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Review

Molecular mechanisms responsible for formation of Golgi ribbon

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Summary. The formation of the Golgi ribbon takes place in protists and metazoans. It is especially prominent in mammalian cells during interphase. Golgi ribbon formation represents an orchestrated sequence of events based not only on different molecular mechanisms but also on discrete cellular functions. Mechanisms responsible for the generation of the Golgi ribbon include Golgi centralization, cis- and trans-Golgins, molecular machines responsible for the fusion of cargo domains with cisternal rims, and several other less studied factors. Here, we substantiate the hypothesis that cis-Golgins function mostly not as tethering factors, but are responsible for the attachment of the cis-most cisternae to the medial Golgi stacks, whereas trans-Golgins are responsible for the attachment of the trans-most cisterna to the medial Golgi stacks. This hypothesis is tested analyzing predictions derived from it and related to molecular mechanisms responsible for mitotic fragmentation of Golgi stacks.

Key words: Golgi apparatus, Ribbon, Fragmentation, Gongins, Mitosis

Introduction

The Golgi apparatus (GA) is found in all eukaryotes and is the main station along the intracellular secretory pathway. The GA is present in different organisms under very different forms: scattered tubular networks (in *S. cerevisiae* or microsporidia; see Beznoussenko et al., 2007), isolated multiple stacks of cisternae (in *Pichia pastoris*, embryonic cells of *Drosophila*, or plants [Donohoe et al., 2007]), and a continuous pericentrosomal ribbon that is made up of interconnected stacks of flat disk-like cisternae (in mammals).

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The position of the GA also varies among species. In plants, yeast, some protists, some insect cells and some mammalian cells (i.e. oocytes) the stacks remain separated from each other, being dispersed throughout the cytoplasm. In some animal cells the ministacks are unlinked to each other and spread throughout the cell. In most mammalian cells the central Golgi ribbon exists only during interphase. Stacks can be composed of 2-3 or more than a dozen cisternae, as in alga (Donohoe et al., 2007) or in Sertoli cells (Rambourg et al., 1979). At the onset of mitosis the GA first undergoes fragmentation, and then these ministacks are transformed into tubular vesicular clusters (Lucocq et al., 1989). At the end of mitosis, tubular vesicular clusters are transformed into ministacks that undergo centralization. After gathering in the centre the ministacks form a ribbon again.

There are many unresolved questions related to Golgi ribbon formation: how is the ribbon organized, why are Golgi stacks linked within a ribbon, what are the molecular mechanisms responsible for ribbon formation, what are the molecular mechanisms responsible for the fragmentation of the GA during mitosis, apoptosis and under some pathological conditions? Here, we analyze existing information about the Golgi ribbon and describe molecular mechanisms responsible for the formation of Golgi ribbon. However, due to limited space, we will not discuss mechanisms responsible for the increase of the GA during S phase and mechanisms responsible for the mitotic transformation of Golgi ministacks into tubular-vesicular clusters.

Fragmented Golgi apparatus

In many cell types the ribbon is absent. This has been observed in oocytes, in myotubes, mammalian cells completely deprived of microtubules (Polishchuk et al., 1999; Trucco et al., 2004), or some yeast, i.e. *Pichia pastoris* (Rossanese et al., 1999). At the embryonic and

pupal stages (even during interphase) of *Drosophila* development and in S2 culture cells, Golgi cisternae are stacked but the pairs of stacks are not connected to form a ribbon (reviewed in Kondylis and Rabouille, 2009). In a *Drosophila* cell line, depletion of the class B genes leads to Golgi fragmentation into smaller elements (Bard et al., 2006). However, in most differentiated insect cells the GA forms a ribbon (Conti et al., 2010).

Golgi fragmentation and separated fragments have also been observed in mammalian neurons (Horton et al., 2005; Ye et al., 2007; Hanus and Ehlers, 2008; Kondylis and Rabouille, 2009), highly differentiated uroepithelial cells (Kreft et al. submitted), in oocytes (Motta et al., 1995) and some other cells. The common characteristic of many of these cells is the absence of a united microtubule star with one centrosome for the entire cell. In cells where the Golgi ribbon is formed, the GA undergoes fragmentation during mitosis and apoptosis. In protist cells where there is a single centrosome, the GA is usually organized into a ribbon (Sokolova and Mironov, 2008).

An important question is how to prove that the GA has been fragmented. This is especially difficult if Golgi fragments remain near the centrosomes. Criteria for the evaluation of the central fragmentation could be the following: 1) Z-stacking using confocal microscopy; 2) FRAP of a Golgi enzyme (GE) tagged with GFP; 3) measurement of the average length of Golgi stacks on random EM sections. These criteria and several assays, such as washout of brefeldin A or nocodazole after redistribution of the GA into the ER or its complete fragmentation, respectively, are used for the evaluation of the functional role of proteins possibly involved in the formation of the Golgi ribbon.

Structure of the Golgi ribbon

The formation of the Golgi ribbon is one of the most interesting features of the GA in mammalian cells. However, the Golgi ribbon appears only if centralization of stacks takes place. In this case, formation of the Golgi ribbon occurs near the centrosome. In mammals, dozens or even hundreds of Golgi stacks are laterally linked together to form an interconnected, ribbon-like structure as a single organelle in the perinuclear area. The development of the Golgi ribbon varies in different cells. For instance, in fibroblasts and in spinal neurons the ribbon is well developed. In contrast, in CHO cells it is broken into several pieces (reviewed in Polishchuk and Mironov, 2004, Mironov and Pavelka, 2008).

When visualized by immunofluorescence, the Golgi ribbon in most mammalian cells appears as a lacy structure occupying a volume of 5-7 μm in length, 1-2 μm in width, and 3-5 μm in depth (Fig. 1a,b) surrounding the centrosome or microtubule (MT) organizing centre. The Golgi ribbon could be flat (Fig. 1a) or form a thick structure (Fig. 1c-f). However, the space close to the centrosome does not contain elements of the GA. For instance, in RBL cells, most of the Golgi elements are situated at a distance of 1-3 μm from the centrosome (our unpublished observations, Fig. 1c,d). One of the mechanisms preventing complete centralization of the GA could involve GMAP-210, a protein participating in attachment of the GA to MT (Rios et al., 2004). It is also possible that the MTs growing from the centrosome at a high density "push" the Golgi membranes out, forcing them to stay at some distance from the cell centre (reviewed in Polishchuk and Mironov, 2004; Mironov and Pavelka, 2008). Finally, the balance between the activity of the minus-end motor dynein, and the plus-end motor kinesin determines the final positioning of the GA.

The formation and positioning of the Golgi ribbon depends on the organization of MT star and the position of the centrosome. The centrosome contains g-tubulin ring complexes that nucleate polarized microtubule assembly. This creates a radial array of microtubules with minus ends embedded in the centrosome and plus ends extended in all directions towards the cell periphery. Golgi membrane-associated minus end-directed microtubule motors, mainly dynein, move the Golgi stacks inward and cause concentration of the Golgi stacks around the centrosome (Rios et al., 2004; Barr and Egerer, 2005).

The position of the centrosome and the GA depends on cell polarity. In many polarized epithelial cells, the centrosome is positioned near the apical portion of the cell surface (Ojakian et al., 1997), where the Golgi also resides. Even in non-polarized cells, conditional polarization of the cell in relation to the direction of cell movement leads to the reorientation of the centrosome and a relocation of the Golgi. For example, both the GA and the centrosome are oriented towards the leading edge of migrating fibroblasts (Kupfer et al., 1982) and towards the immune synapse in cytotoxic T-lymphocytes when they kill their targets (Kupfer et al., 1983). It is important to stress that formation of Golgi ribbon is not related to Golgi stacking (see below).

