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Biochimica et Biophysica Acta 1404 (1998) 139–151

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Inheritance of the mammalian Golgi apparatus during the cell cycle

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Received 17 November 1997; accepted 11 December 1997

Abstract

The creation and propagation of the intricate Golgi architecture during the cell cycle poses a fascinating problem for biologists. Similar to the inheritance process for nuclear DNA, the inheritance of the Golgi apparatus consists of biogenesis (replication) and partitioning (mitosis/meiosis) phases, in which Golgi components must double in unit mass, then be appropriately divided between nascent daughter cells during cytokinesis. In this article we focus discussion on the recent advances in the area of Golgi inheritance, first outlining our current understanding of the behaviour of the Golgi apparatus during cell division, then concluding with a more conceptual discussion of the Golgi biogenesis problem. Throughout, we attempt to integrate ultrastructural and biochemical findings with more recent information obtained using live cell microscopy and morphological techniques. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Biogenesis; Cell cycle; Membrane fusion; Golgi apparatus; Mitosis; Organelle inheritance

1. Partitioning of the mammalian Golgi apparatus during cell division

1.1. Ultrastructural view of Golgi morphology during the cell cycle

The interphase Golgi apparatus in most mammalian cells occupies a juxta-nuclear, usually pericentriolar, location [1]. The unit of the Golgi is comprised of a stack of disc-shaped membranes, termed cisternae, bounded on each face by extensive tubular-reticular networks termed the *cis*-Golgi network (CGN) and the *trans*-Golgi network (TGN) [2]. Adjacent stacks are joined by tubules, forming a ribbon which bifurcates and intersects, yielding an interconnected reticulum [3] (Fig. 1A).

Ultrastructural observations laid the foundation for an initial understanding of the alterations to the Golgi ribbon in animal cells during mitosis. During prophase, the interphase Golgi ribbon is initially fragmented into a collection of stacks which are dispersed in the cytoplasm [4]. Later, during the transition from late prophase to metaphase, the stereotypical architecture of the animal Golgi stack is transformed into a collection of membrane elements, termed the mitotic cluster. The precise morphological composition of membranes in the mitotic clusters appears to vary for different cell types: in HeLa cells [5], NRK cells [6], PtK-1 cells [7] (Fig. 1B), parotid acinar cells [8] and thyroid epithelia [9], the clusters consist of a heterogeneous collection of 50–70 nm diameter vesicles, larger vesicles and short tubules; in L929 fibroblasts [10], chondrocytes [11] and mela-

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PII: S0167-4889(98)00051-2

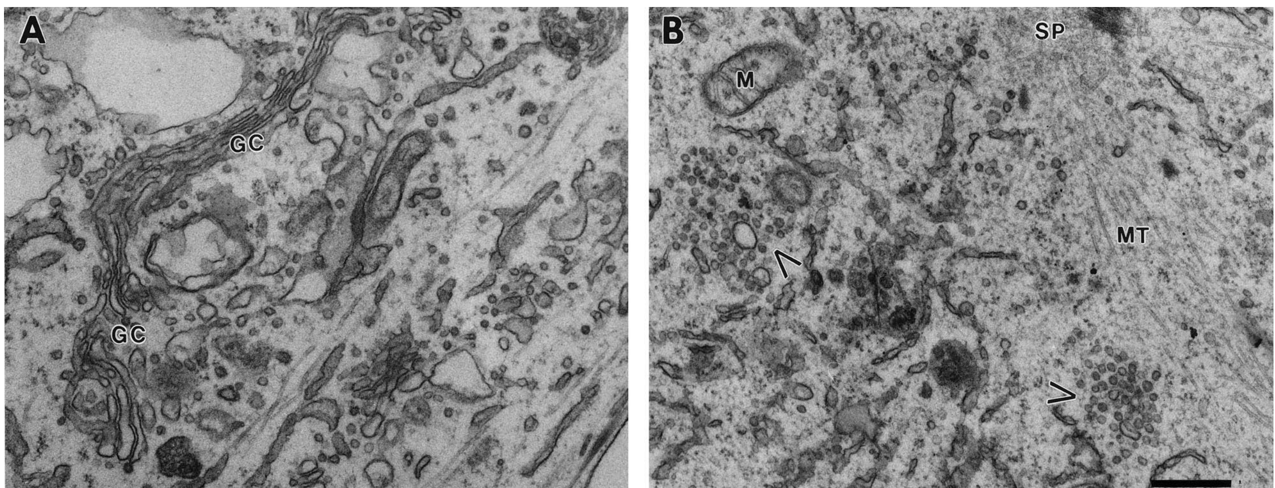

Fig. 1. Ultrastructural appearance of the Golgi during interphase and mitosis in PtK1 cells. (A) An ultrastructural view of the interphase Golgi complex. The typical Golgi ribbon consists of a collection of Golgi stacks and tubular reticular networks, designated GC. (B) The ultrastructural appearance of the Golgi during mitosis: numerous vesicles and tubules constitute the mitotic cluster, arrowheads; spindle pole, SP; spindle microtubules, MT; mitochondria, M. Note that each clustered tubulo-vesicular element corresponds to one of the numerous mitotic fragments observed in the metaphase fluorescence image in Fig. 2 (micrographs kindly provided by Eija Jämsä and Rose Watson). Bar = 0.3 μm.

