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VIEWPOINT

GRASPing for consensus about the Golgi apparatus

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Cisternae of the Golgi apparatus adhere to each other to form stacks, which are aligned side by side to form the Golgi ribbon. Two proteins, GRASP65 and GRASP55, previously implicated in stacking of cisternae, are shown to be required for the formation of the Golgi ribbon.

Introduction

The Golgi apparatus is an intermediate organelle along the secretory pathway that receives proteins and lipids ("cargo") from the endoplasmic reticulum, covalently modifies them, and then exports them via transport vesicles for trafficking to the plasma membrane or other organelles. In most eukaryotic cells, disc-shaped membrane cisternae, each containing a distinct repertoire of cargo-processing enzymes, are stacked one on top of another to form the "Golgi stack," a visual hallmark of the organelle (Fig. 1). The cisternae of the Golgi stack are polarized, with the compartment receiving endoplasmic reticulum–derived cargo termed the cis cisterna followed by the medial; trans; and finally, the trans-Golgi network. The physiological advantages conferred by stacking of Golgi cisternae are unclear, but it is thought to enhance the efficiencies of the sequential chemical modifications of glycoproteins and glycolipids during secretion. Cultured mammalian cells may possess more than 100 Golgi stacks, which are aligned side by side about the centrosome to form the "Golgi ribbon" (Fig. 1). Vesicles and tubules span the intervening, "noncompact" zones between stacks of cisternae, connecting analogous cisternae across the ribbon and thereby ensuring a homogeneous distribution of Golgi resident proteins among all cisternae. During mitosis, the Golgi ribbon is unlinked, the stacks are disassembled, and the cisternae are converted to vesicles and tubules; after cytokinesis, the process is reversed, and the Golgi is rebuilt. The dynamic nature of Golgi structure in interphase and mitotic cells implies the existence of a reversible mechanism that tethers Golgi cisternae to each other to form the stack and a mechanism that aligns and links the stacks into the ribbon.

GRASP proteins tether Golgi cisternae in vitro

Investigations into the molecular basis of Golgi cisterna stacking have ultimately focused attention on a handful of cytoplasmic proteins called "Golgins" and "GRASPs" that are associated with specific Golgi cisternae and interact with each other. Of particular interest are two related proteins GRASP65 and GRASP55 (respective systematic names GORASP1 and GORASP2), discovered by Warren and colleagues via in vitro reconstitution experiments, as capable of mediating stacking of Golgi cisternae (Barr et al., 1997; Shorter et al., 1999). Whereas GRASP65 localizes to the cis cisterna, GRASP55 localization favors medial/trans Golgi cisternae (Shorter et al., 1999); hence, these proteins could, in principle, tether cisternae to form a minimal Golgi stack. In these in vitro assays, perturbations (mutations, antibody interference) to either GRASP65 or GRASP55 inhibited stacking of reformed Golgi cisternae. Moreover, GRASP proteins are phosphorylated in mitosis just before vesiculation of Golgi cisternae, and preventing phosphorylation impairs the disassembly of the Golgi apparatus and mitotic progression (Wang et al., 2003). These findings underpin models of the Golgi stack where GRASP65 and GRASP55, along with Golgin proteins, constitute the core components of a cytoplasmic "matrix" of proteins that surround the cisternae, mediating their stacking as well as the tethering of transport vesicles to cisternae. Curiously, plant cells contain stacked Golgi cisternae, yet they do not express any GRASP or GRASP-related proteins. And some nonvertebrate organisms with stacked Golgi cisternae express just one GRASP-related protein, while the Golgi cisternae are not stacked in other nonvertebrate organisms (e.g., yeast) that express a single GRASP (Glick and Malhotra, 1998). Apparently, the presence or number of GRASP proteins expressed does not correlate with stacked cisternae.

