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Insight

Sweet control: intracellular sorting of glycoproteins in plants

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This article comments on:

Nagashima Y, Sharma V, Reekers LF, von Schaewen A, Koiwa H. 2025. Kifunensine-sensitive *ADP-ribosylation factor A1E^{G69R}* mutant reveals coordination of protein glycosylation and vesicle transport pathways. Journal of Experimental Botany **76**, https://doi.org/10.1093/jxb/eraf017

***N*-glycosylation is a vitally important post-translational modification in eukaryotic cells. Recent studies reveal that protein glycosylation plays essential roles in cellular protein targeting and function, developmental programs, and stress responses in plants. However, the mechanisms underlying the compartmental organization of *N*-glycan processing enzymes and subsequent sorting of glycoproteins to their destinations via intracellular trafficking routes remain poorly understood in plants. Now, Nagashima *et al.* (2025) have found that the ARF GTPase ARFA1E is essential for the function of secretory organelles and post-Golgi trafficking of the glycoprotein KORRIGAN1 under salt stress. Thus, the endosomal regulators are involved in glycoprotein dynamics and function required for plant development and stress tolerance.**

Protein glycosylation can be broadly classified into two principal types, *N*-linked and *O*-linked glycosylation characterized by different glycan attachment sites. The most extensively studied form is *N*-glycosylation (Zhang and Wang, 2024). In plants, most cell wall proteins found in the apoplast and secretory proteins within the lumen of the endomembrane compartments undergo *N*-linked glycosylation (Ruiz-May *et al.*, 2012). In all eukaryotic cells, the process of *N*-glycosylation of proteins starts in the endoplasmic reticulum (ER). After correct folding of polypeptide backbones, glycoproteins exit the ER and are transported to the Golgi apparatus, where the *N*-glycan moieties undergo further processing and maturation to produce characteristic complex *N*-glycans (Rosnoblet *et al.*, 2013). The phenotypic consequences of mutations in enzymes involved in glycan trimming in the ER and downstream Golgi *N*-glycan maturation highlight their biological significance for plant development and tolerance mechanisms (Nagashima *et al.*, 2018).

Glycosylation enzyme sorting at the Golgi apparatus

It is widely accepted that the Golgi apparatus serves as the primary site of maturation for protein *N*-glycans, exhibiting a non-uniform distribution of *N*-glycan processing enzymes along its *cis*-to-*trans* axis. The cisternal maturation model of Golgi transport, which includes the retrograde vesicle trafficking of resident proteins from late Golgi cisternae to early cisternae, accounts for the retention and spatial organization of glycosylation enzymes (Schoberer and Strasser, 2011). In this scenario, the targeting of transport vesicles is likely to be regulated by a distinct but overlapping protein machinery that facilitates vesicle tethering and subsequent fusion with Golgi cisternal membranes (Fig. 1). The conserved oligomeric Golgi (COG) complex acts as an essential tethering factor that maintains the Golgi structure and regulates intra-Golgi retrograde trafficking. The COG complex probably serves as a major interaction hub that physically and functionally interacts with various classes of trafficking-associated molecules, including SNAREs, Rab GTPases, coiled-coil tethers (golgins), and vesicle coats (Fig. 1) (Blackburn *et al.*, 2019). In animal cells, defects in COG and golgins have been linked to mislocalized glycosylation enzymes and trafficking abnormalities (Struwe and Reinhold, 2012). In plants, the Golgi transport components, including the COG homologous subunits, have been identified based on evolutionarily conserved protein sequences (Rui *et al.*, 2022). A recent study reported that loss of function

