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Minireview

Molecular mechanisms in the disassembly and reassembly of the mammalian Golgi apparatus during M-phase

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Abstract The mitotic disassembly and reassembly of the mammalian Golgi apparatus is an ideal system to study the molecular mechanisms involved in biogenesis and maintenance of membranous organelles. As cells enter M-phase, Golgi stacks are converted into Golgi clusters of small membrane fragments, which are dispersed throughout the cytoplasmic space during metaphase. Disassembly is dependent on the action of cdc2-kinase and at least two distinct pathways contribute to the fragmentation: one involves the budding of COP I-coated vesicles from Golgi cisternae, the other is a less well characterised COP I-independent pathway. During telophase, the Golgi fragments reassemble and fuse into a fully functional Golgi stack, using at least two distinct ATPase-mediated fusion pathways.

Key words: Golgi apparatus; Mitosis; COP I-vesicle; p97; Inhibition of fusion

1. Introduction

The mammalian Golgi apparatus is typically made up of a number of stacks each consisting of 3–5 flattened, closely apposed membranous cisternae about 1–2 μm in diameter. Cisternae within a stack are distinct in their content of resident enzymes, conferring overall polarity to the stack [1–3]. Equivalent cisternae within stacks are connected by tubules to form a Golgi reticulum, thus making the mammalian Golgi apparatus a single copy organelle [4,5]. Due to its unique position at the cross-roads of exocytic and endocytic membrane transport pathways, the Golgi apparatus is one of the most dynamic organelles in the mammalian cell. During interphase, its morphological appearance is determined by the balance between the amount of incoming membrane (transport vesicles from the endoplasmic reticulum, intra-Golgi transport vesicles, membrane from the endosomal compartments) and exiting membrane (vesicles leaving the organelle for the ER or the plasma membrane, intra-Golgi transport vesicles). Disturbance of this equilibrium by either inhibition of vesicle budding or vesicle fusion results in severe alteration of Golgi morphology [6–9].

The most dramatic alteration of Golgi morphology occurs during M-phase. Golgi cisternae are consumed in a vesiculation process during prophase and metaphase. The fragmentation is a simple means to ensure partitioning of this vital organelle between the two daughter cells by a stochastic process [10]. A reversal of this process occurs during telophase, when the Golgi apparatus reassembles into the interphase Golgi reticulum [11]. Both disassembly and reassembly have recently been reconstituted in cell-free systems [12,13]. This approach, combined with biochemical and morphological techniques, has provided important insights into the molecular mechanisms involved in these processes. Additionally, the results suggest the existence of novel membrane fission/fusion mechanisms, which most likely play an essential role in protein transport and organelle biogenesis in mammalian cells.

2. The process of Golgi disassembly and reassembly during M-phase

Disassembly of the Golgi reticulum starts immediately upon entry of cells into M-phase. The Golgi apparatus loses its juxta-nuclear localisation to assume a more dispersed, perinuclear localisation [14,15]. During prophase Golgi stacks become increasingly shorter and vesicles of 50–70 nm diameter accumulate at the periphery of the stacks (Fig. 1). In vivo and in vitro, small tubular networks appear as intermediates at this stage [16–18]. By the time cells enter late prophase, stacks have completely disappeared and instead 50–300 Golgi clusters can be seen [16,19]. The clusters are typically ~200 nm–~1 μm in diameter and consist of aggregates of small vesicles (~50 nm in diameter), larger vesicles (~200 nm) and tubular elements which are occasionally branched and interconnected (Fig. 1). The clusters become smaller in size during anaphase and some of their content is dispersed throughout the cytoplasmic space [16,19].

Reassembly of the fragments is essentially a reversal of the disassembly process beginning at the onset of telophase [11]. It involves two overlapping events: fusion of membrane fragments and reorganisation of the fused fragments into an ordered stack. Golgi fragments of 60–90 nm in diameter associate with each other and fuse into larger vesicular structures. These appear to act as nucleation centres for the formation of small Golgi cisternae to which more membrane is added by fusion of fragments (Fig. 1). The fragments fuse with the periphery of the cisternae, giving rise to transient small tubular networks [13,20]. Eventually, the tubular networks are flattened and stacked to give the final product of the reassembly process, a stack of flattened Golgi cisternae.