noma cells [12] the clusters appear to be a collection of tubules, more closely resembling dilated cisternae. These early morphological observations were eventually augmented by a more quantitative analysis of mitotic Golgi membranes in HeLa cells [13,14], which suggested that by metaphase, the low copy number interphase Golgi ribbon is converted to hundreds of mitotic clusters, which could continue shedding membrane vesicles into the surrounding cytoplasm to yield numerous, well dispersed Golgi membranes. As cells progress from metaphase to early telophase, the Golgi stacks are reassembled by the inverse process: dispersed tubulo-vesicular membranes rapidly coalesce, fuse and rearrange to give rise to intact and functional Golgi stacks before the end of cytokinesis [15]. Finally, after cell division, Golgi stacks congregate in the pericentriolar region to re-form the interphase Golgi ribbon [10,16] (Fig. 2).

1.2. Experimental systems for mimicking alterations in Golgi membrane morphology during mitosis

Recent advances in our understanding of the molecules and mechanisms involved in the regulation of Golgi apparatus structure and function are due, in major part, to the ability to model the mitotic disassembly/reassembly process in a cell free assay, and to the application of drugs which mimic certain aspects of the disassembly/reassembly process in intact or permeabilised cells.

1.2.1. Cell-free mitotic disassembly/reassembly

The morphological transformation of Golgi membranes during mitosis has been mimicked in a cell-free system, using highly purified rat liver Golgi stacks and cytosol derived from prometaphase arrested HeLa cells to provide mitotic conditions. This has permitted a detailed analysis of membrane intermediates and the identification of potential components involved in the cycle of disassembly and reassembly [17,18] (Fig. 3). In this system, 50–65% of Golgi stacks are disassembled by the continuous budding of COPI-coated vesicles, presumably in a manner similar to the mechanism of transport vesicle formation. The involvement of COPI in disassembly of the Golgi stacks provided the first direct evidence that the formation of mitotic membranes may utilise the same class of components used for vesicle-based transport. A COPI-independent pathway has also been implicated in the production of a separate class of mitotic fragments, including tubular networks and larger, heterogeneously sized vesicles and tubules [18]. Interestingly, the composition of Golgi resident

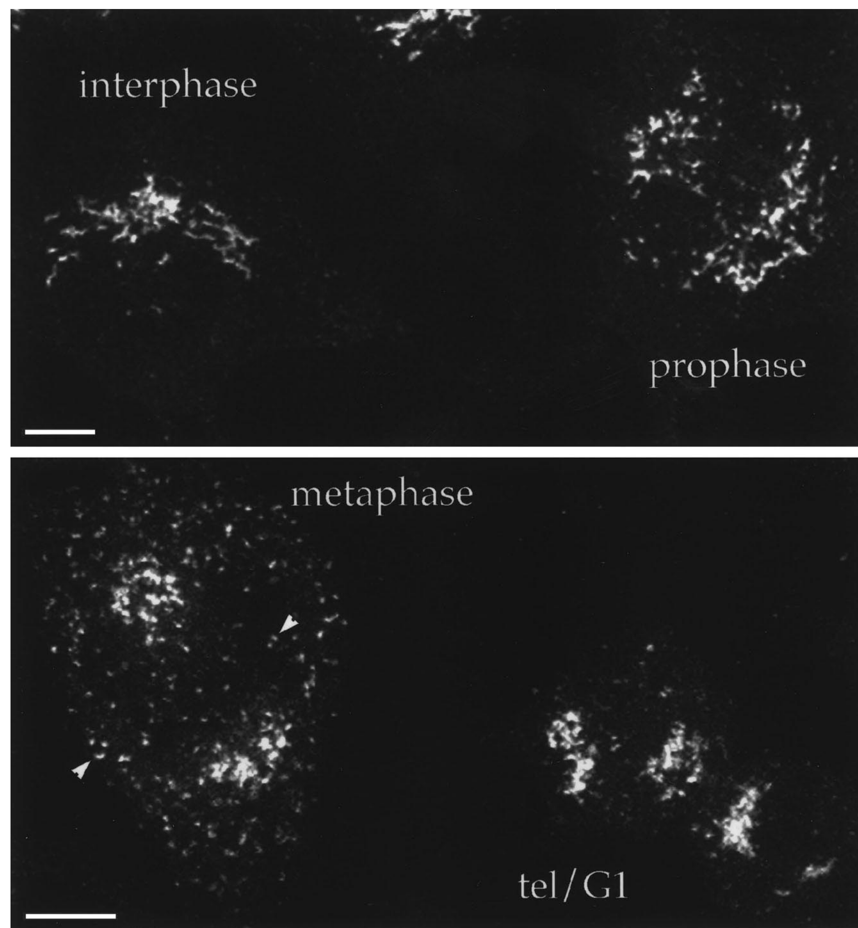

Fig. 2. Morphological changes to the Golgi apparatus in mitosis. Golgi morphology during different stages of the cell cycle in L929 fibroblasts, visualised by immunostaining the *cis*-Golgi marker, GM130. Arrowheads point to mitotic Golgi clusters in the metaphase cell. Bar = 5 μm.

