Signaling pathways controlling mitotic Golgi breakdown in mammalian cells

Inmaculada López-Sánchez and Pedro A. Lazo

Experimental Therapeutics and Translational Oncology Program Instituto de Biología Molecular y Celular del Cáncer, Consejo Superior de Investigaciones Científicas (CSIC), Universidad de Salamanca, Campus Miguel de Unamuno, E-37007 Salamanca, Spain.

*Correspondence to:
Dr. P. A. Lazo, Centro de Investigación del Cáncer, CSIC- Universidad de Salamanca, Campus Miguel de Unamuno, E-37007 Salamanca, Spain.
Tel: 34 923 294 804
Fax: 34 923 294 795
Email: plazozbi@usal.es
Abstract

In mitosis, each daughter cell must receive a complete and equal set of cellular components. Cellular organelles that are single copy, such as endoplasmic reticulum, nuclear envelope and Golgi apparatus, have to break down to allow their correct distribution between daughter cells. The mammalian Golgi is a continuous membranous system formed by cistern stacks, tubules and small vesicles that are located in the perinuclear area. At the onset of mitosis, the Golgi apparatus undergoes a sequential fragmentation that is highly coordinated with mitotic progression and in which reversible phosphorylation plays a critical regulatory role. In fact, several kinases have been implicated in each stage of this fragmentation process. Before mitotic disassembly, the lateral connections between the stacks are severed resulting in the formation of isolated cisternae. Several kinases such as mitogen-activated protein kinase kinase 1 (MEK1), Raf-1, ERK1c, ERK2, Plk3, VRK1, several Golgi matrix proteins (GRASP65 and GRASP55) and the membrane fission protein BARS have been shown to mediate signals in this first step that takes place in late G2 phase. As prophase progresses, the isolated cisternae are first unstacked followed by its breakage into smaller vesicles and tubules that accumulate around the two spindle poles at metaphase. Unstacking and vesiculation are triggered by several proteins including kinases (Plk1 and Cdc2), the GTPase ARF-1 and inactivation of membrane fusion complexes (VCP and NSF). Post-mitotic Golgi reassembly consists of two processes: membrane fusion mediated by two ATPases, VCP and NSF; and cistern restacking mediated by dephosphorylation of Golgi matrix proteins (GRASP65 and GM130) by phosphatase PP2A (Bα). Apart from the tight regulation by reversible phosphorylation, it seems that mitotic Golgi membrane dynamics also involves a cycle of ubiquitination during disassembly and deubiquitination during reassembly in part regulated by the VCP-mediated pathway.
1. Introduction

The functions of the Golgi apparatus are conserved throughout eukaryotic evolution but its morphology and spatial organization vary among organisms. In *Saccharomyces cerevisiae* individual Golgi cisternae are scattered throughout the cytoplasm (1); whereas in most eukaryotic organisms, such as in plants, cisternae are arranged into ordered stacks and the Golgi is made of many individual dispersed stacks (2). In mammalian cells, Golgi stacks are laterally linked forming the characteristic ribbon-like complex that is located in the perinuclear region next to the centrosome (3).

In mammalian cells, cellular organelles that exist as a single copy such as the endoplasmic reticulum, the nuclear envelope and the Golgi apparatus have to break down in mitosis to allow its correct partitioning between daughter cells. In the case of the Golgi complex, its continuous membranous system is first laterally severed at late G2 phase, generating isolated Golgi stacks (4, 5). Then, cisternae unstack and break down into many smaller vesicles and tubules, and disperse around the spindle poles at metaphase forming the “Golgi mitotic haze” (6-9) (Fig. 1). After completion of Golgi mitotic fragmentation and partitioning into daughter cells, Golgi vesicles reassemble in a two step process: membrane regrowth and cisternal restacking.