The connectivity between individual stacks has been shown in fixed cells using scanning EM and observation of the complementary fractures of the GA (Inoue, 1992) and in living cells using FRAP of Golgi resident proteins tagged with GFP (Cooper et al., 1990; Cole et al., 1996). However, how individual Golgi stacks are connected with each other and which part of the stack is common for several stacks is not clear. In general, Golgi stacks could be connected: 1) by tubules or non-compact zones or 2) by single cisternae. In human fibroblasts and in RBL cells, neighbouring stacks are connected by a single cisterna that sometimes extends out of stacks and is transformed into a tubule connecting corresponding cisternae of the neighbouring stacks. Within a Golgi ribbon, stacks are connected by cisternae (Marsh et al., 2001), and more rarely by tubules or non-compact zones. Judging from experiments with FRAP, Man-II, GalT and STF positive compartments can be part of the ribbon whereas the Man-I-positive compartment is not (our unpublished observations). The TGN also does not form

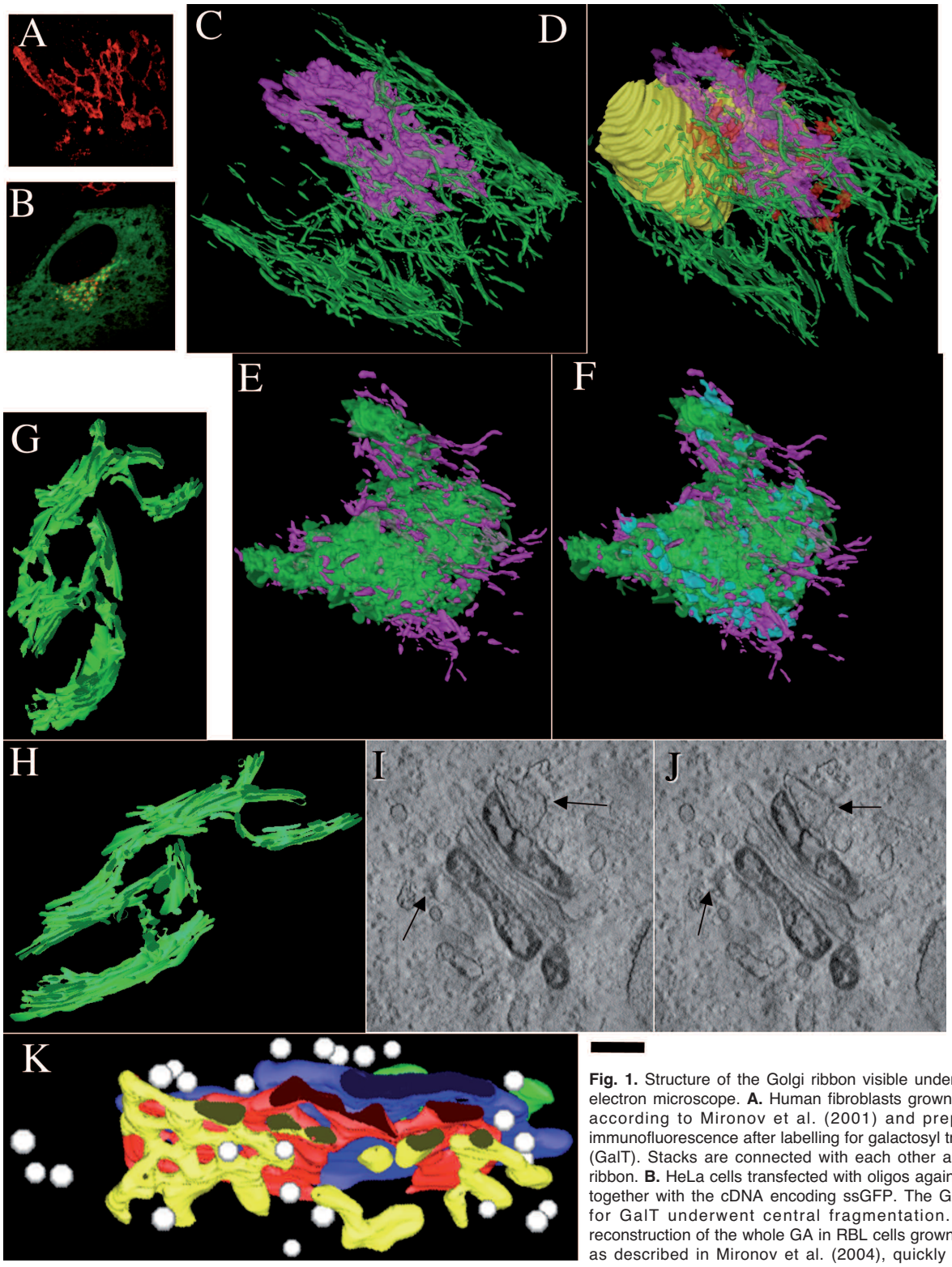

Fig. 1. Structure of the Golgi ribbon visible under light and electron microscope. **A.** Human fibroblasts grown in culture according to Mironov et al. (2001) and prepared for immunofluorescence after labelling for galactosyl transferase (GalT). Stacks are connected with each other and form a ribbon. **B.** HeLa cells transfected with oligos against GM130 together with the cDNA encoding ssGFP. The GA labelled for GalT underwent central fragmentation. **C-F.** 3D reconstruction of the whole GA in RBL cells grown in culture as described in Mironov et al. (2004), quickly frozen as described in Mironov et al. (2001). Serial sections of this cell were used for the 3D reconstruction. The ER is coloured in green **(C, D)** or in magenta **(E, F)**. The GA is coloured in magenta **C, D**) or in green **(E, F)**. The nucleus (yellow) is shown in **D** and the ER exit sites are shown in **D** (red) and **F** (light-blue). **G, H.** 3D reconstruction of the ribbon formed by the Golgi cisternae containing ManII in intact cells. RBL cells were prepared for immuno EM using the HRP-based technique to label ManII as described by Colanzi et al. (2000). ManII-positive cisternae form a ribbon. (**I, J**) Structure of Golgi fragments in mitotic cells. RBL cells were shaken in order to enrich the amount of mitotic cells and then prepared for immuno EM using the HRP-based technique to label Man II as described by Colanzi et al. (2000). Cells without a nucleus were selected at the stage of prophase and EM tomography of Golgi fragments was performed. Two consecutive virtual sections are shown. Mitotic Golgi fragments do not contain the cis-most and the trans-most cisternae and lost their polarity. The trans ER cisternae (arrows show ribosomes) are attached to the medial Golgi stack from both sides. In the centre, cisternae are not labelled for ManII (black precipitate). **K.** 3D reconstruction of the GA that is formed after depletion of SPCA1 by siRNA interference. The cis-most cisterna (yellow) is present whereas the trans-most cisterna is poorly developed. Scale bars: A, 3 μm; B, 5 μm; C-D, 1 μm; E-H, 500 nm; I-K, 220 nm.

a ribbon (Clermont et al., 1995). Additional analyses are necessary in order to find out how the ribbon is organized. Such an analysis should include a total reconstruction of the Golgi ribbon with high resolution EM tomography.

