Principal Investigator: Yanzhuang Wang, PhD
Grant Title: Golgi cisternal stacking is required for accurate glycosylation

Abstract

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Objectives: How and why Golgi cisternae form stacks? The Golgi apparatus is a key cellular membrane organelle in the secretory pathway that processes a wide variety of proteins, including antibodies that protect us against infections, enzymes that help digest our food, and protein factors that help our cells to grow and even die when appropriate. To perform these functions, the Golgi membranes need to form a unique, stacked structure. However, the mechanism and significance of cisternal stacking for normal cell physiology remains largely untested. In an increasing number of diseases, including cancer, viral infections and multiple types of neurodegenerative diseases, the Golgi apparatus is fragmented abnormally, although the underlying mechanism is so far unexplored. Our research aims to understand how the stacked Golgi structure is formed and why its formation is important for Golgi function under normal and disease conditions (*1, 2*).

Methods: A unique multidisciplinary approach. We have developed a unique multidisciplinary approach employing biochemistry, cell biology, electron microscopy, and proteomics and glycomics, combined with a novel *in vitro* reconstitution assay, to provide a mechanistic explanation for Golgi structure formation and function. This allowed us to reveal that stack formation directly involves the Golgi stacking protein GRASP65 and GRASP55, which play complementary and essential roles in Golgi cisternal stacking by forming mitotically regulated *trans*-oligomers (*3-5*).

Results: Golgi cisternal stacking is required for accurate glycosylation. We have explored the functional consequences of Golgi unstacking by the depletion of GRASP55, GRASP65 or both. We found that Golgi cisternal unstacking stimulates COPI vesicle budding and thus enhances protein transport. Golgi fragmentation, however, impairs protein sorting and alters the glycosylation of cell surface proteins. Subsequently, cell adhesion and migration were reduced when the Golgi was unstacked. Furthermore, total protein synthesis and the proliferation of cells with unstacked Golgi were enhanced. We propose that Golgi stack formation is a flux regulator for protein trafficking and thereby functions as a quality control mechanism for protein sorting and modifications, e.g. glycosylation (Figure 1) (*6, 7*). This work has significant impact in understanding human diseases in which the Golgi becomes abnormal. For example, cancer cells have fragmented Golgi, which may be the reason why these cells have reduced cell attachment, changes on cell surface glycan, and secretion of lysosomal enzymes, all of which could be caused by Golgi dysfunction.

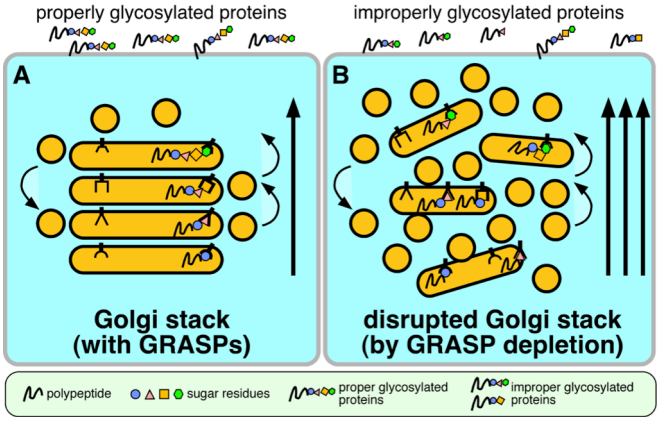

Figure 1. Golgi destruction accelerates protein trafficking and impairs accurate glycosylation. When Golgi cisternae are stacked (**A**), vesicles can only form and fuse at the rims. This slows down trafficking, but enforces accurate glycosylation. Once the cisternae are unstacked (**B**), more membrane area becomes accessible for vesicle budding and fusion, thereby increasing cargo transport. This, however, causes glycosylation and sorting defects.

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