proteins differs in the two classes of membrane intermediates [19]: Golgi residents, including several oligosaccharide processing enzymes, are selectively enriched in the COPI-independent membrane populations; in contrast, mitotic COPI vesicles are enriched for components involved in transport between the endoplasmic reticulum (ER) and Golgi apparatus, such as the putative retrieval receptor for proteins escaped from the ER, the KDEL receptor [20], and a mannose-binding lectin thought to be involved in cargo transport, ERGIC53/p58 [21]. Taken together, these data suggested that during mitotic disassembly of the Golgi stack, the COPI pathway acts to disassemble the cisternal rims, i.e., regions specialised for transport, and the COPI-independent pathway is responsible for the transformation of the remaining cisternae into a heterogeneous collection of tubular membranes which harbour the majority of Golgi resident proteins [19].

A working model has been proposed which links the mitotic disassembly of Golgi stacks to the general cessation in exocytic membrane traffic that accompanies the onset of mitosis, and is based on the postulate that membrane fusion is inhibited during mitosis. This model could explain the inhibition of protein secretion, which relies on both the budding of transport vesicles from donor membranes (e.g. ER) and fusion of these vesicles with downstream acceptor membranes (e.g. Golgi apparatus). Moreover, the constant budding of Golgi-derived vesicles in the absence of fusion of new input vesicles would eventually lead to the consumption of Golgi cisternal rims, explaining the COPI-dependent morphological changes to the Golgi membranes during mitosis [22].

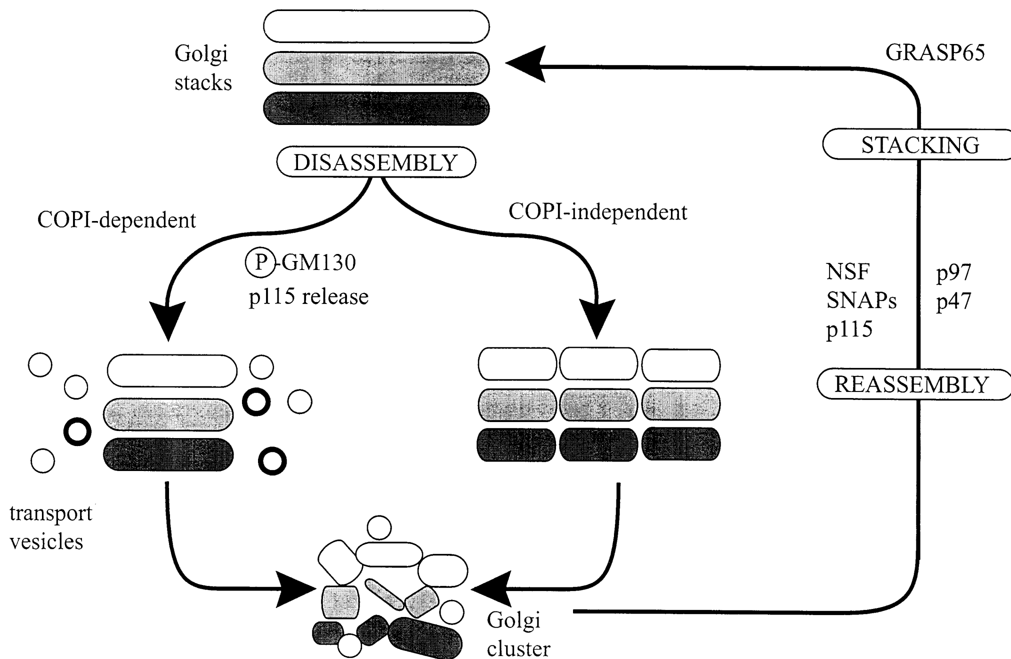

Fig. 3. Mitotic Golgi disassembly/reassembly model. Diassembly of the Golgi during mitosis occurs via COPI and COPI-independent pathways. Similarly, reassembly of the Golgi utilises two different pathways, p97/p47 and NSF/SNAPS/p115. Final assembly of stacked cisternae involves the NEM-sensitive activity of GRASP65. See text for details.

Recent biochemical evidence suggests a role for the putative vesicle docking protein, p115, in the inhibition of COPI vesicle fusion during mitosis [23]. p115 is required for the fusion of transport vesicles with acceptor membranes in a cell-free intra-Golgi transport assay [27], and the yeast counterpart of p115, termed Uso1p, is essential for transport between the ER and Golgi apparatus [28]. Purified p115 is a myosin-shaped molecule with two globular head domains linked by a long, coiled-coil rod, a structure which has led many to suggest a role for p115 as a vesicle tether or 'velcro' factor for capturing transport vesicles [23–26]; however, this attractive scheme still awaits experimental confirmation.

p115 localises to the *cis* face of the Golgi stack during interphase; during mitosis, p115 is released from Golgi membranes, consistent with the idea that it plays a role in the inhibition of vesicle fusion, and thus, the cessation of membrane traffic and disassembly of the Golgi apparatus in mitosis [29,30]. Early studies also suggested that the reason for the release of p115 from Golgi membranes was due to the mitotic modification of its binding partner(s) [30].