The functional role of Golgi ribbon

An important question is whether fragmentation of the Golgi ribbon affects intracellular transport. Depolymerization of MT affects Golgi function only during the early time of treatment when the GA is not completely fragmented (Cole et al., 1996b). Indeed, if the intra-Golgi transport is measured immediately after the addition of a drug that depolymerizes microtubules, for instance nocodazole, then intracellular transport is inhibited (Cole et al., 1996b; Thyberg and Moskalewski, 1999). The transport of cargo to, through, and out of the GA in cultured cells can take place efficiently when the fragmentation of Golgi ribbon is finished and a plateau is reached. At steady state, when the process of Golgi ribbon fragmentation reaches a plateau (when fragmentation of the ribbon is completed) the function of the Golgi remains normal (Trucco et al., 2004). In some insect cells, disruption of Golgi architecture is not accompanied by the inhibition of anterograde transport either (Kondylis et al., 2005). Thus, intra-Golgi transport does not depend on Golgi centralization and ribbon-like organization of the GA. The transport is affected only during the transition from the Golgi ribbon to Golgi fragments.

These "ministacks" allow the GA to perform all its functions (Trucco et al., 2004). Studies of glycosylation in the Golgi have also shown that sugar nucleotide transporters and many glycosyltransferases are located within a single cisterna (Opat et al., 2001; Young, 2004). For glycosylation and protein sorting, even a single cisterna is sufficient (Varki, 1998). This suggests that either a ministack or even a single medial cisterna could be considered as the minimal Golgi unit.

If this is the case, what could be the role of the ribbon? A number of possibilities have been invoked. The one that is most frequently considered is related to GA and cell "motility": directing the movement of a continuous organelle (such as the Golgi ribbon) would be easier and more effective than co-ordinately moving multiple unlinked organelles (the isolated Golgi stacks). This might be required during cell migration when the GA is reoriented towards the leading edge to provide membranes for the advancing cell front. Alternatively, the ribbon could facilitate the equal distribution of Golgi enzymes among the stacks. If the amount of an enzyme in one cisterna is lower than necessary then diffusion-based delivery along the ribbon might give better possibility for function of Golgi enzymes. The integrity of the Golgi ribbon may represent a sort of "signal", for instance for a Golgi-based mitotic checkpoint. It is not clear how cells without a centralized GA can provide membranes for the leading edge during migration and whether such a migration is possible for cells without a Golgi ribbon.

Mechanisms responsible for Golgi fragmentation

In order to understand why mammalian and some other animal cells form a Golgi ribbon it is necessary to examine experiments in which the Golgi ribbon was broken by impairing or eliminating the function of some proteins or organelles. However, the sensitivity of cells from different species to the deletion of different genes is different. For instance, in humans the loss of one allele of SPCA1, the main Golgi [Ca^{2+}] pump induces Hailey-Hailey disease, whereas in mice this loss does not (Callewaert et al., 2003). Therefore, mice are less sensitive to deletion of SPCA1 (Okunade et al., 2007). The method of protein inhibition is also important. One could imagine three main types of protein inhibition: 1) sudden influence, for instance, by antibody or with CALI (Hoffman-Kim et al., 2007) or FALI effects (Heerssen et al., 2008), 2) intermediate influence by protein depletion with siRNA, 3) chronic influence by knock out of the gene.

The fragmented GA is able to reform the ribbon after restoration of centralization. For instance, upon nocodazole washout, when already fragmented GA is placed in conditions where the MT star with a united centrosome is restored, the Golgi fragments immediately undergo centralization, and the ribbon of interconnected stacks is rebuilt (Ho et al., 1989). Sometimes only 65 min is necessary to re-form the Golgi ribbon. During ministack centralization, membrane tubules connecting approaching ministacks are formed and disrupted (Jiang et al., 2006).

Role of microtubules

In general, all mechanisms involved in formation of the Golgi ribbon can be divided into extra-Golgi and intra-Golgi. Extra-Golgi factors are molecular machines that ensure centralization of the GA (coatomer II, SNAREs of the intermediate compartment, MTs, dynein). Elimination of these factors will induce peripheral Golgi fragmentation. The intra-Golgi protein machineries and conditions that are necessary for the formation of the Golgi ribbon could be divided into three main groups: 1) conditions and machines that ensure entrance of cargo into the GA; 2) factors that provide mechanisms for the correct function for Golgi exit; 3) conditions necessary for the correct function of the fusion machinery within the entire GA (this works only if the GE and cargos are present already within the GA). Impairment of these groups induces central fragmentation of the GA.

The first condition that is important for the formation of the Golgi ribbon is centralization of the GA. Golgi ribbon formation and localization depend on the microtubule network (Rios et al. 2004). Centralization can be blocked by several methods: 1)

depolymerization of MTs by specific drugs (e.g. nocodazole) (Trucco et al., 2004) or impairment of their dynamics (for instance, treatment of cells with low concentrations of nocodazole, Minin, 1997); 2) stabilization of MTs, for instance by taxol (Polishchuk et al., 1999); 3) impairment of the function of dynein and proteins regulating MT function (see below), 4) blockage of fusion at the level of the ER exit sites (Mironov et al., 2003). A blockage of MT polymerization induces transformation of the Golgi ribbon into many peripheral fragments (Thyberg and Moskalewski, 1999). Even when MT dynamics are lowered by addition of very low concentrations of nocodazole (Minin, 1997) or the addition of taxol that preserves the MT star thus blocking their depolymerization (treatment of cells with taxol results in polymerization of microtubules independently of the centrosome and formation of microtubule bundles predominantly localized in the cell periphery [Wehland et al., 1983]) the Golgi shows a peripheral type of fragmentation.

GMAP-210 and Alpha-TURC participate in MT-dependent regulation of Golgi ribbon formation (Rios et al. 2004). GMAP-210 binds both the GA and microtubules and thus is involved in pericentriolar positioning of the Golgi (Rios et al. 2004; Barr and Egerer 2005). GMAP-210 captures short MT seeds formed at the centrosome by their minus ends and, together with the Golgi-localized pool of the CLASPs that attach to and stabilize the MT plus ends, generates a meshwork of short MTs associated with adjacent Golgi stacks and links them to form a ribbon (Rios et al., 2004). Cells expressing high levels of GMAP-210 show some recruitment of gamma-tubulin to the GA.

Thus, the first prerequisite for the formation of the Golgi ribbon is the presence of a single MT star with one centrosome for the entire cell and unimpaired MT dynamics. In plants and in yeast the MT pattern is different than in mammalian cells and, thus, these cells have no Golgi ribbon. In contrast, if a protist cell has a MTOC, the Golgi is usually organized into a Golgi ribbon (Sokolova and Mironov, 2008).

Dynein and Golgi centralization

The function of MT motors, and especially dynein, is involved in the centralization of ER-to-Golgi carriers and Golgi ministacks and thus they are important for the formation of the Golgi ribbon. Cells lacking dynein are unable to concentrate organelles such as the GA and lysosomes in the perinuclear region (Harada et al., 1998). Dynein function is affected by several proteins/regulators such as dynactin, p50/dynamitin (Roghi and Allan, 1999), Bicaudal-D (Matanis et al., 2002), CLIPR- 59 (Perez et al., 2002), p150 (Watson et al., 2004), and some others. Dynactin is concentrated at MT plus ends and can transiently capture Golgi membranes that are then transported toward the minus ends by dynein (Vaughan et al., 2002).

Several observations suggest that inhibition of dynein-dependent centralization of ER-to-Golgi carriers induces peripheral fragmentation of the GA. Inhibition of dynein function by impairment of its interaction with regulatory proteins [i.e. expression of the p50 subunit of dynactin (Burkhardt et al., 1997), expression of the COPII-binding domain of p150 causing a delay in the formation of ER-Golgi vesicular tubular transport intermediates (Watson et al., 2004), the over expression of p50/dynamitin that results in the loss of cytoplasmic dynein heavy chain from the membrane of peripheral Golgi elements (Roghi and Allan, 1999)] leads to peripheral fragmentation of the GA.

