

Golgi inheritance: shaken but not stirred

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Our view of what happens to the Golgi and ER during mitosis in mammalian cells has been shaken once more. Rather than the Golgi contents being recycled through, or mixed with the ER, two recent studies taking complementary approaches, find that the contents of these organelles remain separate throughout mitosis.

What is the fate of the mammalian Golgi apparatus during mitosis? This seemingly simple question has become part of the wider debate concerning the nature of the Golgi apparatus and the mechanism of secretory protein transport. At the heart of this debate is the question of whether the Golgi is an organelle with its own separate identity, or a complex transport intermediate containing secretory cargo that is populated by enzymes rapidly recycling to and from the ER (Glick, 2002). In recent years, this debate has been propelled by the use of microscopy techniques that allow fluorescent protein tagged Golgi enzymes to be detected in living cells, and quantitative measurements of their localization, rates of transport between the ER and Golgi, and their diffusion rates in these two compartments to be made (Cole et al., 1996). The initially surprising results from the use of such techniques were that Golgi enzymes showed high diffusional mobility and are recycling between the ER and Golgi, so that at steady state over 30% of β 1, 4-galactosyltransferase, a medial/trans-Golgi enzyme, was present in the ER (Cole et al., 1996; Zaal et al., 1999). Meanwhile, other observations showed that imposing a block on the COPII vesicle formation pathway used by cargo molecules exiting the ER by introducing dominant-negative forms of the Sar1 GTPase into cells lead to the redistribution of Golgi enzymes back into the ER (Storrie et al., 1998). This is similar to the phenotype of brefeldin A (BFA)-treated cells, where the Golgi fuses with the ER due to deregulation of the ARF1 GTPase and its associated coat proteins (Lippincott-Schwartz et al., 1989). These findings lead to the proposal that Golgi proteins might accumulate in the ER during mitosis as a result of their normal interphase recycling pathway (Fig. 1), combined with the mitotic block in protein transport between the ER and Golgi (Zaal et al., 1999; Lippincott-Schwartz and Zaal,

2000). Older observations had also suggested that such a pathway might exist in mitotically arrested cells where Golgi enzymes were found in the ER (Thyberg and Moskalewski, 1992). This was an alternative to a previous model (Fig. 1) in which the Golgi was said to directly fragment into many small vesicles and tubular remnants (Lucocq et al., 1987; Shima et al., 1997). Various lines of evidence were provided to support the conclusion that in mitosis Golgi enzymes and lipids were present within a large continuous membrane system, namely the ER, rather than in unconnected fragments derived from the Golgi (Zaal et al., 1999). These findings have now been reinvestigated by two groups taking complementary approaches for determining the fate of the Golgi in mitosis (Axelsson and Warren, 2004; Pecot and Malhotra, 2004).

Trapping ER and Golgi structures in mitosis

Malhotra and colleagues (Pecot and Malhotra, 2004) have directly tested the hypothesis that Golgi enzymes are mixed with the ER contents in mitosis with an ingenious trapping assay. For this assay, the human invariant chain and sialyltransferase, residents of the ER and Golgi apparatus, respectively, were tagged with domains that rapidly and tightly interact in the presence of the drug rapamycin. The engineered proteins localized to the expected compartments, and sialyltransferase showed a reversible relocation to the ER on BFA treatment and subsequent washout. However, if cells were treated with rapamycin before BFA washout, sialyltransferase was found trapped in the ER presumably by interaction with the invariant chain. Having established this system, the authors then used it to ask if Golgi enzymes become mixed with the ER contents in mitotic cells. Cells synchronized at the G1/S-phase boundary with aphidicolin were released and allowed to progress through mitosis in the presence of rapamycin. If Golgi enzymes are delivered to the ER as cells enter mitosis then, in the presence of rapamycin, sialyltransferase should be trapped in the ER by interaction with the invariant chain. Once the cells exit mitosis, when the ER and Golgi reform, the localization of sialyltransferase can be visualized. Rather than sialyltransferase being relocated to the ER, it was found in the Golgi apparatus, indicating that it was not recycled to the ER at any point during mitosis. When cells with ER and Golgi compartments merged by BFA treatment were allowed to go through mitosis, sialyltransferase could be trapped in

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Abbreviation used in this paper: BFA, brefeldin A.