A cytoskeletal-like Golgi matrix protein, termed GM130 [31], has recently been identified as a mitotically regulated binding partner for p115 [32]. Like p115, GM130 localisation appears to be restricted to the *cis* side of the Golgi stack, implying that additional components must be involved in modulating the assembly of the remaining Golgi cisternae. Good candidates would be other, large peripheral membrane proteins that, like GM130, have been implicated in the maintenance of Golgi architecture [33],

The reassembly of mitotic Golgi fragments into stacked Golgi cisternae has also been mimicked using a cell-free assay [34], and has led to the view that two distinct fusion pathways are involved in mitotic membrane reassembly [35]. The first is dependent on the function of the *N*-ethylmaleimide-sensitive fusion (NSF) ATPase, a component of the SNARE-dependent membrane fusion machinery implicated in a wide range of vesicle fusion events, including ER to Golgi transport [36], intra-Golgi transport [37] and synaptic transmission [38]. The second fusion pathway requires p97, an abundant cytosolic protein and member of the NSF family of AAA ATPases [39]. The yeast homologue of p97, cdc48, was first identified as a cell cycle mutant [40] and has

subsequently been implicated in nuclear membrane fusion required for yeast karyogamy [41]. Interestingly, p97 appears to be the more ancient of the two fusion ATPases, as a homologue has recently been identified in archaebacteria (*S. acidocaldarius*) [42]. The fact that these primitive organisms have no internal membranes suggests that p97 either plays a more general cellular role in addition to its function in intracellular membrane fusion, or that it may possibly be involved in plasma membrane fusion during cytokinesis. In eukaryotes, p97/cdc48 has been implicated in ER membrane fusion [41], clathrin assembly [43], T-cell receptor signalling [44] and ubiquitin-mediated degradation [45], supporting the idea that this ATPase may play several distinct cellular roles. Recent work on mitotic Golgi membranes has led to the identification of p47, a cofactor required for p97-mediated cisternal regrowth, and the first candidate for a protein which may target the activity of p97 to mitotic Golgi membranes [46].

Why the cell would utilise more than one fusion ATPase for reassembly of intact Golgi stacks following mitotic disassembly is still unclear. Morphological evidence from Golgi reassembly following ilimaquinone (IQ) [47] and mitosis-induced fragmentation [35] suggests that p97 and NSF may be involved in the fusion of distinct Golgi membrane populations. However, in contrast to the mitotic system, there is a sequential requirement for NSF and p97 for proper reassembly following IQ-induced Golgi fragmentation [47].

A novel Golgi stacking factor, termed GRASP65, has recently been identified by exploiting the NEM sensitivity of stack formation in the cell-free reassembly system described above [48]. This protein interacts with the Golgi matrix protein GM130 to form a stable, membrane-bound complex that is maintained under mitotic conditions. GRASP65 function is required for the stacking of membranes during reassembly in the cell free assay, however, it is not yet clear how the GRASP65/GM130/p115 complex functions in the rebuilding of Golgi stacks, or if this complex is required for the maintenance of steady-state interphase Golgi architecture. Based on the proposed function of GM130 and p115 in vesicle docking, one could speculate that the process of stacking may be a specialised form of docking, which results in the establishment of stable intercisternal bridges rather than acting as a prelude to membrane fusion.

1.2.2. Microtubule disruption as a model system for Golgi ribbon disassembly

The ultrastructural and biochemical studies described above suggested that during mitosis, Golgi stacks progressively disassemble to produce a heterogeneous collection of membranes which diffuse to redistribute throughout the cell. However, recent investigation of the mechanism of Golgi fragmentation/dispersal following nocodazole-induced microtubule disruption [49] has led to the suggestion that Golgi disassembly results from the retrograde transport of Golgi residents to the periphery via the ER, followed by the reorganisation of Golgi membrane fragments at ER exit sites. It has also been proposed that this mode of Golgi retrograde transport could represent a plausible explanation for the disassembly and redistribution of Golgi membranes during mitosis [49]. Since the ER network occupies a pervasive region within the cytoplasm, retrograde transport of Golgi residents to the ER would serve as a very effective means for distributing Golgi proteins so that cytokinesis alone could result in equal Golgi partitioning [50]. Though intriguing, the Golgi-to-ER disassembly hypothesis is currently based on inferences derived from evidence on the behaviour of the Golgi apparatus in nocodazole, and therefore the applicability of this model to the mitotic situation awaits more direct experimentation.