Whereas the results of in vitro biochemical assays underpin our conceptions of GRASP protein function, probing their roles in vivo has proven to be quite challenging. First, depletion/deletion of each individual GRASP protein is largely without effect on Golgi stack or ribbon formation, but a very complex phenotype results from depletion/deletion of both GRASP proteins. Thus, some reports conclude that the GRASP proteins function redundantly to stack cisternae (Bekier et al., 2017), while others conclude that the Golgi ribbon, not the stack per se, is perturbed upon loss of GRASP proteins (Puthenveedu et al., 2006; Feinstein and Linstedt, 2008; Xiang and Wang, 2010;

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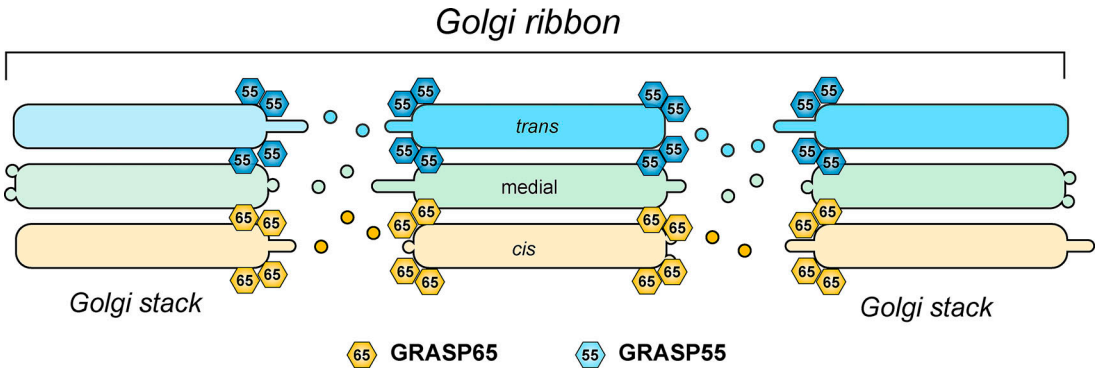

Figure 1. **The organization of the Golgi apparatus in vertebrate cells.** Individual stacks of Golgi cisternae are aligned side to side to form the Golgi ribbon. The GRASP65 and GRASP55 proteins are depicted to be enriched on the rims of the indicated cisternae within individual stacks of cisternae, where they are required to maintain the arrangement of stacks into the ribbon.

Lee et al., 2014; Veenendaal et al., 2014). Recently, two papers published in the *Journal of Cell Biology* employed different methodologies to perturb GRASP protein functions in vivo (Grond et al., 2020; Zhang and Seemann, 2021), providing the most conclusive insight to date into the roles of GRASP proteins in Golgi structure.

The Golgi ribbon is unlinked upon loss of GRASP proteins

Rabouille and colleagues used traditional mouse gene knockout technology to delete GRASP65, finding that such mice are viable with no apparent physiological deficits or gross morphological perturbations of the Golgi (Veenendaal et al., 2014). In their recent study (Grond et al., 2020), GRASP55 was deleted in the GRASP65 null background, but double-knockout mice could not be obtained, consistent with GRASP proteins being at least partially physiologically redundant. Next, using a conditional knockout approach, double GRASP null cells were produced postnatally in the small intestine, and the Golgi of intestinal epithelial cells was examined. In these cells, stacked Golgi cisternae were observed, but their arrangement into a ribbon was compromised, a result corroborated by more detailed analysis of cells in organoid cultures. These findings are at odds with the conclusions of Wang and colleagues (Bekier et al., 2017), who used CRISPR-Cas9 gene editing technology to construct cultured mammalian cell lines that do not express GRASP65 and GRASP55. They found that the appearance of Golgi cisternae was grossly altered, resembling clusters of tubules and vesicles ("tubulovesicular clusters") about swollen cisterna remnants that debatably appeared to be stacked. One possible reason for the disparities between these two studies is that Bekier et al. (2017) documented that loss of GRASP proteins in cultured mammalian cells also resulted in depletion of a subset of Golgin proteins (e.g., GM130, Golgin-45) from Golgi cisternae, so it was not possible to parse the specific contributions of GRASP proteins to Golgi structure.

