM, Scheller R, Slot JW, et al. 2001. Peri-Golgi vesicles contain retrograde but not anterograde proteins consistent with the cisternal progression model of intra-Golgi transport. *J. Cell Biol*. 155:1213–24

Maul GG, Brinkley BR. 1970. The Golgi apparatus during mitosis in human melanoma cells in vitro. *Cancer Res*. 30:2326–35

Mayer A, Wickner W, Haas A. 1996. Sec18p (NSF)-driven release of Sec17p (alpha-SNAP) can precede docking and fusion of yeast vacuoles. *Cell* 85:83–94

Mayr PS, Allan VJ, Woodman PG. 1999. Phosphorylation of p97(VCP) and p47 in vitro by p34CDK1 kinase. *Eur. J. Cell Biol*. 78:224–32

McNew JA, Parlati F, Fukuda R, Johnston RJ, Paz K, et al. 2000a. Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. *Nature* 407:153–59

McNew JA, Weber T, Parlati F, Johnston RJ, Melia TJ, et al. 2000b. Close is not enough:

SNARE-dependent membrane fusion requires an active mechanism that transduces force to membrane anchors. *J. Cell Biol.* 150:105–17

Mellman I. 1996. Endocytosis and molecular sorting. *Annu. Rev. Cell Dev. Biol.* 12:575–625

Mellman I, Simons K. 1992. The Golgi complex: in vitro veritas? *Cell* 68:829–40

Meyer HH, Kondo H, Warren G. 1998. The p47 co-factor regulates the ATPase activity of the membrane fusion protein, p97. *FEBS Lett.* 437:255–57

Meyer HH, Shorter JG, Seemann J, Pappin D, Warren G. 2000. A complex of mammalian ufd1 and npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. *EMBO J.* 19:2181–92

Miles S, McManus H, Forsten KE, Storrie B. 2001. Evidence that the entire Golgi apparatus cycles in interphase HeLa cells: sensitivity of Golgi matrix proteins to an ER exit block. *J Cell Biol.* 155:543–55

Mironov AA, Beznoussenko GV, Nicoziani P, Martella O, Trucco A, et al. 2001. Small cargo proteins and large aggregates can traverse the Golgi by a common mechanism without leaving the lumen of cisternae. *J. Cell Biol.* 155:1225–38

Misteli T. 1996. Molecular mechanisms in the disassembly and reassembly of the mammalian Golgi apparatus during M-phase. *FEBS Lett.* 389:66–69

Misteli T. 2001. The concept of self-organization in cellular architecture. *J. Cell Biol.* 155:181–85

Misteli T, Warren G. 1994. COP-coated vesicles are involved in the mitotic fragmentation of Golgi stacks in a cell-free system. *J. Cell Biol.* 125:269–82

Misteli T, Warren G. 1995a. Mitotic disassembly of the Golgi apparatus in vivo. *J. Cell Sci.* 108:2715–27

Misteli T, Warren G. 1995b. A role for tubular networks and a COP I-independent pathway in the mitotic fragmentation of Golgi stacks in a cell-free system. *J. Cell Biol.* 130:1027–39

Misura KM, Scheller RH, Weis WI. 2001. Self-association of the H3 region of syntaxin 1A. Implications for intermediates in SNARE complex assembly. *J. Biol. Chem.* 276:13273–82

Mollenhauer HH, Morre DJ. 1978. Structural compartmentation of the cytosol: zones of exclusion, zones of adhesion, cytoskeletal and intercisternal elements. *Subcell. Biochem.* 5:327–59

Morin-Ganet MN, Rambourg A, Deitz SB, Franzusoff A, Kepes F. 2000. Morphogenesis and dynamics of the yeast Golgi apparatus. *Traffic* 1:56–68

Moskalewski S, Thyberg J. 1990. Disorganization and reorganization of the Golgi complex and the lysosomal system in association with mitosis. *J. Submicrosc. Cytol. Pathol.* 22:159–71

Moskalewski S, Thyberg J, Hinek A, Friberg U. 1977. Fine structure of the Golgi complex during mitosis of cartilaginous cells in vitro. *Tissue Cell* 9:185–96

Moyer BD, Allan BB, Balch WE. 2001. Rab1 interaction with a GM130 effector complex regulates COPII vesicle cis-Golgi tethering. *Traffic* 2:268–76