The Golgi disassembly and reassembly is tightly coordinated with cell cycle progression, and is regulated by two types of protein modifications, reversible phosphorylation and monoubiquitination. The proteins already associated with control of Golgi in mitosis include Ser-Thr kinases, phosphatases, and membrane proteins (Table 1). Among them are for example, Raf-1 (10), MEK1 (5, 11-13), ERK1c (14), Cdc2 (15-17), Plk1 (16, 18, 19), ERK2 (5, 20), Plk3 (21), VRK1 (22) and PP2A (23 , 24); Golgi matrix proteins like GM130 (23), GRASP55 (20, 25) and GRASP65 (16, 26, 27), the fission protein CtBP3/BARS (4 ), the GTPase ARF-1 (28, 29) and proteins related with fusion events such as VCP (30) (Table 1). But all these proteins and their role in Golgi dynamics have to be coordinated with regulatory signals implicated in the control of cell division.
2. Unlinking of the Golgi ribbon in late G2 phase

Apart from the fact that Golgi complex disassembly is necessary for its correct partitioning between daughter cells (6, 7, 31), recently, several groups have suggested that the first modification that takes place in late G2 phase and that results in the isolation of Golgi stacks is a prerequisite for mitotic entry (5). Moreover, it has been proposed that this unlinking of the Golgi ribbon is a novel non-DNA checkpoint that is required for G2/M transition, and requires a connection with signaling pathways controlling cell division (5, 9, 32).

MAP kinases canonical functions are regulation of proliferation, differentiation and apoptosis (33). However, it has been demonstrated that they are not only activated in mitogenic cascades related with cell cycle entry, but also during mitosis to regulate several processes. The MEK/ERK signaling pathway, in addition to its classical role, is specifically activated in G2/M transition independently of extracellular growth factor stimulation (10, 34, 35). Raf-1, for example, seems to be activated by a different mechanism that does not include classical proteins such as Ras, 14-3-3 or Src (36); in this case MEK1 activation requires other components apart from Raf-1 to be activated in mitosis, since different MEK1 phosphorylations are detected when comparing phosphopeptides from mitotically activated MEK1 with those from Raf-1 activated MEK1 (10, 12). These differences might represent a subpopulation with different signaling characteristics.

Several kinases downstream of MEK1 have been implicated in mitotic Golgi fragmentation, such as ERK proteins (ERK2 and ERK1c) (14, 20, 37), Plk3 (21) and VRK1 (22) although their exact temporal and spatial role has not yet been completely established. Currently, the role of MEK1 in Golgi ribbon unlinking, occurring in G2, is well characterized. Depletion by RNA interference or inhibition of MEK1 delays, but does not block, mitotic entry, suggesting that MEK1 signaling is required for timely G2/M transition. In accordance with this idea, in MEK1-knockdown cells, treatment with brefeldin A (BFA), a non-competitive inhibitor of ARF-1 that induces dispersal of the Golgi membranes (38, 39), and depletion of GRASP65 can bypass the MEK1 requirement (5). Therefore, the MEK1-dependent delay is abrogated in cells with Golgi complex already dispersed either by BFA or siGRASP65 (5). Plk3 is downstream of MEK/ERK cascade
since ectopic expression of activated MEK1 results in activation of Plk3. Moreover, activated MEK1 stimulates Golgi breakdown in the presence of Plk3 but not the kinase-defective Plk3<sup>K52R</sup> (21). Plk3 transmits part of the signal from MEK1 through VRK1 to induce Golgi breakdown (22) (Fig.2). In agreement with this notion, Plk3 induces Golgi breakdown in the presence of VRK1 but not the kinase-dead VRK1<sup>K179E</sup> or the active kinase VRK1<sup>S342A</sup> that is not phosphorylated by Plk3 (22). These data confirm that VRK1 is downstream of Plk3 and that VRK1 serine 342 is the residue phosphorylated in this signaling pathway (22). Besides, knocking-down endogenous VRK1 protein also blocks Golgi fragmentation induced either by MEK1 or Plk3, which means that VRK1 is a new step in the already known MEK1-Plk3 signaling cascade located to the Golgi membranes (22, 40, 41) (Fig. 2).