1.3. Partitioning of mitotic Golgi membranes

The identification of numerous Golgi-derived tubules and vesicles as end products of mitotic disassembly led to the idea that the interphase Golgi ribbon fragments to increase Golgi copy number and dispersal, hence assuring the accuracy of stochastic partitioning: since accurate cytokinetic mechanisms exist to divide the mother cell into two equally sized daughters, any organelle, present in multiple, evenly distributed units, could be inherited solely based on the laws of chance [51]. However, more recently, the direct observation of the behaviour of the Golgi apparatus during cell division has raised doubts about the stochastic nature of Golgi membrane partitioning.

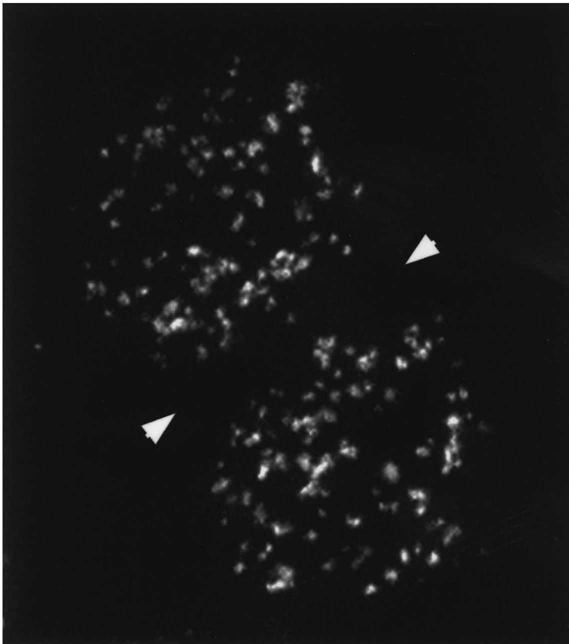

Fig. 4. Accuracy of mitotic Golgi partitioning. The distribution of the GFP-tagged Golgi marker, NAGFP, in a telophase cell. The arrowheads mark the position of the developing cytokinetic furrow. Image analysis suggests that mitotic Golgi clusters are segregated into nascent daughter cells more accurately than predicted for a stochastic partitioning strategy [29].

1.3.1. Visualisation of mitotic Golgi membranes using GFP

The process of mitotic Golgi partitioning has recently been examined in living cells by exploiting the properties of the green fluorescent protein (GFP) [52]. Appending the GFP to the retention signal for the Golgi resident glycosyltransferase *N*-acetylglucosaminyltransferase (NAGT I) localises fluorescence to the medial/*trans* compartments of the stack and has permitted us to follow the fate of Golgi membranes in single cells continuously through mitosis [29,53].

The disassembly of the Golgi ribbon during early stages of mitosis appears to be highly regulated, consisting of two distinct phases of fragmentation, the later phase occurring coincident with a brief period of Golgi membrane dispersal and reorganisation [53]. The major products of disassembly are regulated in number, and consist of polarised, tubulo-vesicular mitotic clusters, which were originally thought to be merely intermediates, not the end products in the pathway (see above and Fig. 2). Definitive evidence for the role of the mitotic cluster as the partitioning unit came from the visualisation of single cells undergoing mitosis, which directly showed that clusters are partitioned into daughter cells (Fig. 4) and, following cytokinesis, assemble to give rise to an interphase Golgi ribbon [29]. While these findings do not exclude a role for free vesicles in the disassembly process, they nevertheless demonstrate that the shedding of Golgi-derived vesicles into the cytoplasm does not go to completion, consistent with previous studies documenting the presence of mitotic clusters throughout the mitosis period [8–10,13,54]. Moreover, the recent data in living cells are also consistent with findings derived from experimental models of the disassembly process [19,55] suggesting that Golgi residents are enriched in the tubulo-vesicular cisternal remnants following disassembly. Together, these findings have led us to speculate that a remaining core of Golgi residents, the mitotic cluster, persist throughout mitosis to facilitate reassembly of the interphase Golgi apparatus during cytokinesis.

The existence of mitotic Golgi clusters as a unit of partitioning, and the precise regulation of their number, position and compartmentation suggest that an active mechanism exists to coordinate the partitioning of Golgi membranes at mitosis. Further support for this concept came from a comparison of partitioned GFP-Golgi membranes in late telophase daughter cell pairs (see Fig. 4), which demonstrated that there is a far greater accuracy of partitioning Golgi membranes than expected from a solely stochastic process [29]. Together, these findings provide strong evidence for the involvement of cellular mechanisms to increase Golgi membrane partitioning exactness. Nevertheless, definitive evidence for the active partitioning of mitotic Golgi membranes awaits the identification of the partitioning machinery.

1.3.2. Partitioning strategies

Cell constituents such as ATP, soluble proteins and perhaps certain organelles, are likely to achieve accurate segregation passively as a consequence of cytokinesis. The probability of equal partitioning solely relies upon the copy number of the component during mitosis, its distribution throughout the cytoplasm, and the ability of the cytokinetic mechanism to divide the cell into two equally sized daughter

cells. This is referred to as a stochastic partitioning strategy [51,56]. In contrast, an ordered partitioning strategy refers to the active segregation of cellular components to ensure equal distribution in nascent daughter cells. The extreme example of this strategy is the complex mechanisms evolved for appropriate segregation of chromosomes, based on the mitotic spindle [57].