3. Mechanisms of disassembly

3.1. COP I-dependent disassembly

COP I-coated vesicles were first identified morphologically based on their distinct coat structure compared to clathrin-coated vesicles [21,22]. The coat consists of seven coat pro-

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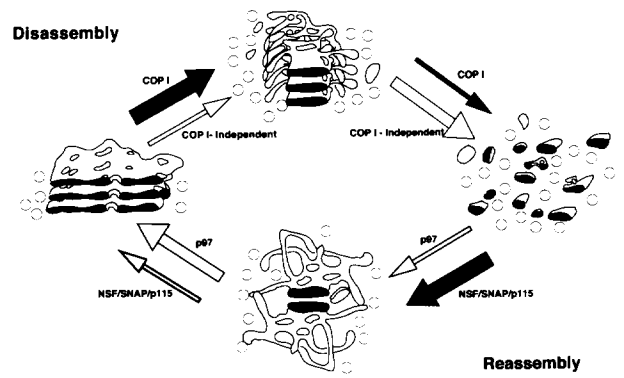

Fig. 1. Schematic representation of the molecular mechanisms involved in mitotic Golgi disassembly and reassembly. During disassembly, COP I vesicles consume cisternae, at the same time COP I-independent fragmentation contributes to the formation of tubular networks. Unstacking occurs rapidly and COP I vesicle formation ceases soon after. Golgi resident enzymes are predominantly associated with COP I-independent fragments. During reassembly, COP I vesicles fuse to give core cisternae to which membrane fragments fuse at their periphery giving rise to tubular networks. Stacking occurs and the tubular networks are reorganised into flattened cisternae. Note that the size of the arrows indicates the estimated relative contribution of each pathway.

teins (COPs) and a small GTP-binding protein, ADP ribosylation factor (ARF) [23,24]. COP I vesicles bud from the endoplasmic reticulum and Golgi cisternae and have been implicated in forward transport between the ER and the Golgi apparatus [25], transport between cisternae within the Golgi apparatus [26,27], and in retrograde transport between the Golgi apparatus and the ER [26,28–30].

COP I-coated vesicles are also the major product of mitotic Golgi fragmentation. In vitro and in vivo, vesicles with the appearance of COP I vesicles bud from cisternae at all stages of disassembly, although their production is strongly decreased in the late stages [17,18]. In vitro, addition of GTPγS, which blocks uncoating of COP I-coated vesicles [31], results in the accumulation of ~60% of total Golgi membrane in COP I vesicles. As the rate of formation of COP I vesicles and the rate of uncoating are the same in mitotic cytosol as in interphase cytosol or in intact cells [6,12], the accumulation of COP I vesicles must be caused by continued budding of COP I vesicles while the fusion with their target membranes is inhibited [12]. Inhibition of vesicle fusion is consistent with the observed general cessation of exocytic and endocytic transport during M-phase [10]. Work on endocytosis suggests that the inhibited step in the transport event is the fusion of vesicles with their target membrane [32,33]. In the case of the Golgi apparatus, inhibition of vesicle fusion would, of course, result in the accumulation of COP I vesicles in the vicinity of Golgi stacks.

A possible mechanism for the inhibition of fusion is suggested by the recent finding that p115, a vesicle fusion/docking protein involved in intra-Golgi [34,35], ER-Golgi transport [36] and transcytosis in MDCK cells [37] binds with ~20-fold lower affinity to Golgi membranes under mitotic conditions than under interphase conditions [38]. A functional relationship between p115 and disassembly was established by the finding that excess p115 reduced the extent of Golgi fragmentation in vitro. Inhibition of binding of p115 to membranes, just like inhibition of intra-Golgi transport in vitro [39] and disassembly itself [12], is dependent on cdc2 kinase, the mitotic master regulator, and additionally a downstream kinase/phosphatase system. The target of phosphorylation appears to be a putative p115 membrane-receptor, which in its phosphorylated state does not bind p115 and, therefore, prevents docking and fusion of COP I vesicles, resulting in their accumulation in the vicinity of stacks.