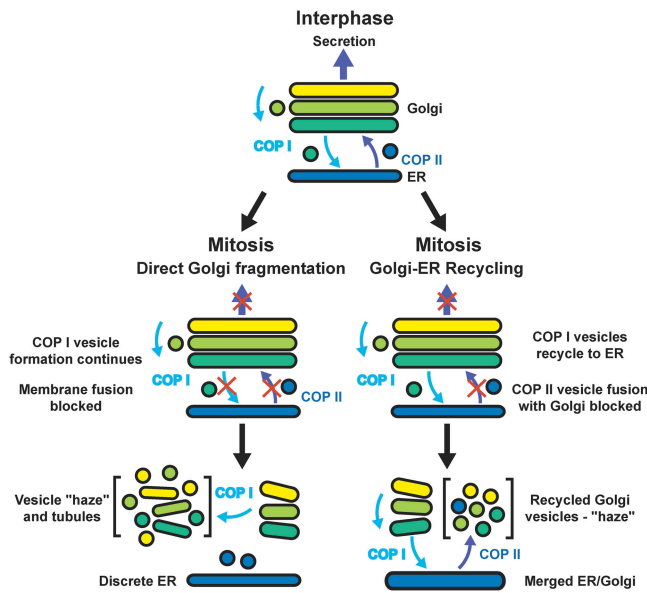


Figure 1. Golgi fragmentation in mitosis. During interphase, the Golgi receives secretory cargo from the ER via the COP II vesicle formation pathway. Depending on their nature, secretory cargo then travel through the Golgi apparatus by a combination of vesicular transport involving COP I vesicles and cisternal maturation. A retrograde COP I vesicle pathway connects Golgi cisternae and the ER to allow recycling of Golgi enzymes and integral membrane proteins of the transport machinery such as the SNAREs. Two models have been proposed to explain the fate of the Golgi in mitosis. The first is the direct fragmentation model, where Golgi cisternae are consumed by continued COP I vesicle budding in the absence of membrane fusion, which is blocked in mitosis. This generates a pool of tubules and vesicles (Golgi haze) containing Golgi enzymes and structural proteins. The second is the ER recycling model, where forward transport into the Golgi by COP II vesicles is blocked, but COP I recycling back to the ER continues until Golgi proteins are redistributed to this compartment. Some Golgi proteins may then exit the ER via the COP II pathway, but these vesicles are unable to fuse with a Golgi compartment during mitosis, and remain in equilibrium with the ER.

the ER, showing that the rapamycin trapping assay does work as expected in mitotic cells.

These findings support previous biochemical studies from a number of groups, where markers of the ER and Golgi could be separated using cell fractionation techniques (Jesch and Linstedt, 1998; Jesch et al., 2001; Seemann et al., 2002). The rapamycin trapping-assay complements these cell fractionation approaches because it shows that the compartment containing Golgi enzymes in mitotic cells is unlikely to be generated via a recycling pathway involving the ER. One caveat to this is the assumption that Golgi contents should become sufficiently mixed with the ER during recycling to allow trapping to occur, which is not necessarily the case if recycling occurs at subdomains of the ER from which ER-retained proteins are excluded and to which proteins recycling to the Golgi are restricted. However, this seems unlikely given the high diffusional mobility observed for proteins within the ER, and measurements suggesting they have access to the entire ER volume (Ellenberg et al., 1997).