The situation is different in polarized epithelial cells where MTs are primarily oriented with their plus ends located near the GA and their minus-ends in the apical cytoplasm. Here, the distribution of MT motors is also different and a selected kinesin isoform (KIFC3) has been shown to play a role in the MT-dependent centralization and positioning of the GA in some polarized epithelial cells (Xu et al., 2002b).

On the other hand, inactivation of kinesins, proteins that move towards the plus end of MTs, in non-polarized cells results in the collapse of the GA and the ER around the centrosome (Feiguin et al., 1994) and, strangely, microinjection of an anti-kinesin antibody inhibits centralization of ER-to-Golgi carriers (Lippincott-Schwartz et al., 1995). Also, if actin is depolymerized the Golgi shifts to the centre (Valderrama et al., 1998). The mechanisms that lead to these two last phenomena are not clear.

Thus, not only MTs but also the dynein-based machinery that is specialized for the delivery of membranes to the minus end of MTs participates in Golgi centralization.

Role of ER-to-Golgi transport for Golgi ribbon formation

The centralization of Golgi stacks filled with enzymes is not sufficient for the formation of the Golgi ribbon. If the centralization of ER-to-Golgi carriers is blocked or if ER-to-Golgi carriers are not formed, the Golgi ribbon is not generated. Indeed, the Golgi ribbon dissociates into isolated stacks with shorter cisternae as soon as the input of membranes from the ER is slowed down or interrupted (Marra et al., 2007). Two hours after removal of the litter, when intra-Golgi transport was blocked, accumulation of secretory progranules in prolactin cells has been observed. As one can see comparing Fig. 1 and Fig. 7 in Rambourg et al. (1993) newly formed Golgi ministacks are devoid of the cis-most and the trans-most cisternae. The Golgi ribbon became fragmented in HepG2 cells after blocking protein synthesis (treatment with cyclohexamide); the GA became shorter (our unpublished observations). Thus, for the generation of the Golgi ribbon, constant delivery of ER-to-Golgi carriers filled with membranes and secretory cargoes to the centrally located Golgi

stacks could be necessary. It seems that the delivery of ER-to-Golgi carriers is important for gluing together Golgi stacks already present in the central zone.

Role of cis- and trans-Golgins

In addition to Golgi centralization and the necessity for delivery of ER-to-Golgi carriers to the centrally located GA, several proteins are important for ribbon formation. Cis-Golgins have been implicated in Golgi ribbon formation. Golgi fragmentation is induced by depletion of p115, (Sohda et al., 2005, Rejman Lipinski et al., 2009), giantin (Rejman Lipinski et al., 2009), GRASP55 (Feinstein and Linstedt, 2008), GRASP65 (Puthenveedu et al. 2006; Marra et al., 2007), GM130 (Puthenveedu et al. 2006), TMF/ARA160 (Fridmann-Sirkis et al., 2004). Of interest, depletion of p115 causes Golgi fragmentation regardless of Rab6A and Rab11A depletion (Rejman Lipinski et al., 2009). Not only inhibition of their function but also impairment due to overexpression could induce Golgi fragmentation in some cells. For instance, overexpression of GRASP65 induces fragmentation in hippocampal neurons (Horton et al., 2005). Thus, these proteins are involved in an early stage of stack formation and in particular are responsible for the attachment of the cis-most cisterna to the medial GA.

The process of formation of the Golgi ribbon also depends on some trans-Golgins and regulators of their function. For instance, depletion of GCC185 in HeLa cells (Derby et al., 2007), golgin-84 (Diao et al., 2003; Rejman Lipinski et al., 2009), Golgin-245 (Yoshino et al., 2005) or impairment of function of ARL1 or Golgin-97 by microinjection of inhibitory antibodies or siRNA interference (our unpublished observations) induce Golgi fragmentation. Of interest, in contrast to the depletion of p115, depletion of Rab6 and Rab11 suppressed fragmentation of the GA in golgin- 84-depleted cells (Rejman Lipinski et al., 2009). The function of Golgins is regulated by phosphorylation. For instance, GRASP55 functions by forming oligomers through its N-terminal GRASP domain by phosphorylation within the C-terminal serine/proline-rich domain. Phosphorylation at these sites of GRASP55 is required for mitotic Golgi disassembly and cell cycle progression (Duran et al., 2008). Expression of nonphosphorylatable GRASP55 mutants enhances Golgi stacking in interphase cells and inhibits Golgi disassembly during mitosis (Xiang and Wang, 2010).

Membrane fusion and Golgi ribbon formation

It seems that protein machineries involved in membrane fusion of Golgi membranes, such as SNAREs are important for the generation of the Golgi ribbon. For instance, microinjection of an inhibitory antibody against GOS-28 (our unpublished observations) or Ykt6 (Zhang and Hong, 2001) induces central Golgi fragmentation. Overexpression of a GS15 mutant lacking the trans-membrane domain that inhibits the function of GS15 induces central fragmentation of the GA (see Fig. 8n in Xu et al., 2002a). P115 can catalyze SNARE complex formation for membrane fusion (Dirac-Svejstrup et al., 2000; Shorter et al. 2002).

If we take into consideration that Rabs can regulate SNARE function (Claas et al., 2008) the following experiments become understandable. Microinjection of Rab1a (S25N) (GDP-restricted mutant) causes peripheral Golgi fragmentation (Wilson et al., 1994). Depletion of Rab1a and b induces Golgi fragmentation (Monetta et al., 2008; Fig. 2E Rab1 Si/GM130 in Razi et al., 2008). Rabenosyn- 5-depletion induces Golgi fragmentation (Rahajeng et al., 2010). However, not all Rabs are involved in Golgi fragmentation. In cells where Rab6A and Rab11A are depleted, the GA becomes more compact in comparison to control cells (Rejman Lipinski et al., 2009). Silencing of Rab proteins blocked Chlamydia-induced and golgin-84 knockdown-stimulated Golgi disruption, whereas Golgi fragmentation was unaffected in p115 depleted cells (Rejman Lipinski et al., 2009). Rabs could be involved in the formation of the Golgi ribbon due to their interaction with trans-Golgins (Burguete et al., 2008).

On the other hand, Golgi membranes can release Ca(2+) from the lumen during intra-Golgi transport (Micaroni et al., 2010a). When this release was affected by knockdown of SPCA1, the protein that facilitates pumping of Ca(2+) and localizes at the cisternal rims, the GA undergoes central fragmentation (Micaroni et al., 2010b). The ability of Golgi membranes to fuse could be affected also by inhibition of phospholipase A2 and as it is expected this inhibition induces central Golgi fragmentation (San Pietro et al., 2009).

Formation of Golgi ribbon and other proteins

There are several facts that are very difficult to explain on the basis of existing knowledge. Golgi fragmentation is observed after Fas receptor-activation of cells (Mukherjee et al., 2007), transfection of cells with constitutively active Rho kinase (Orlando and Pittman, 2006), expression of Gb1g2 or Gb3g2 (Diaz Anel and Malhotra, 2005), depletion of the Golgi-associated conserved oligomeric complex (COG) (Shestakova et al., 2006), depletion of Crn7, a novel cytosolic mammalian WD-repeat protein (Rybakin et al., 2006), depletion of retromer components (Seaman, 2004), or overexpression of Golgi-localized SR-containing protein-56 (GSRP-56, Kobayashi et al., 2006). Increased levels of sphingosine induce Golgi fragmentation (Hu et al., 2005).