3.2. COP I-independent disassembly

Several observations indicate that the COP I-dependent pathway is not the sole fragmentation mechanism. First, the formation of COP I vesicles cannot account for the entire fragmentation process. About 40% of total membrane is incorporated into membrane structures which are distinct from COP I vesicles and appear as a population of larger, more heterogeneous membrane fragments [18]. Second, the amount of resident Golgi enzymes found in COP I vesicles is not increased in vesicles derived from mitotic Golgi membranes and resident enzymes must therefore be predominantly contained in non-COP-coated membrane fragments (B. Sönnichsen and G. Warren, personal communication). The existence of a COP I-independent pathway is also clearly demonstrated by the fact that Golgi membranes can be disassembled into a heterogeneous population of fragments in vitro by mitotic cytosol which has been immuno-depleted of coat proteins [18].

Nothing is known about the molecular mechanisms involved in the COP I-independent fragmentation pathway. A candidate mechanism is suggested by the observation that small tubular networks are formed as intermediates during Golgi fragmentation in vitro and in vivo [17,18]. These tubular networks might be formed by the same fission activity that ultimately gives rise to the more heterogeneous membrane fraction [18]. In order to bud a vesicle from the Golgi membrane the inner layers of the membrane bilayer must come in close proximity and fuse, possibly aided by lumenal fusion proteins. This event is termed periplasmic fusion as opposed to cytoplasmic fusion, which denotes the fusion of cytoplasmic layers of the bilayer as it occurs in the fusion of a vesicle with its target membrane [40]. During initial stages of fragmentation structural restrictions imposed by cytoskeletal elements or stacking matrices are released and the membranes of the Golgi cisternae have more freedom for dynamic movement. This increases the chance of random collisions between the inner layers of the Golgi membranes. Each such event results in the formation of a 'hole', or fenestration, in the cisternae. With time an increasing number of periplasmic fusion events takes place and the fenestrations eventually fuse with each other to give rise to tubular networks. These tubular networks are then consumed by the COP I-independent and, in part, also by the COP I-dependent pathway (Fig. 1). It is not yet clear what the relative contribution of each of these two pathways is in vivo and to what degree they are redundant.

4. Mechanisms of reassembly

4.1. SNAP/NSF/p115-dependent reassembly

Mitotic Golgi fragments reassemble into stacked Golgi cisternae in the absence of cytosolic components in vitro, demonstrating that all components for the fusion reactions involved in reassembly are present in a mitotic membrane fraction and no additional cytosolic factors are needed for

reassembly [41]. As the majority of mitotic fragments are COP I vesicles, it is not surprising that several membrane-associated proteins which mediate the fusion of COP I vesicles to Golgi membranes are also involved in Golgi reassembly. The complex which mediates COP I vesicle fusion is made up of an ATPase called NSF (**N**-ethyl-maleimide **S**ensitive **F**actor), SNAPs (**S**oluble **N**SF **A**ttachment **P**rotein), p115, two cytosolic components which have not been characterised and two trans-membrane receptors, termed SNAREs (**SNAP-Re**ceptor), of which one is present in the vesicle membrane (**v**-SNARE) and the other in the target membrane (**t**-SNARE) [26]. Addition of NSF, SNAP and p115 to NEM-treated, and therefore fusion incompetent, mitotic membranes rescues the reassembly process [41]. This is consistent with the proposed model of inhibition of fusion of COP I vesicles due to a mitotic modification of a member of the fusion machinery. As cells enter telophase, the mitotic phosphorylation is reversed and NSF-dependent fusion resumes, allowing the COP I vesicles to reassemble into cisternae (Fig. 1).