Müller JM, Rabouille C, Newman R, Shorter J, Freemont P, et al. 1999. An NSF function distinct from ATPase-dependent SNARE disassembly is essential for Golgi membrane fusion. *Nat. Cell Biol.* 1:335–40

Müller JM, Shorter J, Newman R, Deinhardt K, Sagiv Y, et al. 2002. Sequential SNARE disassembly and GATE-16/GOS-28 complex assembly mediated by distinct NSF activities drives Golgi membrane fusion. *J. Cell Biol.* 157:1161–73

Munro S. 1998. Localization of proteins to the Golgi apparatus. *Trends Cell Biol.* 8:11–15

Nagahama M, Orci L, Ravazzola M, Amherdt M, Lacomis L, et al. 1996. A v-SNARE implicated in intra-Golgi transport. *J. Cell Biol.* 133:507–16

Nakamura N, Lowe M, Levine TP, Rabouille C, Warren G. 1997. The vesicle docking protein p115 binds GM130, a *cis*-Golgi matrix

protein, in a mitotically regulated manner. *Cell* 89:445–55

Nakamura N, Rabouille C, Watson R, Nilsson T, Hui N, et al. 1995. Characterization of a *cis*-Golgi matrix protein, GM130. *J. Cell Biol.* 131:1715–26

Nebenführ A, Frohlick JA, Staehelin LA. 2000. Redistribution of Golgi stacks and other organelles during mitosis and cytokinesis in plant cells. *Plant Physiol.* 124:135–51

Nigg EA. 2001. Mitotic kinases as regulators of cell division and its checkpoints. *Nat. Rev. Mol. Cell Biol.* 2:21–32

Nilsson T, Hoe MH, Slusarewicz P, Rabouille C, Watson R, et al. 1994. Kin recognition between medial Golgi enzymes in HeLa cells. *EMBO J.* 13:562–74

Nilsson T, Pypaert M, Hoe MH, Slusarewicz P, Berger EG, Warren G. 1993. Overlapping distribution of two glycosyltransferases in the Golgi apparatus of HeLa cells. *J. Cell Biol.* 120:5–13

Nilsson T, Rabouille C, Hui N, Watson R, Warren G. 1996. The role of the membrane-spanning domain and stalk region of N-acetylglucosaminyltransferase I in retention, kin recognition and structural maintenance of the Golgi apparatus in HeLa cells. *J. Cell Sci.* 109:1975–89

Nobes CD, Hall A. 1999. Rho GTPases control polarity, protrusion, and adhesion during cell movement. *J. Cell Biol.* 144:1235–44

Norman TC, Smith DL, Sorger PK, Drees BL, O'Rourke SM, et al. 1999. Genetic selection of peptide inhibitors of biological pathways. *Science* 285:591–95

Nunnari J, Walter P. 1996. Regulation of organelle biogenesis. *Cell* 84:389–94

Oliver JM, Seagrave JC, Pfeiffer JR, Feibig ML, Deanin GG. 1985. Surface functions during mitosis in rat basophilic leukemia cells. *J. Cell Biol.* 101:2156–66

Orci L, Amherdt M, Ravazzola M, Perrelet A, Rothman JE. 2000a. Exclusion of Golgi residents from transport vesicles budding from Golgi cisternae in intact cells. *J. Cell Biol.* 150:1263–70

Orci L, Perrelet A, Rothman JE. 1998. Vesicles on strings: morphological evidence for processive transport within the Golgi stack. *Proc. Natl. Acad. Sci. USA* 95:2279–83

Orci L, Ravazzola M, Volchuk A, Engel T, Gmachl M, et al. 2000b. Anterograde flow of cargo across the Golgi stack potentially mediated via bidirectional "percolating" COPI vesicles. *Proc. Natl. Acad. Sci. USA* 97:10400–5

Orci L, Stamnes M, Ravazzola M, Amherdt M, Perrelet A, et al. 1997. Bidirectional transport by distinct populations of COPI-coated vesicles. *Cell* 90:335–49

Owen DJ, Schiavo G. 1999. A handle on NSF. *Nat. Cell Biol.* 1:E127–38

Parlati F, McNew JA, Fukuda R, Miller R, Söllner TH, Rothman JE. 2000. Topological restriction of SNARE-dependent membrane fusion. *Nature* 407:194–98