Central fragmentation of the GA has been observed after impairment of function of the Golgi localized GPI proteins. For instance, depletion of GREG leads to fragmentation of the GA. Also, in cells expressing the GPI-GREG construct, partial fragmentation of the GA is observed. Expression of 23TM-GREG, a fusion protein composed of GREG and the transmembrane domain of

p23, a type I transmembrane protein at the GA, results in Golgi fragmentation (Xueyi Li et al., 2007). Golgi fragmentation is observed in CHO cell lines deficient in biosynthesis of the GPI-anchor (Abrami et al., 2001).

The mechanisms underlying several phenomena are difficult to explain, such as the central fragmentation of the GA after depletion of cells of Zeste White 10 (ZW10), a mitotic checkpoint protein implicated in Golgi/endoplasmic reticulum (ER) trafficking/transport, Sun et al., 2007) or the central fragmentation of the GA marked with GAlNac-T2 after treatment of cells with NH4Cl for 40 h (Axelsson et al., 2001). The protein machinery responsible for tubule fission is also involved in the formation of Golgi ribbon. For instance, in fibroblasts from BARS (a protein that is considered as a fission machine, see Weigert et al., 1999)-knockout embryos, the GA is fragmented (Hidalgo Carcedo et al., 2004). However, the common sense suggests that in the absence of fission machinery the Golgi ribbon should be more stable higher because tubules connecting stacks will not be sectioned by the fission machine. Additional experiments are needed to resolve these contradictions.

One of the elements of the fission machinery is coatomer I. Therefore it is not surprising that depletion of ß-COP with siRNA causes peripheral fragmentation of the GA (Fig. 2B in Styers et al., 2008; Fig. 2C ßCOP/Si/GM130 in Razi et al., 2009). The p24 family are functionally linked with COPI proteins and therefore depletion of p28 (Koegler et al., 2010), p25 (Mitrovic et al., 2008), Surf4 together with ERGIC-53 (Mitrovic et al., 2008) or overexpression of p23 (Rojo et al., 2000) leads to fragmentation of the GA.

Thus, cis-Golgins and Trans-Golgin, together with proteins regulating membrane fusion between Golgi compartments, are important for Golgi ribbon formation. The role of some other proteins in Golgi ribbon formation needs to be clarified.

Structure of Golgi fragments

Peripheral and central Golgi ministacks differ from each other. In a GA that under normal conditions forms a ribbon and undergoes fragmentation due to impairment of centralization, the structure of ministacks is almost normal. These Golgi fragments (for instance, in cells deprived of MTs) are situated near the ER exit sites, contain the cis-most and the trans-most cisternae and usually are located very close to the plasma membrane. The ER exit sites and ministacks usually form complexes after MT depolymerization.

The ministacks have a typical stacked organization. In favourable sections, the Golgi ministacks formed after depletion of cells of microtubules were observed in close association with ER exit sites (Storrie et al., 1998; Polishchuk et al., 1999; Trucco et al., 2004). In contrast, the centrally located GA fragments are devoid of the cis-most and the trans-most cisternae, and integration of cargo domain into cisternae is minimal (Marra et al., 2007). For instance, in ldlG cells where the function of GM130 is impaired after arrival of cargo to the GA, no aCGN and aTGN (sometimes called as the cis-most and the trans-most cisternae correspondingly) were formed

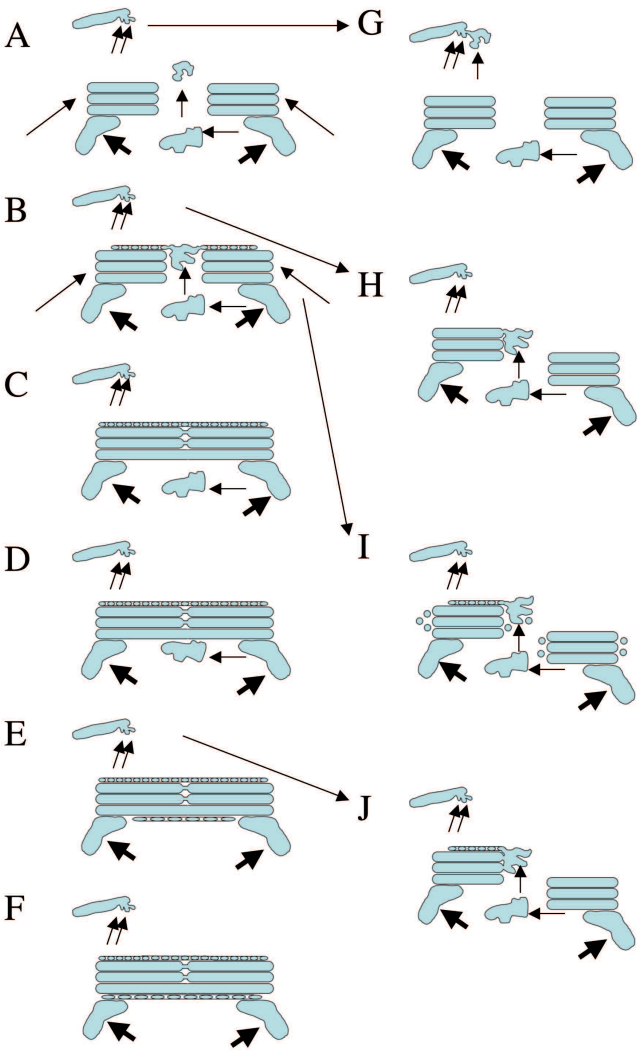

Fig. 2. Mechanisms of Golgi ribbon formation. **A.** Golgi stacks (long thin arrows) that are formed near the ER exit sites (double arrow) should be centralized. These stacks do not contain the cis-most and the trans-most cisternae but the trans ER (thick arrows) is attached to the trans-side of the stack. ER-to-Golgi carriers (vertical arrow) and the TGN (horizontal arrow) are not integrated into the Golgi ribbon. **B.** ER-to-Golgi carriers (vertical arrow) form the cis-most cisterna. **C.** Transformation of ER-to-Golgi carriers into Golgi cisternae. **D.** The TGN approaches the medial GA. **E.** Transformation of the TGN into the trans-most cisterna. **F.** Insertion of the trans-most cisterna between the medial GA and the trans ER. **G.** In the absence of centralization of ER-to-Golgi carriers, Golgi stacks remain separated. **H.** In the absence of function of cis- Golgins, Golgi stacks remain separated in the centre of the cell. **I.** Impairment of membrane fusion induces Golgi vesiculation and prevents formation of the Golgi ribbon. **J.** In the absence of function of trans-Golgins, the cis-most cisterna is formed but the stacks remain separated.

(Fig. 4b in Marra et al., 2007). Interestingly, in plants, the trans-most cisterna is poorly developed (Kang and Staehelin, 2008).

If the Golgi fragments are situated in the cell centre, there is still a necessity to deliver post-Golgi carriers to the plasma membrane. When the GA is fragmented the main problem is the delivery of post-Golgi carriers to the plasma membrane. Therefore, if the GA is fragmented with the formation of peripheral ministacks, these fragments should be close to the PM. In oocytes these GA are close to invaginations of the plasma membrane (Motta et al., 1995). Deep tubular invaginations of the PM are described in cells with a fragmented GA: T-tubules in myofibers (Rahkila et al., 1996), long PM invaginations surrounded by Golgi stacks and reaching the central Golgi stacks in oocytes (Motta et al., 1994), in cells massively secreting collagen (Canty and Kadler, 2005; Kadler et al., 2008) and in endothelial cells (Bendayan and Rasio, 1997).