Previous observations on the accuracy of partitioning organelles, starting with the work of Wilson on scorpion spermatocyte mitochondria [58] and expanded more recently by Birky and colleagues using algal chloroplasts [56,59], have led to the puzzling conclusion that these organelles are partitioned with an accuracy that falls somewhere in between that expected for an ordered or a stochastic partitioning scheme. Birky describes this phenomenon as stochastic partitioning with a tendency to equality [56]. The implication is that for at least some organelles, there are mechanisms in place to ensure an accuracy better than would be achieved solely through a passive, stochastic partitioning.

1.3.3. A working model for mitotic Golgi membrane partitioning

From the analysis of cells using the GFP-tagged Golgi marker emerges strong support for an active, though error-prone partitioning strategy, which we have termed semi-ordered partitioning [60]. The prerequisite for such a mechanism is that the cell must be able to count and segregate mitotic Golgi membranes, however, it is difficult to envision how the cell accomplishes this task, given the number and complex nature of partitioning units that must be accounted for during mitosis.

A paradigm for a cellular counting mechanism during mitosis exists in the form of the centrosome. In G2/early prophase, the centrosome duplicates, and each of the resulting pair nucleates a rich astral array of microtubules. As a cell progresses into prometaphase, the centrosomes move to opposite poles of the cell to form a mitotic spindle; then, during anaphase and telophase, each centrosome is partitioned into a daughter cell along with attached microtubules and one pair of daughter chromosomes [61].

A reasonable hypothesis to explain the semi-ordered partitioning of organelles during mitosis involves the exploitation of centrosome duplication and separation to facilitate organelle segregation into the daughter cells. Thus, if in addition to the well-known organisation of the Golgi ribbon by microtubules in interphase [16,49,62–64], mitotic Golgi membranes remain organised by microtubules during centrosome separation in mitosis, they could be fairly equally distributed to either side of the metaphase plate, and subsequently partitioned into nascent daughter cells during cytokinesis [60]. Simultaneous observation of Golgi membrane and microtubule behaviour during the early stages of mitosis in living cells should provide a means to test and further clarify this current working model.

2. Golgi apparatus biogenesis

The replication of the Golgi apparatus in proliferating [50] or activated cells [65] relies upon several factors, including: (i) the coordinate expression of individual Golgi components, a prerequisite to double the number of Golgi constituents; (ii) the regulated input/export and retention of nascent membranes arriving at the Golgi complex; (iii) the association of Golgi membranes with various peripheral proteins provided by the cytoplasm; and, (iv) the assembly of resident components into functional Golgi units.

Little is known about the coordination of Golgi resident synthesis during the cell cycle. Feedback mechanisms may exist which monitor Golgi apparatus growth/composition in relation to cell cycle phase, then transmit this information into the cell nucleus, thereby regulating the synthesis of new Golgi components. Alternatively, Golgi components may be constitutively expressed during the cell cycle, and regulation of Golgi stack size, or perhaps unit number, may only be limited by disassembly and partitioning at mitosis. Indirect experimental data supporting the latter hypothesis come from experiments where cells were repeatedly blocked in G1/S phase of the cell cycle by the DNA synthesis inhibitor aphidicolin. In the presence of this drug, cells continue to grow in size, despite their inability to proceed through the cell cycle [66]. After two rounds of aphidicolin arrest and subsequent progression into M-phase, cells are approximately twice the size of their unsynchronised counterparts. Interest-

ingly, the number of Golgi mitotic fragments keeps pace with the gain in cell size, increasing in number from ∼65 in normal cycling cells to ∼130 clusters in cells subjected to the aphidicolin treatment [29]. Therefore, similar to the plasma membrane, Golgi membranes may constitutively grow in mass during the cell cycle, until the cells divide. Interestingly, the decoupling of the organelle growth cycle and the cell cycle has also been observed for chloroplasts [67]. The pathways responsible for regulating Golgi resident gene expression during the cell cycle are currently unknown.

The growth of the Golgi apparatus during the cell cycle is dependent on ER-derived material, which arrives at the entry face of the Golgi stack, and, in one scheme, is thought to move sequentially through the cisternal compartments in the form of transport vesicles [68,69]. Secreted cargo, or proteins destined for downstream membranous cytoplasmic compartments transit through the Golgi stack. In contrast, Golgi resident components, such as glycosyltransferases, are thought to be maintained within the appropriate Golgi membranes by a combined process of protein retention and retrieval [70,71]. ER residents which have escaped from their proper location, and proteins of the transport machinery involved in ER-to-Golgi and post-TGN transport are recycled back to the appropriate location via retrograde transport pathways [72–75]. The net outcome of this complex bi-directional traffic is a Golgi stack exhibiting both morphological and biochemical polarity [69,76,77], and interference with any of the membrane transport pathways communicating with the Golgi complex seems to result in impaired Golgi morphology [78,79].