GRASP55 and GRASP65 are two peripheral Golgi proteins localized to the medial-trans and cis cisternae membranes, respectively (42, 43). Both GRASP proteins form homodimers which are able to maintain lateral cisternae connections and stacks by establishing trans-oligomers with adjacent membranes homodimers in order to hold the Golgi ribbon-like structure (Fig. 3A). In mitosis, trans-oligomers are inhibited by phosphorylation in their C-terminal serine/proline rich domain (SPR). Thus, when phosphorylation takes place, trans-oligomers are prevented (Fig. 3A) and, as a consequence, the Golgi complex is disassembled (19, 27, 44-46). Consistent with the role of GRASP proteins in Golgi G2 phase unlinking, antibodies against GRASP65, expression of GRASP domains mutants or non-regulatable mutants block mitotic Golgi fragmentation and delay mitotic entry (26, 27). Similarly, microinjection of GRASP55 protein fragments or C-terminal mutants also leads to G2 block and decrease in mitotic index (25), suggesting that these proteins are both implicated in cisternae G2 phase unlinking in a cell cycle phosphorylation dependent manner. GRASP proteins are likely target candidates for regulatory signals. In mitosis, GRASP55 is phosphorylated by ERK2 downstream MEK1 (5, 20) whereas GRASP65 is phosphorylated by Plk1 (16, 18, 19) and Cdc2 (16, 19). Besides, experimental evidences also suggest the existence of at least one additional kinase targeting GRASP65, since metabolically labeled cells arrested in mitosis by nocodazole show several phosphopeptides for Cdc2 and Plk1, but there is one additional phosphopeptide not accounted for them (16). The sequential order of these
phosphorylations is not known. These data indicate that during mitosis GRASP proteins are phosphorylated and, as a result, Golgi complex first unlinks and then unstacks thus facilitating vesicles and tubules formation. However, it is still unknown how Plk1 and Cdc2 are coordinated and organized at this stage of the process.

The membrane fission protein named CtBP3/BARS is involved in several membrane trafficking steps such as retrograde transport of KDEL receptor by COPI vesicles (47). Its relevance in Golgi G2 unlinking phase has been demonstrated by its depletion, effect of dominant-negative mutants and antibodies against BARS experiments, all of which supports that BARS is required for G2/M transition and Golgi breakdown (4, 48). Moreover, BARS is also phosphorylated in mitosis by an unknown kinase (49), all suggesting it is a potential regulatory target in early stages of the process.

3. Golgi stacks disassembly and vesiculation

From prophase to metaphase Golgi apparatus further breaks down into many smaller vesicles and tubules that accumulate around the spindle mitotic poles (50, 51). Thereby, GRASP65 protein is phosphorylated so that trans-oligomerization and cisternal stacking are disrupted (Fig. 3A) (19). This unstacking facilitates COPI vesicle formation by increasing the amount of membrane surface, which is required for Golgi complex disassembly in mitosis (45). Plk1 and Cdc2 are both involved in unstacking as they phosphorylate GRASP65 (16, 18, 19, 44), whereas GRASP55 is phosphorylated by ERK2 (5, 20). Given that GRASP65 and GRASP55 are localized in different regions of the Golgi complex, it has been hypothesized that they play complementary functions in Golgi stacking and unstacking in a cell cycle phosphorylation dependent manner (25, 46, 52).

Apart from unstacking, it is also required that vesicle budding remains active whereas membrane fusion complexes are inhibited, and thus smaller vesicles can be formed. The GTPase ARF-1 (Active ADP-ribosylation factor-1) is involved in recruitment of COPI protein complex implicated in membrane traffic (53). In mitosis, some evidence indicates that ARF-1 remains active so that there is a continuous COPI vesicle formation and, as a consequence, fragmentation of Golgi membranes (24, 29, 54). But this is still controversial since other authors have shown that ARF-1 is inactivated in mitosis (28, 31).
On the other hand, Cdc2 phosphorylates GM130 (15) and p47 (17) which results in the inactivation of NSF and VCP-dependent fusion pathways, respectively.

