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Grant Title: Control of COPII vesicle trafficking by intracellular protein glycosylation

Abstract

One third of all eukaryotic proteins pass through the secretory pathway for targeting to specific locations, including the endoplasmic reticulum (ER), Golgi, plasma membrane or extracellular milieu. Since misdirected proteins cannot function, the secretory pathway plays a critical role in establishing and maintaining normal cell and tissue physiology. In particular, the COPII coat protein complex, which mediates vesicle trafficking between the ER and Golgi, is a key control point for protein targeting. Moreover, mutations in COPII genes cause a range of human diseases, including hematological disorders and several types of hereditary spastic paraplegia. Detailed knowledge of COPII vesicle trafficking is required to understand its role in cell physiology and to devise new treatments for disorders in which it is disrupted. However, while the core COPII machinery is well defined, little is known about how mammalian cells regulate COPII activity in response to developmental, metabolic or pathological cues.

Recently, we and others found that several COPII proteins are modified by O-linked β-*N*-acetylglucosamine (O-GlcNAc), a dynamic form of intracellular glycosylation. However, the mechanistic and functional impacts of O-GlcNAc on the COPII pathway remain unclear. In our Mizutani Foundation-funded project, we used a chemical biology approach to show that at least four COPII components engage in O-GlcNAc-mediated protein-protein interactions in human cells, and that pharmacological inhibition of O-GlcNAc cycling hinders COPII trafficking. These results indicate that O-GlcNAc regulates COPII activity through the modification of specific pathway components. During the project period, we focused on the essential COPII protein Sec23A. We used mass spectrometry (MS) and mutagenesis to identify eight sites of O-GlcNAcylation on Sec23A. Next, we used chemical capture and MS to identify a novel coiled-coil protein that interacts with Sec23A specifically by contacting O-GlcNAc moieties at four of these sites. Overexpression of the coiled-coil protein enhanced COPII-dependent protein trafficking in human cells, suggesting a functional connection with Sec23A. We are currently using CRISPR/Cas9 technology to test the impact of genetic loss-of-function of both Sec23A glycosylation and the expression of the coiled-coil protein in human cells and in an intact vertebrate model organism. Our results shed light on the role of O-GlcNAc in regulating the COPII pathway and may reveal new opportunities for future therapeutic intervention in human diseases of protein trafficking.