Patel S, Latterich M. 1998. The AAA team: related ATPases with diverse functions. *Trends Cell Biol.* 8:65–71

Patel SK, Indig FE, Olivieri N, Levine ND, Latterich M. 1998. Organelle membrane fusion: a novel function for the syntaxin homolog Ufe1p in ER membrane fusion. *Cell* 92:611–20

Patil C, Walter P. 2001. Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals. *Curr. Opin. Cell Biol.* 13:349–55

Paumet F, Brugger B, Parlati F, McNew JA, Söllner TH, Rothman JE. 2001. A t-SNARE of the endocytic pathway must be activated for fusion. *J. Cell Biol.* 155:961–68

Pelham HR. 2001a. SNAREs and the specificity of membrane fusion. *Trends Cell Biol.* 11:99–101

Pelham HR. 2001b. Traffic through the Golgi apparatus. *J. Cell Biol.* 155:1099–102

Pelletier L, Jokitalo E, Warren G. 2000. The effect of Golgi depletion on exocytic transport. *Nat. Cell Biol.* 2:840–46

Pelletier L, Stern CA, Pypaert M, Sheff D, Ngô H, et al. 2002. Golgi biogenesis in *Toxoplasma gondii*. *Nature*. In press

Peters C, Bayer MJ, Buhler S, Andersen JS,

Mann M, Mayer A. 2001. Trans-complex formation by proteolipid channels in the terminal phase of membrane fusion. *Nature* 409:581–88

Pevsner J, Hsu SC, Braun JE, Calakos N, Ting AE, et al. 1994. Specificity and regulation of a synaptic vesicle docking complex. *Neuron* 13:353–61

Pfeffer SR. 1999. Transport-vesicle targeting: tethers before SNAREs. *Nat. Cell Biol.* 1: E17–22

Pfeffer SR. 2001. Constructing a Golgi complex. *J. Cell Biol.* 155:873–75

Piel M, Nordberg J, Euteneuer U, Bornens M. 2001. Centrosome-dependent exit of cytokinesis in animal cells. *Science* 291:1550–53

Prescott AR, Farmaki T, Thomson C, James J, Paccaud JP, et al. 2001. Evidence for prebudding arrest of ER export in animal cell mitosis and its role in generating Golgi partitioning intermediates. *Traffic* 2:321–35

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

Preuss D, Mulholland J, Franzusoff A, Segev N, Botstein D. 1992. Characterization of the *Saccharomyces* Golgi complex through the cell cycle by immunoelectron microscopy. *Mol. Biol. Cell* 3:789–803

Puthenveedu MA, Linstedt AD. 2001. Evidence that Golgi structure depends on a p115 activity that is independent of the vesicle tether components Giantin and GM130. *J. Cell Biol.* 155:227–38

Pypaert M, Nilsson T, Berger EG, Warren G. 1993. Mitotic Golgi clusters are not tubular endosomes. *J. Cell Sci.* 104:811–18

Rabouille C, Kondo H, Newman R, Hui N, Freemont P, Warren G. 1998. Syntaxin 5 is a common component of the NSF- and p97-mediated reassembly pathways of Golgi cisternae from mitotic Golgi fragments in vitro. *Cell* 92:603–10

Rabouille C, Levine TP, Peters JM, Warren G. 1995a. An NSF-like ATPase, p97, and NSF mediate cisternal regrowth from mitotic Golgi fragments. *Cell* 82:905–14

Rabouille C, Misteli T, Watson R, Warren G. 1995b. Reassembly of Golgi stacks from mitotic Golgi fragments in a cell-free system. *J. Cell Biol.* 129:605–18

Rambourg A, Clermont Y. 1997. Three-dimensional structure of the Golgi apparatus in mammalian cells. In *The Golgi Apparatus*, ed. EG Berger, J Roth, pp. 37–61. Basel: Birkhauser

Rizzuto R, Brini M, Pizzo P, Murgia M, Pozzan T. 1995. Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells. *Curr. Biol.* 5:635–42

Robinson DN, Spudich JA. 2000. Towards a molecular understanding of cytokinesis. *Trends Cell Biol.* 10:228–37