GM130 is a coiled-coil protein anchored by its C-terminus extreme to GRASP65 (Fig. 3B) and thus to cis-Golgi stacks (42, 55, 56). Besides, GM130 interacts by its N-terminus with the tethering factor p115 which mediates the initial vesicle tethering between COPI vesicles, through Giantin, and membranes, bridged by GM130, forming a p115-GM130-Giantin complex (Fig. 3B) (56-58). Next, it is formed the docking SNARE complex which defines the specificity of vesicle targeting (59, 60). The role of NSF is to break the SNARE complex up, a process that needs ATP hydrolysis, and that results in vesicle fusion (61). Cdc2 phosphorylates GM130 and prevents its binding with p115 and, therefore, it is blocked the initial vesicle tethering and also the fusion process dependent on NSF ATPase (15) (Fig. 3B).

Less is known about VCP (Valosin-Containing Protein) mechanism of action in the fusion pathway. VCP is a member of the type II AAA ATPase family (ATPases associated with various cellular activities) that is implicated in a variety of cellular processes including membrane fusion, transcription activation, cell cycle control and apoptosis (62). Similar to NSF, VCP interacts with the SNARE pair, in this case through the p47 cofactor, and break it up after ATP hydrolysis. Unlike NSF, VCP does not need the tethering process, but instead requires another cofactor named VCIP135 during the membrane fusion (60, 63, 64). The phosphorylation of p47 by Cdc2 in mitosis inhibits its binding to the Golgi complex, thus the fusion is dependent on VCP (17).

In addition to reversible phosphorylation, it has been suggested a novel regulatory level that involves a cycle of ubiquitination and deubiquitination, which is also coordinated with Golgi membrane dynamics. In fact, the fusion pathway regulated by VCP is also controlled by an ubiquitination cycle; since two cofactors, p47 and VCIP135 are connected with ubiquitin modifications. p47 contains an ubiquitin binding motif (UBA) recognizing monoubiquitin, and VCIP135 is a deubiquitinating enzyme so that ubiquitinated proteins seem to be crucial in the VCP-mediated fusion mechanism (62, 65). The attachment of a single ubiquitin, monoubiquitination, to the target protein serves as regulatory modification; in contrast to polyubiquitination, formed by several ubiquitin molecules that are attached into chains linked through Lys48 for proteasome degradation signaling, or Lys63 for DNA
damage repair (66). The fact is that experimental assays carried out with the ubiquitin mutant I44A support the notion of ubiquitination as a new Golgi dynamics controller. The I44A mutant acts as a dominant-negative that conjugates to target proteins but it is not recognized by VCP-p47 protein complex. Therefore, the ubiquitin mutant but not the wild type ubiquitin prevented cisternal regrowth in a reassembly assay since VCP-p47 complex is not able to recognize ubiquitinated Golgi proteins (67); so that some unknown Golgi factors are required to be monoubiquitinated at the onset of mitosis in order to be later deubiquitinated in telophase. However, the possible targets and the exact mechanism by which ubiquitin signal functions in Golgi disassembly and reassembly are still poorly characterized (65, 67, 68).