When the GA is fragmented due to the absence of ER-to-Golgi transport, or in cells depleted of cis or trans-Golgins, centrally located Golgi ministacks are shorter and lack the cis-most and the trans-most cisternae. In the absence of GRASP65, the number of cisternae per Golgi stack is reduced (Sutterlin et al., 2005). The relative number of COPI vesicles increased due to the relative increase of cisternal rims in comparison to the Golgi ribbon (Marra et al., 2007). In the absence of GRASP55 (Feinstein and Linstedt, 2008) or p115 (Sohda et al., 2005), Golgi stack length is shortened but Golgi stacking, compartmentalization, and transport appear normal, although round profiles are visible more frequently. In cells depleted of GM130 and then treated with nocodazole, the cis-most and the trans-most cisternae are absent (Marra et al., 2007). In cells depleted of Golgin-97 or ARL1, the trans-most cisterna is absent (our unpublished observations). After alteration of the fusion machinery, centrally located Golgi ministacks are heavily vesiculated and cannot be assessed for the presence of the cis-most and the trans-most cisternae.

In general, centrally located ministacks that are formed after depletion of cis- and trans-Golgins are similar to the resting Golgi stacks (Mironov and Pavelka, 2008). These stacks contain more vesicles, no cis-, and/or trans-most cisterna (Fig. 2a-d). In cells lacking GM130 and devoid of MTs the cis-most cisterna of the stacks is absent before and after the release of the transport block, whereas in control cells this cisterna is absent in the resting stacks but is visible in transporting stacks (see Fig. 2 in Marra et al., 2007). Without GRASP65, the attachment of the cis-most cisternae to the medial Golgi is affected (see Fig 2Bc in Sütterlin et al., 2005).

Depletion of either GRASP55 or GRASP65 by siRNA reduces the number of cisternae per Golgi stack (Xiang and Wang, 2010). From images presented in this paper it seems that in cells depleted of GRASP55 the cis- and the trans-most cisternae are less developed. Rescue of GRASP55 depletion by expression of exogenous GRASP55 restored the normal structure of the Golgi stacks that acquire both the cis- and the trans-most cisternae. Xiang and Wang (2010) claim that simultaneous knockdown of both GRASP proteins leads to disassembly of the entire stack. However, in Fig. 3D in Xiang and Wang (2010), ministacks are visible and they contain cisternae attached to each other. Most Golgi membranes are attached to each other. Thus, in fact, GRASP55 and GRASP65 play complementary and essential roles in Golgi ribbon formation.

Importantly, isolated stacks that are formed when the ER-to-Golgi transport of cargo is blocked exhibit similar features to stacks that are formed when one of the protein machines responsible for the ribbon formation is inhibited. These ministacks do not have the well-developed cis-most and trans-most cisterna and are surrounded by an increase in the number of COPI-dependent vesicles. In contrast, transporting stacks have both the cis-most and trans-most cisternae, they contain intercisternal connections and only a few COPI-dependent vesicles are visible (Mironov and Pavelka, 2008).

Thus, when the GA is fragmented due to impairment of centralization, peripheral ministacks contain the cis-most and the trans-most cisternae. In contrast, if the GA is fragmented due to impairment of function of cis-Golgins, Golgi fragments do not contain both the cis-most and trans-most cisternae. Finally, if function of trans-Golgin is impaired Golgi ministacks contain the cis-most cisterna but do not contain only the trans-most cisterna.

New function for Golgins

According to the existing consensus, GM130, giantin and p115 are so-called tethering factors that are involved in vesicular transport, whereas GRASP55 and GRASP65 are considered as proteins responsible for stacking and the attachment of cisternae to each other. Tethering is defined as a "formation of physical links, between two membranes that are due to fuse, before the engagement of SNAREs" (Whyte and Munro, 2002). GM130 and p115 are also considered to form part of the Golgi matrix, which maintains the cisternal stacking architecture of the Golgi apparatus (Nakamura et al., 1995; Seeman et al., 2000). Now it becomes clear that in the absence of GRASP55 and GRASP65 Golgi stacks are still formed and, thus, these proteins are not required for Golgi stacking (Sutterlin et al., 2005; Duran et al., 2008). Therefore, these proteins are not responsible for Golgi stacking. On the other hand, if we consider that COPI dependent vesicles cannot be anterograde transport carriers the obvious question would be what is the function of p115, GM130 and giantin other than tethering?

Most Golgins have extensive coiled-coil regions throughout the entire polypeptide, a common protein motif known to form an extended rod-like structure

(Kjer-Nielsen et al., 1999; Burkhard et al., 2001). Coiled-coils proteins have two (α-helices that wrap around each other with a slight left-handed super helical twist forming rod-like structures (Gillingham and Munro, 2003). These proteins have elongated shapes sometimes reaching several dozens of nm. Most Golgins are peripheral membrane proteins (De Matteis et al., 2008). Depending on their localization, Golgins can be subdivided into 2 subfamilies: cis and trans Golgins. Cis-Golgins include p115, GM130/Golgin-95, GRASP65, GRASP55, CASP, Golgin-45, Golgin-67, and Golgin-84. Trans-Golgins include Golgin-97, GCC88, Golgin-160/MEA-2/GCP170, Golgin-245/p230/tGolgin-1, GCC185, GMAP-210, and Grp1p (similar to Golgin-160/GCP170) in yeast, and a related group of proteins - possibly splice variants - GCP372 and GCP364. Giantin/macrogolgin is not restricted to either the cis or the trans pole of the GA (De Matteis et al., 2008).

Golgins are present not only in cells able to form a Golgi ribbon, but also in plants, yeast, and insect cells where the ribbon is absent. P115 can be found in all eukaryotes and GRASPs are found in all eukaryotes except plants, golgin-45 is present only in vertebrates, and GM130 is present only in mammals. Rab1, like p115, is found in all eukaryotes, while Rab2, like its partner golgin-45, is also only present in vertebrates (Short et al., 2005). Thus, in cells where the Golgi ribbon (plants, yeast) is not formed, some Golgins are absent. However, in general this machinery is present in all species. Importantly, cells for which the fragmentation of the ribbon during mitosis is more important have a more enriched family of Golgins.

Our hypothesis proposed recently (De Matteis et al., 2008) poses that cis- Golgins are responsible for the attachment of the cis-most cisternae to the medial GA and that trans-Golgins are responsible for the attachment of the trans-most cisterna to the medial GA. Golgins could also be important for the correct sorting of post-Golgi carriers trafficking to the newly formed cell wall. For instance, the insect Lava lamp (Lva), a golgin protein, is required for cellularization (Papoulas et al., 2005). Giantin could be responsible for the generation of the zone of exclusion or for capturing of cargo domain. It could block attachment of cis cisternae to non-compact zones. Finally, giantin could regulate SNARE interaction.

If we take into consideration the role of Golgi centralization and the peculiarities of the structure of peripheral and central ministacks, an obvious consequence of events that leads to formation of the Golgi ribbon could be envisaged as follows.

1. Immature Golgi stacks that are formed near the ER exit sites are centralized, and this process depends on the presence of MT stars, MT dynamics, and the function of minus end-directed MT motors. If centralization is blocked the GA will appear as a sum of the peripheral fragments possessing all the normal structural features of Golgi stacks. In the absence of transport the medial Golgi is not covered by the cis-most cisterna from the cis-side and by the trans-most cisterna from the trans side.