Rogalski AA, Bergmann JE, Singer SJ. 1984. Effect of microtubule assembly status on the intracellular processing and surface expression of an integral protein of the plasma membrane. *J. Cell Biol.* 99:1101–9

Rossanese OW, Reinke CA, Bevis BJ, Hammond AT, Sears IB, et al. 2001. A role for actin, Cdc1p, and Myo2p in the inheritance of late Golgi elements in *Saccharomyces cerevisiae*. *J. Cell Biol.* 153:47–62

Rothman JE. 1981. The Golgi apparatus: two organelles in tandem. *Science* 213:1212–19

Rothman JE. 1994. Mechanism of intracellular protein transport. *Nature* 372:55–63

Rothman JE, Warren G. 1994. Implications of the SNARE hypothesis for intracellular membrane topology and dynamics. *Curr. Biol.* 4:220–33

Rouiller I, Butel VM, Latterich M, Milligan RA, Wilson-Kubalek EM. 2000. A major conformational change in p97 AAA ATPase upon ATP binding. *Mol. Cell* 6:1485–90

Roy L, Bergeron JJ, Lavoie C, Hendriks R, Gushue J, et al. 2000. Role of p97 and syntaxin 5 in the assembly of transitional endoplasmic reticulum. *Mol. Biol. Cell* 11:2529–42

Rudd PM, Elliott T, Cresswell P, Wilson IA, Dwek RA. 2001. Glycosylation and the immune system. *Science* 291:2370–76

Sagiv Y, Legesse-Miller A, Porat A, Elazar Z. 2000. GATE-16, a membrane transport

modulator, interacts with NSF and the Golgi v-SNARE GOS-28. *EMBO J.* 19:1494–504

Scales SJ, Chen YA, Yoo BY, Patel SM, Doung YC, Scheller RH. 2000. SNAREs contribute to the specificity of membrane fusion. *Neuron* 26:457–64

Scales SJ, Pepperkok R, Kreis TE. 1997. Visualization of ER-to-Golgi transport in living cells reveals a sequential mode of action for COPII and COPI. *Cell* 90:1137–48

Schroeter D, Ehemann V, Paweletz N. 1985. Cellular compartments in mitotic cells: ultrahistochemical identification of Golgi elements in PtK-1 cells. *Biol. Cell* 53:155–63

Sciaky N, Presley J, Smith C, Zaal KJ, Cole N, et al. 1997. Golgi tubule traffic and the effects of brefeldin A visualized in living cells. *J. Cell Biol.* 139:1137–55

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

Seemann J, Jokitalo EJ, Warren G. 2000b. The role of the tethering proteins p115 and GM130 in transport through the Golgi apparatus in vivo. *Mol. Biol. Cell* 11:635–45

Seemann J, Pypaert M, Taguchi T, Malsam J, Warren G. 2002. Partitioning of the matrix fraction of the Golgi apparatus during mitosis in animal cells. *Science* 295:848–51

Sheff D, Pelletier L, O'Connell CB, Warren G, Mellman I. 2002. Transferrin receptor recycling in the absence of perinuclear recycling endosomes. *J. Cell Biol.* 156:797–804

Shima DT, Cabrera-Poch N, Pepperkok R, Warren G. 1998. An ordered inheritance strategy for the Golgi apparatus: visualization of mitotic disassembly reveals a role for the mitotic spindle. *J. Cell Biol.* 141:955–66

Shima DT, Haldar K, Pepperkok R, Watson R, Warren G. 1997. Partitioning of the Golgi apparatus during mitosis in living HeLa cells. *J. Cell Biol.* 137:1211–28

Shima DT, Warren G. 1998. Inheritance of the cytoplasm during cell division. In *Dynamics of Cell Division*, ed. SA Endow, DM Glover, pp. 248–69. Oxford: Oxford Univ. Press

Short B, Preisinger C, Korner R, Kopajtich R, Byron O, Barr FA. 2001. A GRASP55-rab2 effector complex linking Golgi structure to membrane traffic. *J. Cell Biol.* 155:877–83

Shorter J, Beard M, Seemann J, Dirac-Svejstrup AB, Warren G. 2002. Sequential tethering of Golgins and catalysis of SNAREpin assembly by the vesicle tethering protein p115. *J. Cell Biol.* 157:45–62