4. Golgi apparatus inheritance and the mitotic spindle

In metaphase, Golgi small vesicles are scattered throughout the cytoplasm (Fig. 1) and they start to be distributed between daughter cells as the mitotic spindle is assembled. Two different models have been proposed trying to explain how the partitioning occurs. In one view, Golgi vesicles and tubules are absorbed into the endoplasmatic reticulum membranes and then reemerged from it, as it occurs with the nuclear envelope, which means that Golgi inheritance is mediated by the endoplasmatic reticulum (31, 69). The second model suggests that Golgi fragments are partitioned independently of the endoplasmatic reticulum (6, 70-73), and proposes that the mitotic spindle is responsible for the partitioning (50, 51, 72). There are some data supporting this second model. One is the observation of Golgi vesicles and tubules accumulated around the spindle poles (8, 50, 51, 74), whereas the endoplasmatic reticulum is excluded from that area (8, 71). Recently, some experiments carried out by Wei and Seemann have finally demonstrated the link between the Golgi partitioning and the mitotic spindle since they have developed a procedure that induces an asymmetrical cell division so that one daughter cell (karyoplast) receives the entire spindle (centrosomes, chromosomes and spindle microtubules) and the other (cytoplast) lack all these (75). Under these experimental conditions, the ribbon-like Golgi complex reforms in the karyoplasts whereas in the cytoplasts the Golgi stacks are scattered throughout their cytoplasm although they maintain their transport activity (76).
This has led to the proposal that there are two Golgi inheritance mechanisms: the spindle-independent by which elements of functional stacks are fragmented and distributed; and the spindle-dependent by which the ribbon determinants are partitioned between daughter cells (76).

The signals controlling the inheritance are not known but they are likely to be related to the interaction of vesicles with components of the centrosome and synchronized with cell cycle progression.

5. Golgi apparatus reassembly

In telophase, Golgi fragments start to reassemble by two mechanisms: membrane fusion processes mediated by two ATPases, VCP and NSF; and cisternae restacking mediated by dephosphorylation of GRASP proteins and GM130 (Fig. 4).

Vesicle fusion events dependent on NSF ATPase and requires two golgins, p115 and GM130, as well as SNARE pairs (GOS-28/Syntaxin-5). p115 is initially required for membrane regrowth and later for cisternal restacking through the formation of GM130-p115-Giantin complexes (30, 58, 60, 77). The other ATPase implicated is VCP and its cofactors p47 (63), p37 (78) and VCIP135 (64), which form two different types of protein complexes: VCP-p47-VCIP135 and VCP-p37-VCIP135. In the first pathway, the protein complex that binds to Golgi membranes through Syntaxin-5 is disrupted after ATP hydrolysis (60, 64) and monoubiquitination has been demonstrated to be necessary for the process. As has been mentioned, monoubiquitination seems to be another level of regulation in Golgi dynamics at mitosis (67, 68). In the second pathway, the protein complex formed by VCP-p37-VCIP135 uses GS-15 instead of Syntaxin-5 to bind Golgi membranes and, as in NSF pathway, it also requires p115 (78). In contrast to NSF, deubiquitinating activity is not necessary in this case since p37 does not contain an ubiquitin binding motif (UBA) so that the complex VCP-p37 activity is not related with ubiquitination (78).

GM130 (23) and GRASP65 (24) dephosphorylation is mediated by PP2A (Bα). PP2A phosphatases are composed by a series of serine/treonine enzymes that functions as a trimeric complex formed by an invariable catalytic (C) and structural (A) subunits which
bind to a variable regulatory one (B). Among these regulatory subunits, Bα is associated with the Golgi membranes and so that it functions specifically at the Golgi complex (23).

After membranes regrowth, cisternal restacking starts in order to reform the continuous and stacked Golgi membranous system characteristic of interphase. Restacking is initiated by p115 through its interaction with dephosphorylated GM130 and Giantin between adjacent membranes (58). Besides, p115 is phosphorylated by CKII or CKII-like kinase that stimulates Giantin-GM130 binding, and stacking (79). Finally, since GRASP65 homodimers are dephosphorylated by PP2A (Bα) (24), they are able to restore trans-oligomers with adjacent membranes homodimers and to establish lateral connections and Golgi stacks. Although there are not data about GRASP55 dephosphorylation, it is assumed that it is the case since GRASP65 and GRASP55 play complementary roles in Golgi cisternal stacking (46).