4.2. p97-mediated reassembly

Just as the COP I-coated vesicles are not the only product of Golgi disassembly, the NSF/SNAP/p115-mediated pathway of fusion is not the sole fusion mechanism. An ATPase, p97, which was first described as an NSF-related ATPase from *Xenopus laevis* and whose homologue cdc48 is required for fusion of the nuclear envelope in *S. cerevisiae* plays a major role in Golgi reassembly [42,43]. In vitro, p97 is sufficient to reassemble NEM-inactivated Golgi membrane fragments into Golgi cisternae [41,44]. However, their rims appear less fenestrated and dilated than cisternae reassembled in the presence of NSF/SNAP/p115. Only if both fusion pathways are functional, do mitotic fragments reassemble into morphologically normal stacks in vitro. One possibility is that the two fusion mechanisms act at temporally different points in the reassembly pathway (Fig. 1). Acharya et al. suggested that the NSF/SNAP pathway is required for the fusion of vesicles into precursors of cisternae, while the p97 ATPase is involved in the later formation of the stack. This is supported by their observation that addition of NSF and SNAP to Golgi fragments only results in the fusion of small fragments into larger ones. This intermediate can then be rescued to give full stacks by addition of purified p97 [44]. Alternatively, Rabouille et al. have suggested that the two ATPases mediate the fusion of the two fragmentation products: NSF promotes the fusion of COP I-vesicles, and p97 the fusion of the larger, more heterogeneous fraction of membranes [41].

5. Conclusion

The recent analysis of molecular events involved in the mitotic dis/reassembly of the Golgi apparatus has revealed that the same fission and fusion machineries which mediate vesicular transport between organelles also play major roles in the mitotic dis/reassembly process. This finding underlines the importance of the equilibrium between membrane fission and fusion in the maintenance of organelle morphology, both in interphase and M-phase. It is not clear at present whether the continued budding of COP I vesicles is simply a default activity and has as such nothing to do with the mitotic division process or whether it is a means to increase the chance of equal partitioning during M-phase by increasing the fragment number.

While the molecular mechanisms described can explain the control of membrane fusion and fission during M-phase, they do not give an obvious clue as to how the complex and distinct, stacked morphology of the Golgi apparatus is achieved upon reassembly. This question will have to await characterisation of components which are unambiguously involved in cisternal stacking.

Most excitingly, the investigation of the mitotic behaviour of the Golgi apparatus has also revealed the existence of a novel fission mechanism, evident in the COP I-independent disassembly pathway, and a novel fusion mechanism, mediated by p97. It is most likely that these two novel processes, just like the COP I-mediated fusion and fission mechanisms, play essential roles in protein transport and organelle biogenesis during interphase.

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References

[1] Palade, G. (1975) Science 189, 347–358.
[2] Rabouille, C. and Nilsson, T. (1995) FEBS Lett. 369, 97–100.
[3] Nilsson, T., Pypaert, M., Hoe, M.H., Slusarewicz, P., Berger, E. and Warren, G. (1993) J. Cell Biol. 120, 5–13.
[4] Rambourg, A. and Clermont, Y. (1990) Eur. J. Cell Biol. 51, 189–200.
[5] Tanaka, K. and Fukudome, H. (1991) J. Elect. Micr. Tech. 17, 15–23.
[6] Orci, L., Malhotra, V., Amherdt, M., Serafini, T. and Rothman, J.E. (1989) Cell 56, 357–368.
[7] Rambourg, A., Clermont, Y. and Képès, F. (1993) Anat. Rec. 237, 441–452.
[8] Pind, S.N., Nuoffer, C., McCafferey, M.J., Plutner, H., Davidson, H.W., Farquhar, M.G. and Balch, W.E. (1994) J. Cell Biol. 125, 239–252.
[9] Martinez, O., Schmidt, A., Salamero, J., Hoflack, B., Roa, M. and Goud, B. (1994) J. Cell Biol. 127, 1575–1584.
[10] Warren, G. (1993) Annu. Rev. Biochem. Sci. 323, 348.
[11] Lucocq, J.M., Berger, E.G. and Warren, G. (1989) J. Cell Biol. 109, 463–474.
[12] Misteli, T. and Warren, G. (1994) J. Cell Biol. 125, 269–282.
[13] Rabouille, C., Misteli, T., Watson, R. and Warren, G. (1995) J. Cell Biol. 129, 605–618.
[14] Cajal, S.R. (1914) Trab. Lab. Invest. Biol. 12, 134–227.
[15] Burke, B., Griffiths, G., Reggio, H., Louvard, D. and Warren, G. (1982) EMBO J 1, 1621–1628.
[16] Lucocq, J.M. and Warren, G. (1987) EMBO J. 6, 3239–3246.
[17] Misteli, T. and Warren, G. (1995) J. Cell Sci. 108, 2715–2727.
[18] Misteli, T. and Warren, G. (1995) J. Cell Biol. 130, 1027–1039.
[19] Lucocq, J.M., Pryde, J.G., Berger, E.G. and Warren, G. (1987) J. Cell Biol. 104, 865–874.
[20] Souter, E., Pypaert, M. and Warren, G. (1993) J. Cell Biol. 122, 533–540.
[21] Orci, L., Glick, B.S. and Rothman, J.E. (1986) Cell 46, 171–184.
[22] Harter, C. (1995) FEBS Lett. 369, 89–92.
[23] Serafini, T., Orci, L., Amherdt, M., Brunner, M., Kahn, R.A. and Rothman, J.E. (1991) Cell 67, 239–253.
[24] Serafini, T., Stenbeck, G., Brecht, A., Lottspeich, F., Orci, L., Rothman, J.E. and Wieland, F.T. (1991) Nature 349, 215–220.
[25] Hosobuchi, M., Kreis, T.E. and Scheckman, R. (1992) Nature 360, 603–605.
[26] Rothman, J.E. and Wieland, F.T. (1996) Science 272, 227–234.
[27] Ostermann, J., Orci, L., Tani, K., Amherdt, M., Ravazzola, M., Elazar, Z. and Rothman, J.E. (1994) Cell 75, 1015–1025.

