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Article Addendum

# Spindle-dependent partitioning of the Golgi ribbon

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**During mitosis, the Golgi apparatus needs to be divided into the daughter cells. To achieve successful division, the single continuous Golgi ribbon is disassembled in early mitosis into vesicular and tubular membranes, which upon segregation fuse to reform a functional Golgi complex in telophase. Although the process of Golgi division has been well described, the underlying mechanisms remain largely unknown. The observation that Golgi membranes accumulate around the spindle poles implies a role of the mitotic spindle in Golgi partitioning. By inducing asymmetrical cell division where the spindle goes into only one of the daughter cells, we have recently shown that the inheritance of a continuous Golgi ribbon critically relies on the mitotic spindle, while membranes sufficient to reassemble polarized, functional Golgi stacks are inherited independently.**

Like all other cellular organelles, the Golgi apparatus grows in interphase and is divided into the daughter cells in mitosis. During cell division, the single continuous Golgi ribbon in mammalian cells is disassembled in early mitosis and reformed upon partitioning in both daughter cells.[1,2] The nuclear envelope is also dissolved at the onset of mitosis to allow chromosome segregation. For the purpose of partitioning, the nuclear membranes are first absorbed into the ER and re-emerge out of the ER at the end of mitosis to assemble a nuclear envelope around the decondensing chromosomes.[3,4] The Golgi has been suggested to follow the same fate as the nuclear envelope during mitosis, where the disassembled Golgi membranes fuse with the ER.[5,6] However, several recent reports presented compelling evidence against this mode of partitioning. Instead of merging with the ER, the Golgi membranes remain distinct and separated from the ER throughout mitosis.[7-10]

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The spindle instead has been proposed to play a key role in the Golgi division process, based on two major observations. First, Golgi partitioning is highly accurate to a comparable extent as chromosome segregation that utilizes the spindle machinery.[11,12] Second, mitotic Golgi membranes are found concentrated around the two spindle poles.[13,14] Whether the spindle is indeed involved in organizing Golgi division was so far difficult to analyze, because Golgi segregation could not be uncoupled from chromosome and centrosome separation.

To dissect the function of the spindle in Golgi partitioning, we recently established an approach by which cytokinesis proceeds in the absence of chromosome segregation.[15] By overriding the spindle checkpoint and triggering cytokinesis, this assay allows to directly test whether the mitotic spindle is necessary for Golgi inheritance. Cells are first treated with an Eg5 kinesin inhibitor (monastrol or trityl cysteine) that blocks centrosome separation in prophase. As a result, the cells become arrested in early mitosis with monopolar spindles.[16] The disassembled Golgi membranes in these cells behave similarly to those in untreated cells and still accumulate around the poles of the monopolar spindles. To induce cell division, the spindle checkpoint is then bypassed by microinjection of Mad1 recombinant protein[17] or by addition of a Cdk1 inhibitor such as roscovitine or purvalanol A.[18,19] Subsequently the cells assemble a cleavage furrow and complete cytokinesis, which gives rise to a karyoplast that receives the entire spindle, chromosomes and centrosomes, and a cytoplast lacking all of these.

In the karyoplast, Golgi stacks reassemble and are laterally linked together into one continuous ribbon in the perinuclear region. In the cytoplast, stacked and polarized Golgi cisternae also reform, which are fully functional in transporting cargo through the secretory pathway. In contrast to the karyoplast, however, these stacks are scattered throughout the cytoplasm and not interconnected into a ribbon. This suggests that the factors required for ribbon formation are partitioned together with the spindle and are therefore not present in the cytoplast. Indeed, by lowering the division temperature, the spindle is positioned closer to the cleavage furrow, which allows some spindle microtubules with associated membranes, but not chromosomes, to be incorporated into the cytoplast. The resulting cytoplast reforms an intact Golgi ribbon, demonstrating that the factors required for ribbon assembly are linked to the spindle. Alternatively, a ribbon can be restored by adding back the missing factors to the cytoplast. Microinjection of

a Golgi detergent extract together with tubulin into the cytoplast fully reestablishes a ribbon. These findings uncover that Golgi partitioning is regulated by two distinct mechanisms. Polarized stacks of Golgi cisternae, the basic units functional in secretion, are segregated into progeny by a spindle-independent process. In contrast, the factors that link the stacks into a continuous ribbon are partitioned together with the spindle.

Previous reports demonstrated that downregulation of the cis-Golgi matrix proteins GM130 and GRASP65 by RNAi disrupts the Golgi ribbon structure,[20] suggesting that the two proteins might play a role in lateral linkage of Golgi stacks into a ribbon under the interphase condition. However, we observed that both GM130 and GRASP65 are present in the cytoplast, indicating that neither GM130 nor GRASP65 is sufficient to reform a Golgi ribbon, at least in post-mitotic cells. Furthermore, upon microinjection of the mRNAs of GM130 and GRASP65 together with purified tubulin into the cytoplast, both proteins are expressed, but a ribbon is not formed and individual Golgi stacks remain scattered. Interestingly, expression of GM130 and GRASP65 causes extensive tubulation of the scattered Golgi stacks. Tubular profiles positive for GM130 have been described to carry cargo proteins between the peripheral intermediate compartment and the centrally located Golgi ribbon,[21] but the structures we observed were more prominent and numerous. The tubulation may reflect an initial step in mobilizing the Golgi stacks along microtubule tracks. If other parts of the Golgi remain static, only tubules are pulled out of the Golgi membranes and the Golgi elements are not brought together.

In addition, the microinjected protein extract that rescues ribbon assembly is depleted of both GM130 and GRASP65 and instead enriched in proteins resident to medial/trans-cisternae. In fact, the majority of cis-Golgi proteins are either absent or highly de-enriched in this extract. Therefore, medial/trans-Golgi proteins contain the information to restore the ribbon. Taken together, our data suggest that post-mitotic ribbon formation depends on a different subset of Golgi proteins (medial/trans proteins) rather than those required for the maintenance of the ribbon in interphase.

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