Whereas numerous advances have been made with respect to membrane traffic in and out of the Golgi apparatus, a major unanswered question is how new Golgi stacks are constructed. Does this process require addition of proteins to pre-existing stack templates; does the stack arise through de novo synthesis; or, does stack construction reflect a combination of these two processes, which we envision as a maturation process (Fig. 5)?

One of the few potential examples of templated biogenesis comes from investigation of plants and some fungi, where the Golgi apparatus exists throughout the entire cell cycle as a collection of tens to thousands of discrete stacks, called dictyosomes [80,81]. This morphological feature has greatly

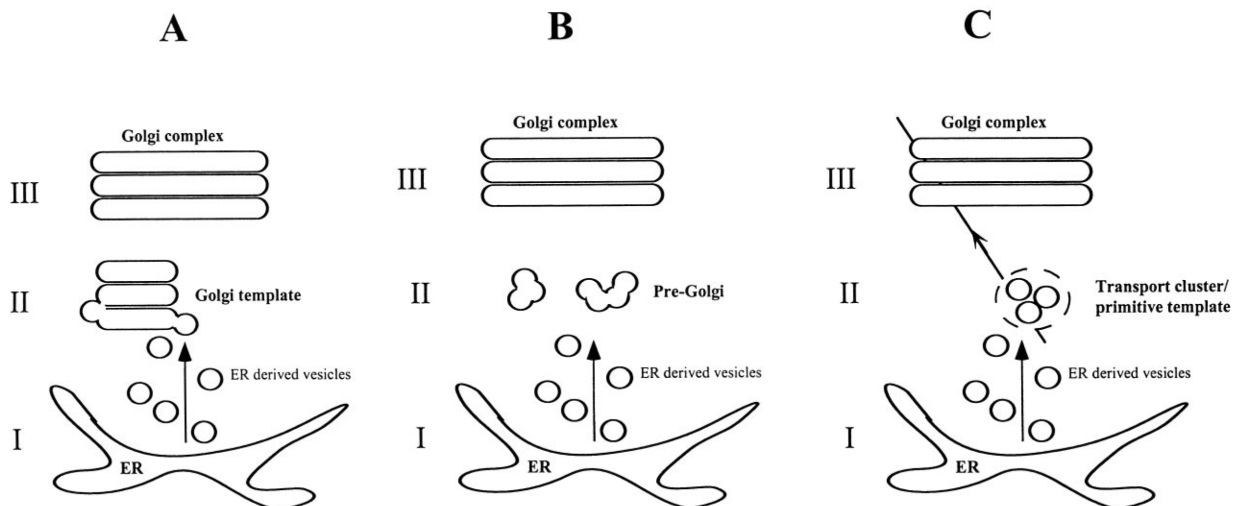

Fig. 5. Alternative models for the construction of new Golgi stacks. (I) The packaging of Golgi residents into ER-derived vesicles; (II) the construction of new Golgi/pre-Golgi membranes leading to (III) a stacked and polarised Golgi unit. (A) Templated biogenesis: ER-derived vesicles are transported and added onto pre-existing Golgi templates. Eventually, Golgi unit replication is achieved through medial stack division. (B) Biogenesis de novo: the construction of polarised Golgi stacks by the accretion and self-assembly of vesicles derived from the ER. (C) A compromise: ER-derived vesicles are transported to Golgi/transport clusters, which serve as primitive pre-Golgi templates. These are able to form a typical Golgi complex through a maturation process, and transport along microtubules into the centrosomal region.

facilitated ultrastructural analysis and quantitation of Golgi stack number and size during different stages of the cell cycle, and has led to the suggestion that plant Golgi biogenesis occurs through lateral dictyosome growth and medial fission [80].

The extreme alternative to using stacks as templates for Golgi apparatus growth is de novo biogenesis, which we define as the construction of polarised Golgi stacks by the accretion and self-assembly of ER-derived vesicles, the smallest membrane units for this and other organelles which obtain membranes from the ER [60] (Fig. 5). One line of evidence for the ability to assemble the Golgi apparatus from vesicular building blocks comes from the study of Golgi membrane behaviour during mitosis. In prophase, Golgi stacks are progressively transformed into a heterogeneous collection of tubules and vesicles, an event believed to facilitate the partitioning process (see previous section). At the onset of cytokinesis, disassembled Golgi membranes rapidly fuse and reorganise into a secretion competent, Golgi stack [15]. Similarly, treatment of cells with the sponge metabolite ilimaquinone (IQ) results in disassembly of the Golgi ribbon into a collection of 60 nm vesicles which are capable of fusing to re-establish functional Golgi stacks upon drug removal [82].

Though these observations provide evidence that the Golgi apparatus can in principle be built from vesiculated starting material, it is important to note that the Golgi vesicles observed during mitosis or IQ treatment are derived from the Golgi complex itself and not from the ER. Therefore, the existence of Golgi vesicles during mitosis and IQ treatment does not necessarily support the de novo Golgi membrane synthesis model. Rather, recent findings suggest that disassembled Golgi membranes may play a role as organisational templates for stack reassembly.