Shorter J, Warren G. 1999. A role for the vesicle tethering protein, p115, in the post-mitotic stacking of reassembling Golgi cisternae in a cell-free system. *J. Cell Biol.* 146:57–70

Shorter J, Watson R, Giannakou ME, Clarke M, Warren G, Barr FA. 1999. GRASP55, a second mammalian GRASP protein involved in the stacking of Golgi cisternae in a cell-free system. *EMBO J.* 18:4949–60

Slusarewicz P, Nilsson T, Hui N, Watson R, Warren G. 1994. Isolation of a matrix that binds medial Golgi enzymes. *J. Cell Biol.* 124:405–13

Sohda M, Misumi Y, Yano A, Takami N, Ikehara Y. 1998. Phosphorylation of the vesicle docking protein p115 regulates its association with the Golgi membrane. *J. Biol. Chem.* 273:5385–88

Söllner T. 2002. Vesicle tethers promoting fusion machinery assembly. *Dev. Cell.* 2:377–78

Söllner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE. 1993. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. *Cell* 75:409–18

Sönnichsen B, Lowe M, Levine T, Jamsa E, Dirac-Svejstrup B, Warren G. 1998. A role for Giantin in docking COPI vesicles to Golgi membranes. *J. Cell Biol.* 140:1013–21

Sönnichsen B, Watson R, Clausen H, Misteli T, Warren G. 1996. Sorting by COP I-coated vesicles under interphase and mitotic conditions. *J. Cell Biol.* 134:1411–25

Souter E, Pypaert M, Warren G. 1993. The Golgi stack reassembles during telophase before arrival of proteins transported from the endoplasmic reticulum. *J. Cell Biol.* 122: 533–40

Stuart RA, Mackay D, Adamczewski J, Warren G. 1993. Inhibition of intra-Golgi transport in vitro by mitotic kinase. *J. Biol. Chem.* 268:4050–54

Sutterlin C, Hsu P, Mallabiabarrena A, Malhotra V. 2002. Fragmentation and dispersal of the pericentriolar Golgi complex is required for entry into mitosis in mammalian cells. *Cell* 109:359–69

Sutterlin C, Lin CY, Feng Y, Ferris DK, Erikson RL, Malhotra V. 2001. Polo-like kinase is required for the fragmentation of pericentriolar Golgi stacks during mitosis. *Proc. Natl. Acad. Sci. USA* 98:9128–32

Szathmary E, Maynard Smith J. 1995. The major evolutionary transitions. *Nature* 374:227–32

Tagaya M, Wilson DW, Brunner M, Arango N, Rothman JE. 1993. Domain structure of an N-ethylmaleimide-sensitive fusion protein involved in vesicular transport. *J. Biol. Chem.* 268:2662–66

Tamaki H, Yamashina S. 1991. Changes in cell polarity during mitosis in rat parotid acinar cells. *J. Histochem. Cytochem.* 39:1077–87

Thyberg J, Moskalewski S. 1992. Reorganization of the Golgi complex in association with mitosis: redistribution of mannosidase II to the endoplasmic reticulum and effects of brefeldin A. *J. Submicrosc. Cytol. Pathol.* 24:495–508

Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. 2000. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* 101:249–58

Troyer D, Cameron ML. 1980. Spermiogenesis in lumbricid earthworms revisited. I. Function and fate of centrioles, fusion of organelles and organelle movements. *Biol. Cell* 37:279–86

Ungermann C, Sato K, Wickner W. 1998. Defining the functions of trans-SNARE pairs. *Nature* 396:543–48

Valsdottir R, Hashimoto H, Ashman K, Koda T, Storrie B, Nilsson T. 2001. Identification of rabaptin-5, rabex-5, and GM130 as putative effectors of rab33b, a regulator of retrograde traffic between the Golgi apparatus and ER. *FEBS Lett.* 508:201–9

van Leyen K, Duvoisin RM, Engelhardt H, Wiedmann M. 1998. A function for lipoxygenase in programmed organelle degradation. *Nature* 395:392–95

Varki A. 1998. Factors controlling the glycosylation potential of the Golgi apparatus. *Trends Cell Biol.* 8:34–40

Vincent JP, Magee T. 2002. Argosomes: membrane fragments on the run. *Trends Cell Biol.* 12:57–60