2. The next step that leads to the formation of Golgi ribbon is the ability of cargo domains to be attached to medial GA and then be integrated into Golgi cisternae. This process depends on the function of cis-Golgins. Cis-Golgins forces the cis- Golgi network to form the cis-most cisterna that facilitates integration of the ER-to-Golgi carriers into the stack. If the function of these Golgins is impaired the GA will be fragmented but the fragments will localize within the central zone. When these proteins are inhibited the fragments do not contain the cis-most and the trans-most cisternae.

3. However, formation of the cis-most cisterna per se is not sufficient for the formation of the ribbon. To finish ribbon formation it is necessary to replace the trans ER from the last medial cisterna by the trans-most cisterna derived from the TGN. This cisterna attaches to the membrane domain containing mostly cargo. If the function of trans-Golgins and proteins regulating their function is affected the GA becomes fragmented and fragments will be localized near the centrosome. These fragments do not have the trans-most cisternae where the cis-most cisternae can be visible.

4. Finally, the Golgi fragments that are delivered to the centrosome need the function of fusion machinery in order to form connections between neighbouring stacks. Delivery of ER-to-Golgi carriers is necessary for this process. If this machinery is blocked the stacks will not fuse with each other and, additionally, they will be vesiculated due to the activity of COPI. This scheme describes new roles for the cis and trans Golgins. These proteins are necessary for gluing the cis-most and the trans-most cisternae to the medial GA. The sequence of events is presented schematically in Fig. 2.

It seems that Golgins have a double function. The reason for such a suggestion is the fact that the budding yeast Saccharomyces cerevisiae has a GRASP homologue, whereas plants have Golgi stacks but no GRASP proteins (Xiang and Wang, 2010). Of interest, in plants the development of the cis-most cisterna and the trans-most cisterna containing clathrin-coated buds are poorly developed (Kang and Staehelin, 2008). In contrast, in Saccharomyces cerevisiae there are no stacks that can form ribbon.

Mitotic Golgi fragmentation

If our hypothesis is correct, we can explain results related to the fragmentation of the Golgi ribbon during the first stage of mitosis. The first prediction is that during mitotic fragmentation of the GA, the function of most proteins involved in Golgi ribbon formation will be impaired. The second prediction derived from our hypothesis is that newly forming ministacks (Golgi fragments that are formed during the first stage of mitotic fragmentation) should not contain either the cis-most or the trans-most cisternae.

Checking our predictions we revealed the following. In mammalian cells at the onset of mitosis, the GA undergoes an extensive disassembly process. The pericentriolar Golgi ribbon of mammalian cells is converted into small fragments, which are dispersed throughout the cytosol. Then, the GA disassembles into tubular vesicular clusters. The first stage of Golgi fragmentation during mitosis is linked with the alteration of the function of the centriole. Each centriole in a centrosome duplicates every cell cycle during S phase to generate a new daughter centriole that arises orthogonally and very close to each mother centriole. During G2, the Golgi ribbon is converted into isolated small stacks that in most cases are isolated (i.e. not interconnected by tubules) and not longitudinally aligned.

In G2-blocked cells, FRAP of GalT-GFP was inhibited, suggesting that stacks are isolated (Hidalgo Carcedo et al., 2004). The number of cisternae that composed single stacks or the average diameter of the stacks does not differ significantly from stacks that form a ribbon. However, small stacks that are formed during the first stage of mitotic Golgi fragmentation do not contain the cis-most and the trans-most cisternae and mostly are not polarized. Often these ministacks contain the trans-ER attached to both sides of the medial Golgi stack. Further, Man-II-positive cisternae can be present at both poles of the stacks, whereas Man-II-negative cisternae are present in the middle of the stack (Fig. 1h,i). Golgi fragments formed during incubation of permeabilized interphase cells with mitotic cytosol are not covered by the cis-most cisterna from the cis side and the trans-most cisterna from the trans side. Instead, from the trans side they are often covered by the trans ER (Fig. 7 in Colanzi et al., 2000).

The reorganization of the MT star during G2 phase seems to be responsible for this peripheral Golgi fragmentation. Indeed, a G2 cell harbours two centrosomes; each containing two closely associated centrioles. During the late G2 phase, centrosome separation is observed. This allows the formation of the bipolar spindle (Pelletier, 2007; Riparbelli et al., 2010). Thus, the initial stage of mitotic fragmentation of the GA involves impairment of Golgi centralization. Most of the above-mentioned proteins are involved in this fragmentation: GRASP65 (Sutterlin et al., 2001, 2005), GRASP55 (Duran et al., 2008), p115 (Sohda et al., 1998), the p97/p47 complex (Rabouille et al., 1995, 1998; Kondo et al., 1997; Wang et al., 2004; Wang, 2008), Zeste White 10 (ZW10, Sun et al., 2007). GRASP65-depleted cells entered mitosis, but accumulated in metaphase with condensed chromatin and multiple aberrant spindles and eventually died (Sutterlin et al., 2005).

During mitosis, the function of proteins involved in Golgi fragmentation is regulated by phosphorylation/dephosphorylation that affects their function. During mitosis, p115 is dephosphorylated (Sohda et al., 1998), whereas GM130 is heavily phosphorylated (Wang et al., 2005). Phosphorylation of p115 enhances its binding to Giantin and Golgi-localized GM130 (Sonnichsen et al., 1998; Sohda et al., 1998) whereas phosphorylation of GM130 inhibits it (Dirac-Svejstrup et al., 2000). Golgin-84 is phosphorylated during mitosis (Diao et al., 2003). GRASP65 is phosphorylated during mitosis (reviewed in Wang, 2008).

The function of GRASP55 is inhibited in late G2 phase causing unlinking of the Golgi ribbon (Feinstein and Linstedt, 2008). Mimicking mitotic phosphorylation of GRASP55 using aspartic acid substitutions induces central Golgi fragmentation (Feinstein and Linstedt, 2008). Phosphorylation of GRASP65 or GRASP55 and activation of BARS promotes Golgi ribbon unlinking, preventing any of these events leading to cell cycle arrest in G2 (reviewed in Collanzi et al., 2007). Expression of a non-phosphorylatable GRASP55 mutant inhibited Golgi ribbon fragmentation in early mitosis (Feinstein and Linstedt, 2007). Inhibition of Plk1 inhibited mitotic Golgi fragmentation in semi-intact NRK cells (Sutterlin et al., 2001). Knockdown of GRASP65 affects spindle dynamics and may cause cell death (Sutterlin et al. 2005).

P47, a cofactor of the AAA ATPase p97 that is involved in post-mitotic Golgi membrane fusion, is also phosphorylated by CDK1 during mitosis (Kondo et al. 1997; Mayr et al. 1999). Mutant forms of p47 that can no longer be phosphorylated by CDK1 prevent complete fragmentation of the Golgi during mitosis (Uchiyama et al., 2002). During mitosis, Rab1 is phosphorylated by CDK1, and this induces increased Rab1 membrane association (Bailly et al., 1991). Usually, inhibition of phosphorylation of these proteins blocks Golgi fragmentation during mitosis.

Several kinases are involved in the phosphorylation of these proteins: a novel isoform of ERK, ERK1c (Aebersold et al. 2004; Shaul and Seger 2006), polo-like kinase 1 (Plk1), MEK1 and CDK1 participate in the initial fragmentation of the Golgi ribbon during early prophase (Acharya et al. 1998; Colanzi et al. 2000; Sutterlin et al. 2001). During mitosis GRASP65 is phosphorylated by cdc2 and plk whereas GRASP55 is mainly phosphorylated by MAPK (Xiang and Wang, 2010). Elevation of tyrosine-phosphorylated ERK2 by increased MEK1 expression disrupts the GA, as observed at the light microscope level (Cha and Shapiro 2001).