Although the Golgi stack does disassemble into a heterogeneous mixture of vesicles/tubules during mitosis, many of these membranes remain grouped into mitotic clusters, whose properties would make them a good template upon which to reorganise the Golgi stack following mitosis [60]. Mitotic clusters persist throughout mitosis, and their number within a given metaphase cell population is remarkably constant, suggesting that they represent a basic structural unit of the Golgi complex. Moreover, confocal fluorescence microscopy confirms earlier ultrastructural observations which suggested Golgi resident proteins within the mitotic cluster remained compartmentalised despite the complete loss of stacked cisternal Golgi structure [5,8,29]. These findings demonstrate that a polarised remnant of the Golgi apparatus persists throughout mitotic disassembly and suggests the existence of an underlying template, a Golgi matrix, which is responsible for organising the biochemical architecture of Golgi membranes. Such a Golgi matrix could potentially exist independent of membrane composition even when Golgi membrane structure is drastically transformed. This would explain how the complex architecture of the Golgi stack can be restored within minutes following mitosis or drug treatments such as BFA and IQ [15,83,84], as a blueprint for reassembly of proper Golgi stacks, the Golgi matrix, would remain.

A dense, cytoskeletal-like matrix has been localised to the intracisternal space of Golgi stacks [85], and also within mitotic cluster membranes [9]. Good candidates for the scaffold include the recently identified stacking factor, GRASP65 [48], a collection of Golgi apparatus localised, cytoskeletal-like proteins such as giantin [86,87], GM130 [32], Golgin-160 [88], Golgin-245 [89] and p210 [90], and two Golgi-localised variants of the actin/spectrin erythrocyte cytoskeleton: ankyrin-G119 [91] and Golgi β-spectrin [92]. The size and structure of these proteins, as well as their localisation to specific compartments within the Golgi complex fits the profile expected of proteins involved in the formation of an organellar scaffold.

An independent line of evidence which may appear to support the notion of de novo Golgi membrane biogenesis from ER-derived units comes from studies of the fungal metabolite, brefeldin A (BFA). BFA treatment of cells results in the rapid, bulk, retrograde transfer of Golgi resident proteins to the ER, and upon drug removal, ER-localised Golgi residents are mobilised from the ER to re-form a morphologically intact and functional Golgi apparatus [83]. Whereas these observations may have initially been a strong argument for the existence of de novo Golgi membrane synthesis, several groups have now demonstrated the existence of tubulo-vesicular Golgi remnants following BFA-induced disassembly of the Golgi ribbon [93–95]. Furthermore, it has been shown that TGN residents do not redistribute to the

ER [83], and fractionation of membranes following BFA treatment demonstrates that 50% of the resident enzyme galactosyltransferase remains associated with the Golgi membrane peak [96]. Similar to the mitotic cluster, the BFA remnants of the Golgi apparatus could serve as a seed to rebuild the stack following drug removal. Although these membrane remnants are morphologically reminiscent of mitotic clusters [94], their biochemical composition and organisation require further examination.

Interestingly, recent work studying transport of a GFP-tagged secretory marker in living cells has demonstrated the importance of 'transport clusters' (TCs) in ER to Golgi transport [97]. These TCs are most likely the vesicular tubular clusters (VTCs), otherwise termed the intermediate compartment (IC) or recycling compartment [74,98–100]. The experiments in living cells show that TCs are the intermediates in ER to Golgi transport. They form at ER exit sites in a step which requires ER derived, COPII-coated transport vesicles and they move in a motor directed, microtubule and COPI-dependent manner to the Golgi complex where they deliver their cargo. The morphological similarities shared by mitotic clusters and the TCs are intriguing, and lead us to include an additional biogenesis model, which holds that TCs could act as a primitive template in Golgi stack formation, a pre-Golgi unit (Fig. 5). Since the ER-Golgi recycling compartment contains representatives of the *cis*-Golgi, it is feasible that these early Golgi proteins provide organisational information for nascent Golgi residents. Clearly, further characterisation of the TCs will be necessary to test this hypothesis.

Definitive identification of a biogenetic pathway for the Golgi apparatus awaits a more direct, continuous observation of the birth of new Golgi units. The recent advent of the green fluorescent protein as a vital tag for Golgi membranes should greatly facilitate our future understanding of this process.

Note added in proof

While this article was in preparation we have been able to provide evidence for a role of the centrosome and mitotic spindle in Golgi partitioning during cell division, which may be found in: D.T. Shima, N. Cabrera-Poch, R. Pepperkok, G. Warren, An ordered inheritance strategy for the Golgi apparatus: visualization of mitotic disassembly reveals a role for the mitotic spindle, J. Cell Biol. 141 (1998) 955–966. A web site containing animations documenting the behavior of Golgi membranes in living cells during mitosis is accessible through www.icnet.uk/axp/cb/.

Acknowledgements

We would like to thank Graham Warren and Christiana Ruhrberg for critical reading of the manuscript, and members of the ICRF Cell Biology Laboratory for insightful discussions.

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