Volchuk A, Amherdt M, Ravazzola M, Brugger B, Rivera VM, et al. 2000. Megavesicles implicated in the rapid transport of intracisternal aggregates across the Golgi stack. *Cell* 102:335–48

Ward TH, Polishchuk RS, Caplan S, Hirschberg K, Lippincott-Schwartz J. 2001. Maintenance of Golgi structure and function depends on the integrity of ER export. *J. Cell Biol.* 155:557–70

Warren G. 1993. Membrane partitioning during cell division. *Annu. Rev. Biochem.* 62:323–48

Warren G. 1995. Intracellular membrane morphology. *Philos. Trans. R. Soc. London Ser. B* 349:291–95

Warren G, Wickner W. 1996. Organelle inheritance. *Cell* 84:395–400

Waters MG, Clary DO, Rothman JE. 1992. A novel 115-kD peripheral membrane protein is required for intercisternal transport in the Golgi stack. *J. Cell Biol.* 118:1015–26

Weide T, Bayer M, Koster M, Siebrasse JP, Peters R, Barnekow A. 2001. The Golgi matrix protein GM130: a specific interacting partner of the small GTPase rab1b. *EMBO Rep.* 2:336–41

Weigert R, Silletta MG, Spano S, Turacchio G, Cericola C, et al. 1999. CtBP/BARS induces fission of Golgi membranes by acylating lysophosphatidic acid. *Nature* 402:429–33

Weimbs T, Low SH, Chapin SJ, Mostov KE, Bucher P, Hofmann K. 1997. A conserved domain is present in different families of

vesicular fusion proteins: a new superfamily. *Proc. Natl. Acad. Sci. USA* 94:3046–51

Weimbs T, Mostov K, Low SH, Hofmann K. 1998. A model for structural similarity between different SNARE complexes based on sequence relationships. *Trends Cell Biol.* 8:260–62

Wendler F, Page L, Urbé S, Tooze SA. 2001. Homotypic fusion of immature secretory granules during maturation requires syntaxin 6. *Mol. Biol. Cell* 12:1699–709

White J, Keller P, Stelzer EH. 2001. Spatial partitioning of secretory cargo from Golgi resident proteins in live cells. *BMC Cell Biol.* 2:19

Whitehead CM, Rattner JB. 1998. Expanding the role of HsEg5 within the mitotic and postmitotic phases of the cell cycle. *J. Cell Sci.* 111:2551–61

Wickner W, Haas A. 2000. Yeast homotypic vacuole fusion: a window on organelle trafficking mechanisms. *Annu. Rev. Biochem.* 69:247–75

Xu Y, Wong SH, Zhang T, Subramaniam VN, Hong W. 1997. GS15, a 15-kilodalton Golgi soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) homologous to rbet1. *J. Biol. Chem.* 272:20162–66

Xu Z, Sato K, Wickner W. 1998. LMA1 binds to vacuoles at Sec18p (NSF), transfers upon ATP hydrolysis to a t-SNARE (Vam3p) complex, and is released during fusion. *Cell* 93:1125–34

Ye Y, Meyer HH, Rapoport TA. 2001. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 414:652–56

Yoshimura SI, Nakamura N, Barr FA, Misumi Y, Ikehara Y, et al. 2001. Direct targeting of *cis*-Golgi matrix proteins to the Golgi apparatus. *J. Cell Sci.* 114:4105–15

Yuan X, Shaw A, Zhang X, Kondo H, Lally J, et al. 2001. Solution structure and interaction surface of the C-terminal domain from p47: a major p97-cofactor involved in SNARE disassembly. *J. Mol. Biol.* 311:255–63

Zaal KJ, Smith CL, Polishchuk RS, Altan N, Cole NB, et al. 1999. Golgi membranes are absorbed into and reemerge from the ER during mitosis. *Cell* 99:589–601

Zeligs JD, Wollman SH. 1979. Mitosis in rat thyroid epithelial cells in vivo. I. Ultrastructural changes in cytoplasmic organelles during the mitotic cycle. *J. Ultrastruct. Res.* 66:53–77

Zerial M, McBride H. 2001. Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Biol.* 2:107–17

Zhang T, Hong W. 2001. Ykt6 forms a SNARE complex with syntaxin 5, GS28, and Bet1 and participates in a late stage in endoplasmic reticulum-Golgi transport. *J. Biol. Chem.* 276:27480–87