Analyses of siRNA-depleted and gene-edited cell lines and modified animals are often complicated by incomplete depletion of a query protein, unintended loss of other proteins, or compensatory processes that obscure loss-of-function effects. Notably, siRNA depletion of GM130, which is associated with GRASP65 on the cis cisterna, impairs secretory traffic from the endoplasmic reticulum to the Golgi apparatus, resulting in a reduction in the size of Golgi cisternae and diminished interstack connectivity possibly due to vesiculation of cisternae (Seemann et al., 2000; Puthenveedu et al., 2006). To minimize these drawbacks, Zhang and Seemann (2021) used gene editing to modify the GRASP65 and GRASP55 loci to append an inducible protein degradation domain to each protein in cultured mammalian cells, which was used to elicit degradation of the GRASP proteins within just 2 h. Hence, the acute effects of GRASP protein depletion could be determined before the onset of potentially confounding effects. Fluorescence recovery after photobleaching assays of a fluorescently tagged Golgi resident protein revealed that acute depletion of both GRASP65 and GRASP55 resulted in decreased mobility of the resident Golgi enzyme within the ribbon, indicating that connectivity of cisternae between stacks was compromised. Stacks of Golgi cisternae with proper cis–trans polarity were observed by electron and light microscopy, both shortly (~2 h) after GRASP protein turnover was initiated, and after mitosis, indicating that GRASP proteins are not required to establish or to maintain the Golgi stacks. Importantly, the authors observed no changes in the levels of GRASP-associated proteins (e.g., GM130) when assayed shortly after initiating GRASP protein turnover, but the amounts of several GRASP-associated proteins were reduced after prolonged growth in the absence of GRASP proteins. The results are in general agreement with experiments by Jarvela and Linstedt (2014), who expressed GRASP65 and GRASP55 fusion proteins appended with "killer RFP" and used chomophore-assisted light inactivation to rapidly (1 min) ablate the proteins in cultured mammalian cells. Similar to Zhang and Seemann (2021), they observed that the Golgi ribbon was disassembled upon inactivation of GRASP proteins, but stacking of cisternae was unaffected. Taken together, these results conclusively show that acute depletion of GRASP65 and GRASP55 impairs lateral linking of stacked Golgi cisternae within the ribbon while not affecting stacking of cisternae.

Conclusions and perspectives

A body of work now more than 20 years old has shown that GRASP65 and GRAPS55 are core structural components of a matrix of cytoplasmic proteins associated with Golgi cisternae; however, the Grond et al. (2020) and Zhang and Seemann (2021) reports now firmly establish that GRASP proteins are dispensable for stacking of Golgi cisterna and indicate that they are required for linking Golgi stacks within the ribbon. These new studies suggest that the integrity of the Golgi matrix critically depends on the presence of GRASP proteins, and their

absence perturbs the balance of cargo flow through the Golgi, reducing the interstack exchange required to maintain connectivity of stacks within the ribbon. How might GRASP proteins facilitate linking of stacks within the Golgi ribbon? When the ribbon is disrupted (using the microtubule depolymerizing reagent nocodazole) and individual Golgi stacks are examined, GRASP65 and GRASP55 appear to be enriched at the rims of Golgi cisternae (Fig. 1; Tie et al., 2018). Hence, the GRASP proteins are positioned at the vesicle-rich interface between adjacent cisternal stacks. Grond et al. (2020) observed reductions in the size of Golgi cisternae in cells deleted of both GRASP proteins and speculated that this may be due to increased coatomer I vesicle formation at the rims of cisternae. In this view, GRASP proteins dampen vesicle flux at the rims of Golgi cisternae, a model supported by the observation that depletion of GRASP proteins leads to an increase in secretion rate (Wang et al., 2008). These new studies firmly shift our view of GRASP protein function away from the stacking of Golgi cisternae, and we look forward to new mechanistic insights into the roles of GRASP proteins in Golgi ribbon formation as well as in non–Golgi-dependent processes, such as unconventional protein secretion (Kinseth et al., 2007).

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