[28] Letourneur, F., Gaynor, E.C., Hennecke, S., Demolliere, C., Duden, R., Emr, S.D., Riezman, H. and Cosson, P. (1994) Cell 79, 1199–1207.
[29] Cosson, P. and Letourneur, F. (1994) Science 263, 1629–1631.
[30] Pelham, H.R.B. (1995) Cell 79, 1125–1127.
[31] Melançon, P., Glick, B.S., Malhotra, V., Weidman, P.J., Serafani, T., Gleason, M.L., Orci, L. and Rothman, J.E. (1987) Cell 51, 1053–1062.
[32] Tuomikoski, T., Felix, M.A., Doree, M. and Gruenberg, J. (1989) Nature 342, 942–945.
[33] Woodman, P.G., Adamczewski, J., Hunt, T. and Warren, G. (1993) Mol. Biol. Cell. 4, 541–553.
[34] Waters, M.G., Clary, D.O. and Rothman, J.E. (1992) J. Cell Biol. 118, 1015–1026.
[35] Sapperstein, S.K., Walter, D.M., Grosvenor, A.R., Heuser, J.E. and Waters, M.G. (1995) Proc. Natl. Acad. Sci. USA 92, 522–527.
[36] Nakajima, H., Hirata, A., Ogawa, Y., Yonehara, T., Yoda, K. and Yamasaki, M. (1991) J. Cell Biol. 113, 245–260.
[37] Barroso, M., Nelson, D.S. and Sztul, E. (1995) Proc. Natl. Acad. Sci. USA 92, 527–531.
[38] Levine, T.P., Rabouille, C., Kieckbusch, R.H. and Warren, G. (1996) J. Biol. Chem. in press.
[39] Mackay, D., Kieckbusch, R., Adamczewski, J. and Warren, G. (1993) FEBS Lett. 336, 549–554.
[40] Rothman, J.E. and Warren, G. (1994) Curr. Biol. 4, 220–233.
[41] Rabouille, C., Levine, T.P., Peters, J.-M. and Warren, G. (1995) Cell 82, 905–914.
[42] Peters, J.-M., Walsh, M.J. and Franke, W.W. (1991) EMBO J. 9, 1757–1767.
[43] Latterich, M., Fröhlich, K.-U. and Scheckman, R. (1995) Cell 82, 885–894.
[44] Acharya, U., Jacobs, R., Peters, J.-M., Watson, N., Farquhar, M.G. and Malhotra, V. (1995) Cell 82, 895–904.