Zhang X, Shaw A, Bates PA, Newman RH, Gowen B, et al. 2000. Structure of the AAA ATPase p97. *Mol. Cell* 6:1473–84

Zorn GA, Lucas JJ, Kates JR. 1979. Purification and characterization of regenerating mouse L929 karyoplasts. *Cell* 18:659–72

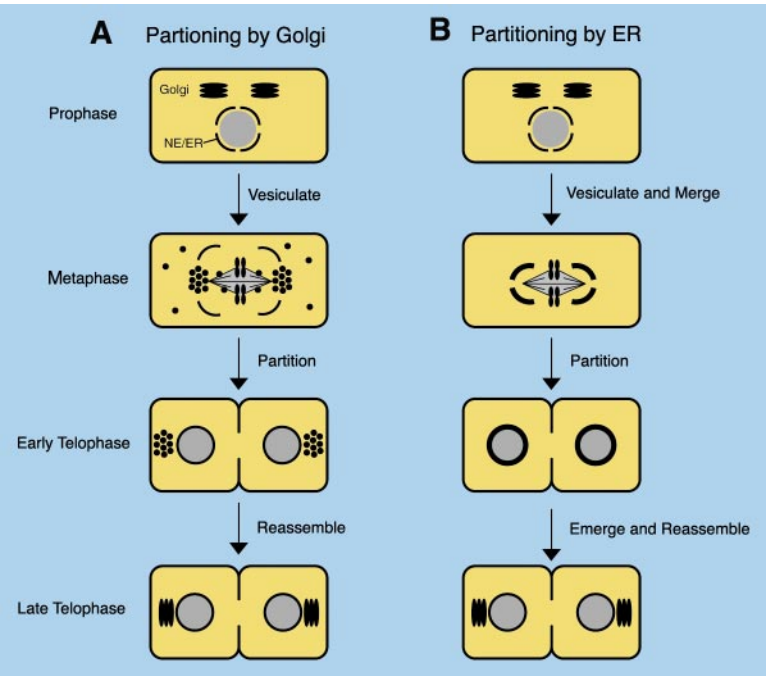

Figure 2 Golgi partitioning models. Two popular models have been posited for the mode of Golgi partitioning during mammalian M-phase. (*A*) The Golgi partitioning model argues that Golgi membranes themselves are the partitioning unit. Early in mitosis the Golgi vesiculates, and these vesicles are evenly distributed between nascent daughter cells by metaphase via clusters of vesicles associated with the mitotic spindle poles and vesicles dispersed into the periphery by astral microtubules. These vesicles then fuse during telophase to reform Golgi stacks. (*B*) The second model argues that the partitioning unit is a merged ER/Golgi compartment. The Golgi vesiculates at the onset of prometaphase, and these vesicles then merge with the ER. Golgi components emerge from the ER during telophase to form Golgi stacks.