6. Conclusion

Mitotic Golgi dynamics is a regulated and coordinated process that requires several molecular elements including kinases, structural Golgi proteins, small GTPases and ATPases (Fig. 4). Phosphorylation is one of the main regulatory mechanisms of this process and although there have been described some kinases and phosphatases that are implicated, the interconnections between different signaling cascades is still not well characterized temporally or spatially. Therefore, the future challenge is to identify and to characterize new signaling pathways implicated and identify kinases, their substrates and timing in mitosis, which will help to understand how the fragmentation and reassembly process is controlled.

Acknowledgements

I.L.-S. was supported by a fellowship from Ministerio de Educación, Ciencia e Innovación. Work in the laboratory was supported by grants from Ministerio de Educación, Ciencia e
REFERENCES


cellular phosphoprotein and important for the negative modulation of T24-ras mediated transformation, tumorigenesis and metastasis. Embo J. 1993;12:469-78.


Table 1. **Proteins involved in the Golgi fragmentation process in mitosis**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Unlinking of the Golgi ribbon in late G2 phase</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Kinases</strong></td>
<td></td>
</tr>
<tr>
<td>Raf-1</td>
<td>(10)</td>
</tr>
<tr>
<td>MEK1</td>
<td>(5, 11-13, 21)</td>
</tr>
<tr>
<td>ERK2</td>
<td>(5, 20)</td>
</tr>
<tr>
<td>ERK1c</td>
<td>(14)</td>
</tr>
<tr>
<td>Plk3</td>
<td>(21)</td>
</tr>
<tr>
<td>VRK1</td>
<td>(22)</td>
</tr>
<tr>
<td><strong>Golgi matrix proteins</strong></td>
<td></td>
</tr>
<tr>
<td>GRASP55</td>
<td>(5, 20, 46, 52)</td>
</tr>
<tr>
<td>GRASP65</td>
<td>(19, 44, 46, 80)</td>
</tr>
<tr>
<td><strong>Membrane fission protein</strong></td>
<td></td>
</tr>
<tr>
<td>BARS</td>
<td>(4, 48)</td>
</tr>
<tr>
<td><strong>2. Golgi stacks disassembly and vesiculation</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Kinases</strong></td>
<td></td>
</tr>
<tr>
<td>Plk1</td>
<td>(16, 19)</td>
</tr>
<tr>
<td>Cdc2</td>
<td>(15-17, 19, 44)</td>
</tr>
<tr>
<td><strong>GTPases</strong></td>
<td></td>
</tr>
<tr>
<td>ARF-1</td>
<td>(24, 28, 29)</td>
</tr>
<tr>
<td><strong>Membrane fusion complexes (ATPases)</strong></td>
<td></td>
</tr>
<tr>
<td>p47 (VCP)</td>
<td>(17)</td>
</tr>
<tr>
<td>GM130 (NSF)</td>
<td>(15)</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

**Figure 1.** Golgi membranes morphology in interphase and metaphase in HeLa cells. In interphase, the Golgi complex is a continuous membranous system. In metaphase, the Golgi complex is dispersed into vesicles and tubules throughout the cytoplasm around the DNA (mitotic Golgi Haze). The Golgi ribbon is labeled with an anti-Giantin antibody in red and DNA is labeled with DAPI in blue.

**Figure 2.** MEK1-Plk3-VRK1 signaling pathway in Golgi fragmentation. Reprinted with permission from American Society for Microbiology (22).

**Figure 3.** A. Cisternae lateral connections and stacks are held together by trans-oligomerization of GRASP proteins. In mitosis, GRASP proteins are phosphorylated so that the Golgi complex unlinks and unstacks. B. Vesicle fusion events. The initial tethering process is mediated by forming p115-GM130-Giantin complexes that are blocked by phosphorylation in mitosis.

**Figure 4.** Diagram of the Golgi fragmentation, assembly and location where different signaling proteins participate.