However, CDK1 does not function alone and a number of other protein kinases of the polo and MAPK family have also been implicated (reviewed in Lowe and Barr, 2006). Plk1 phosphorylates GRASP65 at multiple residues during mitosis (Lin et al. 2000). TrkB-mediated Golgi fragmentation employs a MEK-dependent signalling pathway resembling that implicated in the regulation of Golgi fragmentation in mitotic cells (Schecterson et al., 2010).

Inhibition of Golgi fragmentation delays cell entry into mitosis. Fragmentation of the Golgi ribbon begins before M phase and it depends at least partially on

MEK1 activity. The inhibition of MEK1 kinase in HeLa cells caused a 2 h delay in mitotic entry. This delay was abrogated if the Golgi ribbon was broken down by depletion of the Golgin GRASP65 (Feinstein and Linstedt, 2007). Golgi fragmentation during G2 requires BARS, a well-known fission factor (Weigert et al., 1999; Yang et al., 2005, 2006), involved in cutting Golgi tubules. The precise mechanisms of these transformations should be verified. For instance, if the Golgi fragmentation is the result of the activity of the fission machinery one can envisage that the function of BARS would affect the ribbon-like structure of the Golgi. However, the addition of recombinant BARS alone to permeabilized cells does not induce fragmentation of the Golgi (Hidalgo Carcedo et al., 2004). In permeabilized cells, BARS can induce Golgi fragmentation only in the presence of mitotic cytosol (previously depleted of BARS) and not of interphase cytosol (Hidalgo Carcedo et al., 2004).

Interestingly, the GTP-bound form of Arf1 is important for mitotic Golgi fragmentation (Altan-Bonnet et al., 2003; Xiang et al., 2007). For instance, the expression of the constitutively active ARF1 (Q71L) mutant blocks mitotic Golgi disassembly, as well as cell progression through mitosis (Altan-Bonnet et al., 2003, 2004, 2006). At metaphase, Arf1 and Sec13 detach from membranes (Altan-Bonnet et al., 2006). Arf1 is phosphorylated during mitosis (Altan-Bonnet et al., 2003.

In S2 cells of *Drosophila*, Golgi stack separation occurs physiologically at G2 and is accompanied by a local depolymerization of F-actin around tER-Golgi units, perhaps by the specific inactivation of Abi and/or Scar (Kondylis et al., 2007). Of interest, when Golgi unpairing is inhibited by overexpression of Abi, the mitotic index drops dramatically (Rabouille and Kondylis, 2007). Thus, phosphorylation/dephosphorylation of Golgins and proteins involved in membrane fusion at the level of the GA explains mechanisms underlying the first step of mitotic Golgi fragmentation.

Post mitotic Golgi reassembly

At the end of mitosis the Golgi fragments are gathered close to the centrosome on the proximal side of the nucleus. Then Golgi stacks and the centrosome are both relocated to the distal side of the nucleus and form the pericentriolar ribbon structure (Thyberg and Moskalewski, 1992). Dephosphorylation and re-oligomerization of the cis-Golgins is a prerequisite for reassembly of the Golgi ribbon. GM130 (Lowe et al., 2000) and GRASP65 (Wang et al., 2005; Wang, 2008) restored their normal status at the end of mitosis.

Golgi stacks could be restored from the Golgi vesicles (Misteli and Warren, 1994, 1995a,b), but restored stacks are unpolar, which indicates that additional information or processes are needed to restore stack polarization. *In vitro* reassembly of the GA (fragmented *in vitro* by mitotic cytosol) requires NSF- and p97 (Rabouille et al., 1995). The NSF and p97 pathways of Golgi reassembly contribute non-additively to cisternal regrowth (Rabouille et al., 1998). *In vitro* reassembly of the GA is regulated by a Rab-GTPase (Shorter et al., 2002). Post-mitotic Golgi reassembly depends on phosphorylation of p115 (Sohda et al. 1998). Thus, at the onset of mitosis, phosphorylation/dephosphorylation of Golgins and other proteins participating in Golgi ribbon formation are involved in breaking the Golgi ribbon. Restoration of their status at the end of mitosis leads to restoration of the Golgi ribbon.

Thus, testing the predictions of our hypothesis about the role of Golgi centralization, Golgins and the fusion machinery in mitotic Golgi fragmentation shows that most of the predictions are correct. Our hypothesis explains why in the absence of these proteins or when their normal phosphorylation status is affected the GA undergoes central fragmentation. This represents evidence in favour of our hypothesis.

Cell pathology and Golgi fragmentation

During apoptosis, the GA undergoes irreversible fragmentation (reviewed in Aslan and Thomas, 2009). Fragmentation of the GA during apoptosis is a result of caspase-mediated cleavage of several Golgins, such as GM130, golgin-160, and p115 (Mukherjee and Shields, 2009). In apoptotic PC12 cells, inhibition of Rho kinase blocks Golgi fragmentation (Orlando and Pittman, 2006). Golgi fragmentation is a common pathological feature shared by many human neurodegenerative diseases (Ramamohan et al., 2007).

The GA undergoes fragmentation in several neurodegenerative disorders (Gonatas et al., 2006), such as amyotrophic lateral sclerosis and Alzheimer's disease (Nakagomi et al., 2008). For instance, Golgi fragmentation occurs in neurons with prefibrillar alpha-synuclein aggregates (Gosavi et al., 2002). Golgi fragmentation is confirmed in primary cultures of dorsal root ganglia neurons overexpressing alpha-synuclein. This effect of alpha-syn seems to have some selectivity to the MT system, as actin microfilaments and MT-independent trafficking remain unaffected (Lee et al., 2006). In human epithelial cells, Chlamydia infection also induces Golgi fragmentation to generate Golgi ministacks surrounding the bacterial inclusion, and this enhances Chlamydia replication. Fragmentation of the GA during Chlamydia infection is triggered by the proteolytic cleavage of golgin-84. Inhibition of golgin-84 truncation prevents Golgi fragmentation induced by *C. trachomatis* (Heuer et al., 2009). Thus, Golgi fragmentation in pathology depends on the impairment of the function of Golgins.

Conclusions

Thus, formation of Golgi ribbon represents an

orchestrated sequence of events based not only on different molecular mechanisms but also on discrete cellular functions. Among the mechanisms responsible for the generation of the Golgi ribbon one can mention the ability of cells to induce centralization (concentration of Golgi stacks within a restricted space) that needs a normally functioning MT star and minus end motors, normally functioning Golgins (factors responsible for the attachment of the cis-most cisterna to the medial GA, machines responsible for the attachment of the trans-post cisterna to the medial GA) and SNAREs/Rabs (molecular machines responsible for the fusion of cargo domains with cisternal rims) and several other less-studied factors. Thus, the concerted action of all these factors is sufficient for the generation of the Golgi ribbon and for the normal function of Golgi stacks. Existing experimental observations could be interpreted within the framework of several models of intracellular transport. Within the framework of the vesicular model of intra-Golgi transport, Golgins are considered as tethering factors. Within the framework of the models where vesicles are not considered as transport carriers (Mironov et al., 2005; Mironov and Beznoussenko, 2008) one needs to establish the functional role of Golgins. The assumption that their main function is the attachment of the cis-most and the trans-most cisternae could be useful for explaining the existing results. However, in future experiments it will be necessary to clarify many of aspects related to Golgi ribbon formation.

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