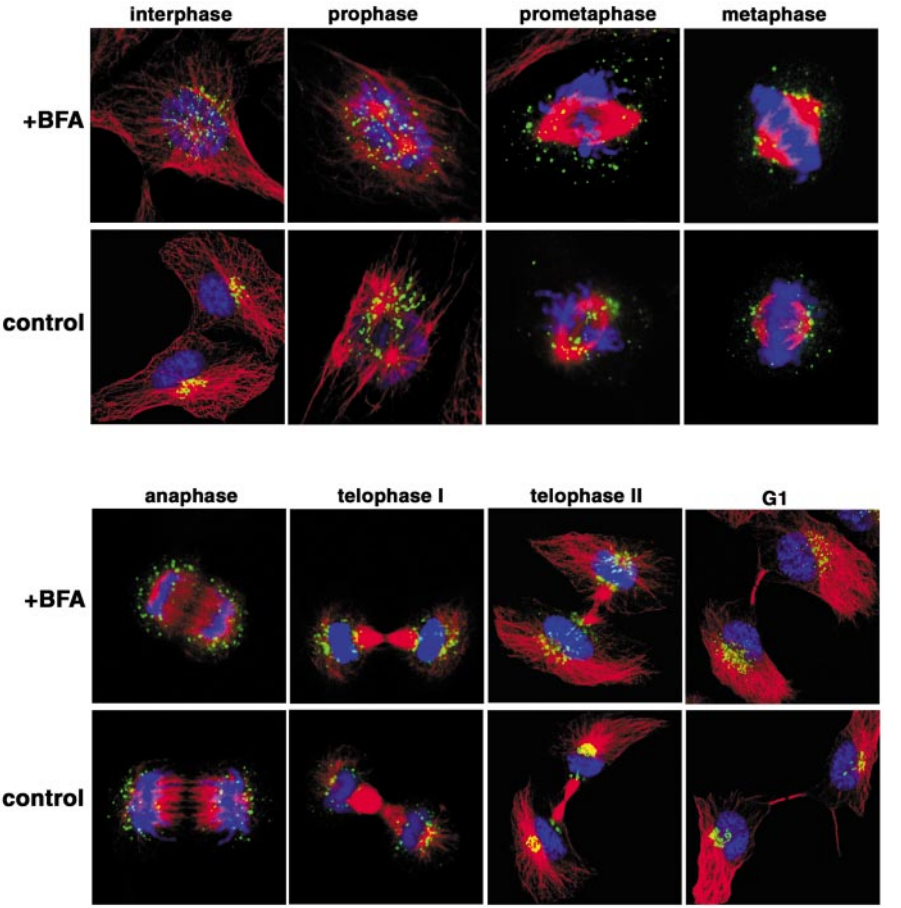

Figure 3 Partitioning of the Golgi matrix in the presence or absence of BFA. Exponentially growing NRK cells were treated with BFA before fixation (*top rows*) or left untreated (*bottom rows*), then triple-labelled for GM130 (*green*), DNA (TO-PRO3 iodine, *blue*) and α-tubulin (*red*). Cells at each stage of mitosis are shown.

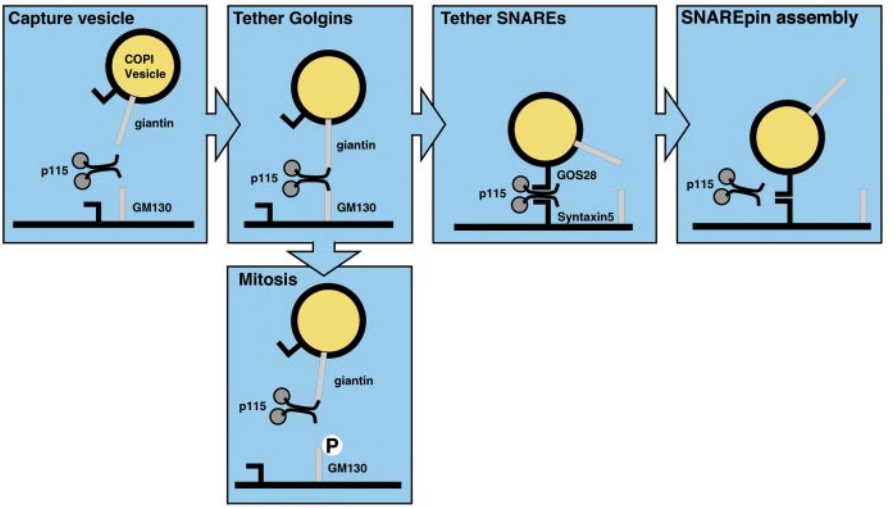

Figure 5 COPI vesicle processing during mitosis and NSF-driven Golgi reassembly. During interphase p115 crosslinks Giantin on COPI vesicles to GM130 on acceptor membranes. At mitosis, cyclin B-CDK1 phosphorylates GM130 and precludes p115 binding. Consequently, COPI vesicles no longer tether and do not fuse with their acceptor compartment. Continued budding in the absence of fusion converts cisternae into COPI vesicles. Dephosphorylation of GM130 at telophase allows the reformation of Giantin-p115-GM130 tethers, which occurs early in NSF-driven Golgi reassembly. After this event, p115 catalyzes the assembly of cognate SNAREpins by first linking the SNAREs together. Assembled SNAREpins then mediate rapid bilayer mixing. Not illustrated here is the NSF/α-SNAP-mediated binding of GATE-16 to the v-SNARE, GOS-28, prior to COPI vesicle capture. Similarly, the phosphorylation of p115 by a CKII-like kinase that may elicit the transition from GM130-p115-Giantin tethers to SNAREpin assembly is not depicted.

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