Seeing through the mitotic Golgi haze

A second study has revisited the fate of both the Golgi and the ER in mitotic cells using stable cell lines expressing fluo-

rescent protein tagged *N*-acetylgalactosaminyltransferase 2 and Sec61 β (part of the translocon), respectively (Axelsson and Warren, 2004). The first surprise from this study is the clear separation of these two markers throughout the cell cycle (Fig. 2), apparently incompatible with the presence of Golgi enzymes in the ER. In mitosis, Golgi enzymes relocated to larger punctate clusters and a diffuse haze spread throughout the cell thought to be due to many small Golgi-derived vesicles, whereas the ER and nuclear envelope existed as a large reticular network surrounding the mitotic spindle. This is consistent with a number of previous studies showing that in mitosis a discrete number of punctate Golgi clusters exist, although varying with cell type, plus a haze composed of numerous small vesicles (Lucocq et al., 1987; Shima et al., 1997). Although the existence of the punctate clusters and diffuse haze is not disputed, their origin is. An alternative to the recycling model introduced previously is the direct fragmentation of the Golgi into small tubular fragments and dispersed vesicles ("Golgi haze") by the COP I vesicle formation pathway. One problem with studying this Golgi haze is that these structures are below the level of resolution of the light microscope, and whereas electron microscopy has shown that these small vesicles containing Golgi enzymes exist (Lucocq et al., 1987), it is less suited to investigating their dynamics and mechanism of formation. Axelsson and Warren (2004) have now addressed the nature of the Golgi haze by asking if it can be distinguished from the ER by diffusion measurements of Golgi and ER markers during mitosis (Axelsson and Warren, 2004). Although there are some subtle differences between diffusion of ER and Golgi markers in mitotic cells, these are not sufficient to conclude that they are separated, rather than in the same compartment. To help discriminate these two possibilities, the ER was fragmented in mitotic cells by treatment with the drug filipin, and the diffusion measurements repeated. The authors argue that the Golgi markers are present in small rapidly diffusing vesicles, whereas the ER markers are rapidly diffusing through a large continuous compartment. Fragmentation of the ER into relatively large but essentially immobile structures, compared with Golgi vesicles, should therefore have a large effect on diffusion of ER markers, whereas having little effect on Golgi markers. As predicted, diffusion of the Golgi marker remained essentially unaltered, whereas that of the ER marker was greatly reduced. When Golgi markers were relocated to the ER by BFA treatment, filipin reduced their diffusion to the same extent as an ER protein. Golgi and ER markers can therefore be distinguished in mitotic cells on the basis of specific diffusion properties, suggesting they are not in the same compartment. One outstanding issue with this argument is the rapid diffusion properties of the mitotic Golgi vesicles compared with the fragmented ER, but this may be partly explained by their difference in size, small compared with large, respectively.

How can these be reconciled with previous findings using fluorescent protein or otherwise tagged markers? One key point is a technical issue concerning the use of protein tagging, where protein folding may be a limiting factor for the transport of the tagged protein from the ER. It has been suggested that this gives rise to an artificially large ER-localized pool of the tagged Golgi enzyme, and thus the appearance of a Golgi recycling pathway feeding an ER pool of these en-

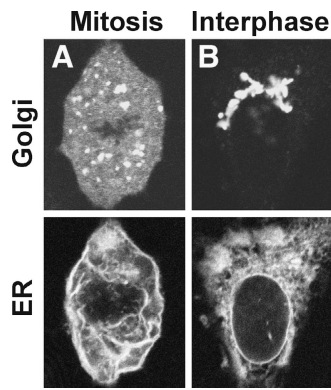


Figure 2. Morphologically distinct ER and Golgi compartments exist in both interphase and mitotic cells. The ER localized translocon component Sec61 β and the Golgi marker *N*-acetylgalactosaminyltransferase 2 were imaged in interphase and mitotic cells. At all time points the Golgi and ER remained morphologically distinct, despite significant differences in their morphology in mitotic compared with interphase cells. Images are courtesy of M. Axelsson, taken from Axelsson and Warren (2004).

zymes that may be exaggerated in cells arrested in mitosis for prolonged periods (Farmaki et al., 1999; Terasaki, 2000; Jokitalo et al., 2001). Obviously, the issue of fluorescent proteins interfering with the behavior of the protein to which they are fused is a criticism that can be applied to all such studies, and simply because the main technique to be used is microscopy based does not obviate the need for proper biochemical characterization of such markers. A number of simple criteria should at least be met concerning the level, localization, and biogenesis of these markers compared with the endogenous protein. Switching to nonprotein markers does not necessarily resolve these issues. Bodipy-ceramide, a fluorescent lipid analogue used in previous studies to mark the Golgi (Zaal et al., 1999), is also present at significant levels in the ER in mitotic cells (Axelsson and Warren, 2004), complicating the interpretation of diffusion measurements made with this probe. Again, this highlights the need for proper characterization of organelle markers used for such studies. Furthermore, there are many technical details that need to be considered when performing photobleaching experiments to measure diffusion, which if ignored can lead to serious misinterpretation of the data (reviewed in Verkman, 2002).

Clarifying the mechanism of Golgi inheritance

The new findings support the vesicle fragmentation model for Golgi inheritance rather than the ER recycling model. This conclusion is similar to that reached by a previous study of the ER and Golgi in early embryonic division sea urchins, showing that the bulk of the Golgi is distinct from the ER during mitosis (Terasaki, 2000). However, it has also been proposed that elements of both models may operate depending on the Golgi protein in question (Prescott et al., 2001), and there are still some uncertainties to be addressed. Although the two new studies provide valuable evidence supporting the idea that the Golgi has its own identity and remains separate from the ER during mitosis in mammalian cells (Axelsson and Warren, 2004; Pecot and Malhotra, 2004), both make use of drug treatments to help discrimi-

nate the ER and Golgi which may give rise to unforeseen problems. At present, a fairly limited range of ER and Golgi markers has been investigated, and it would be valuable to extend the rapamycin trapping approach in particular to a variety of other membrane and luminal proteins of both the ER and various cisternal subcompartments of the Golgi apparatus, and to include biochemical analysis of the trapped complexes. This would make it possible to address whether or not recycling Golgi proteins become sufficiently mixed with the ER to allow trapping to occur, which is not necessarily the case if recycling occurs at subdomains of the ER from which ER-retained proteins are excluded.

Why do the vesicles making up the Golgi haze diffuse so rapidly in mitotic cells, much more rapidly than fragmented ER vesicles? The cytoplasm is crowded with many large protein assemblies and organelles, and this would be expected to have an effect on vesicle diffusion, albeit one that is not necessarily predictable (Seksek et al., 1997; Verkman, 2002). However, at present it is unknown how the properties of the cytoplasm alter measurements of vesicle diffusion, the precise effects of vesicle size, and what volume of the cytoplasm is actually available for vesicle diffusion in living cells and how this differs between interphase and mitosis. All of these points are important for a full interpretation of measurements made on membrane organelles such as the ER and Golgi.

There is also the question if fragmentation of the Golgi during mitosis has any function other than in organelle inheritance. One suggestion is that Golgi disassembly has a function in a form of organelle checkpoint regulating cell cycle progression at the G2/M phase transition (Sutterlin et al., 2002). However, this is an isolated observation and the mechanism is unknown. It is also worth noting that the studies from the Malhotra and Warren labs show that in BFA-treated cells where the Golgi is merged with the ER, progression through mitosis, cytokinesis, and Golgi inheritance are apparently normal (Axelsson and Warren, 2004; Pecot and Malhotra, 2004). The underlying reason, if any, for a Golgi fragmentation in mitosis separate from the ER is therefore still a mystery. One possibility is that the behavior of the Golgi in mitosis mirrors the different modes of cell division seen in fungi, plants, and vertebrate cells (Guertin et al., 2002). Evidence from studies of *Drosophila melanogaster* indicate that the fate of the Golgi mitosis can reflect differences in the growth state of the cells or organism. However, at the present time no real coherent picture has emerged and further studies will be needed to clarify this issue.

One point that all protagonists in this debate can surely agree on is that there are limitations to light microscopy, and there is a need for a time resolved electron microscopy and tomography study to simultaneously follow a variety of ER and Golgi markers through mitosis and definitively establish the fate of these organelles in mammalian cells.

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