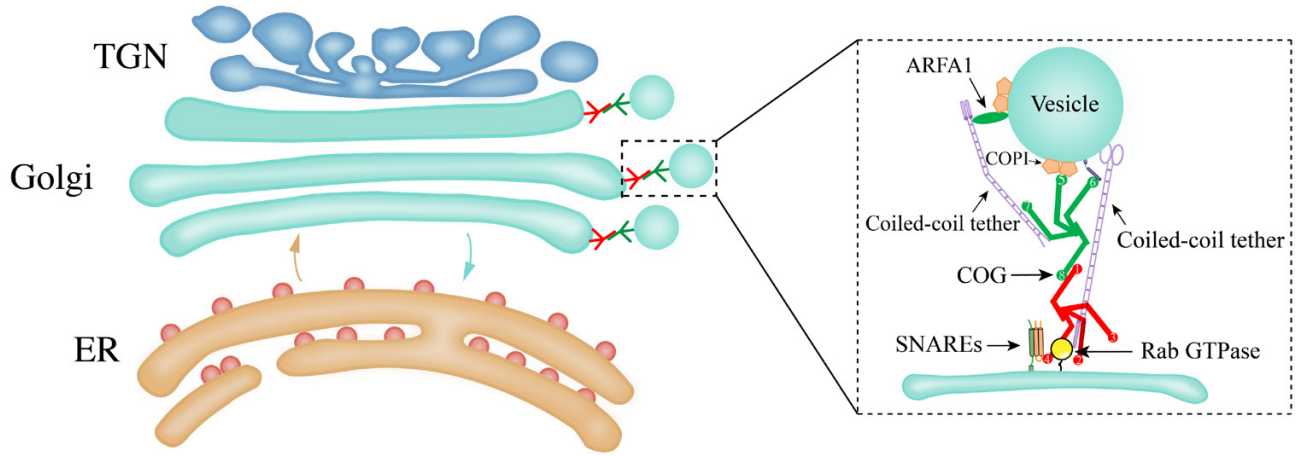

Fig. 1. Hypothetical model of vesicle tethering during intra-Golgi retrograde trafficking. The hetero-octameric COG complex functions as the docking station for incoming retrograde vesicles at the Golgi cisternae. COG subunits coordinate trafficking regulators, including COPI coats, Rab GTPases, coiled-coil tethers (golgins), and SNAREs, to form a pre-tethering complex that facilitates subsequent vesicle tethering and fusion with the Golgi membrane. The roles of COG and golgin components have been demonstrated in the spatial organization of *N*-glycan processing enzymes within the Golgi of animal cells and in the regulation of protein *N*-glycosylation in plants. In addition, the ARFA1 GTPase recruits COPI coat and golgin protein for vesicle transport in Golgi (figure adapted from Blackburn *et al*., 2019).

of the COG7 subunit accelerates dark-induced ubiquitination and autophagy, which is accompanied by increased protein *N*-glycosylation due to the enhanced transcript levels and corresponding protein abundance of Golgi glycosyltransferases (Choi *et al*., 2023). However, the roles of the COG complex in the spatial regulation of glycosylation enzymes during intra-Golgi trafficking remain largely unknown in plants.

Nagashima *et al*. (2025) provide new insights into the regulation of luminal glycoprotein biogenesis by intracellular trafficking. They established an effective forward genetic screening method to identify candidate genes that interact with *N*-glycan modifications based on growth sensitivity to a glycosylation enzyme inhibitor that leads to defective complex *N*-glycan formation. Through next-generation sequencing (NGS)-based mapping, the trafficking regulator ARFA1E, a member of the ADP-ribosylation factor (ARF) GTPase family, was identified, featuring a G69R mutation that impairs GDP–GTP exchange activity, thereby maintaining the protein in the GDP-bound inactive form. Fluorescent imaging revealed that ARFA1E localizes to both the Golgi apparatus and the *trans*-Golgi network (TGN), resembling the subcellular localization of ARFA1C. The overexpression of ARFA1E^{G69R} caused abnormal morphology of the ER and Golgi, as indicated by organelle markers, suggesting a role for ARFA1E in vesicle trafficking between the ER and Golgi. Thus, it seems that ARFA1E plays a crucial role in regulating the spatial organization of glycosylation enzymes. Arabidopsis ARFA1 has been implicated in Golgi-to-ER retrograde trafficking; for instance, expression of the inactive form of ARFA1 disrupted the localization of the COPI subunit at the Golgi (Singh *et al*., 2018), suggesting that ARFA1 is involved in recruiting the COP-I coat for cargo sorting. Furthermore, evidence indicates that Arabidopsis ARFA1 is engaged in recruiting the Golgi matrix protein golgin homolog to the Golgi membrane through direct interaction, consistent with its function in mammalian cells (Matheson *et al*., 2007). Consequently, it is hypothesized that ARFA1E plays a role in Golgi transport for glycosylation enzyme sorting by recruiting COP-I and golgin proteins in plant cells (Fig.1). However, the detailed mechanisms underlying these processes require further elucidation.

Glycoprotein sorting in post-Golgi trafficking

Following maturation in the Golgi apparatus, plant glycoproteins are further transported to their final destinations by sorting at the TGN, where the sorting machinery controls multiple divergent trafficking pathways (Fig. 2). Recent studies have revealed that the nanostructure of the plant TGN encompasses two types: Golgi-associated TGN (GA-TGN), which functions jointly with the *trans*-side of the Golgi apparatus, and Golgi-released independent TGN (GI-TGN), which originates from the GA-TGN (Uemura *et al*., 2019). Super-resolution microscopy has shown that the single GA-TGN separates into two distinct subdomains defined by the SNARE protein VAMP721 and clathrin adaptor protein (AP) complex AP-1 for secretory trafficking, and VAMP727 and AP-4 for vacuolar trafficking in Arabidopsis. The carriers VAMP721 and AP-1 when equipped with clathrin coats at the GA-TGN subdomain transit through the GI-TGN to the plasma membrane (PM) (Fig. 2), while VAMP727 and AP-4 without clathrin coats are involved in cargo transport from the GA-TGN to the vacuole passing through the multivesicular body (MVB) (Fig. 2) (Shimizu *et al*., 2021). Increasing

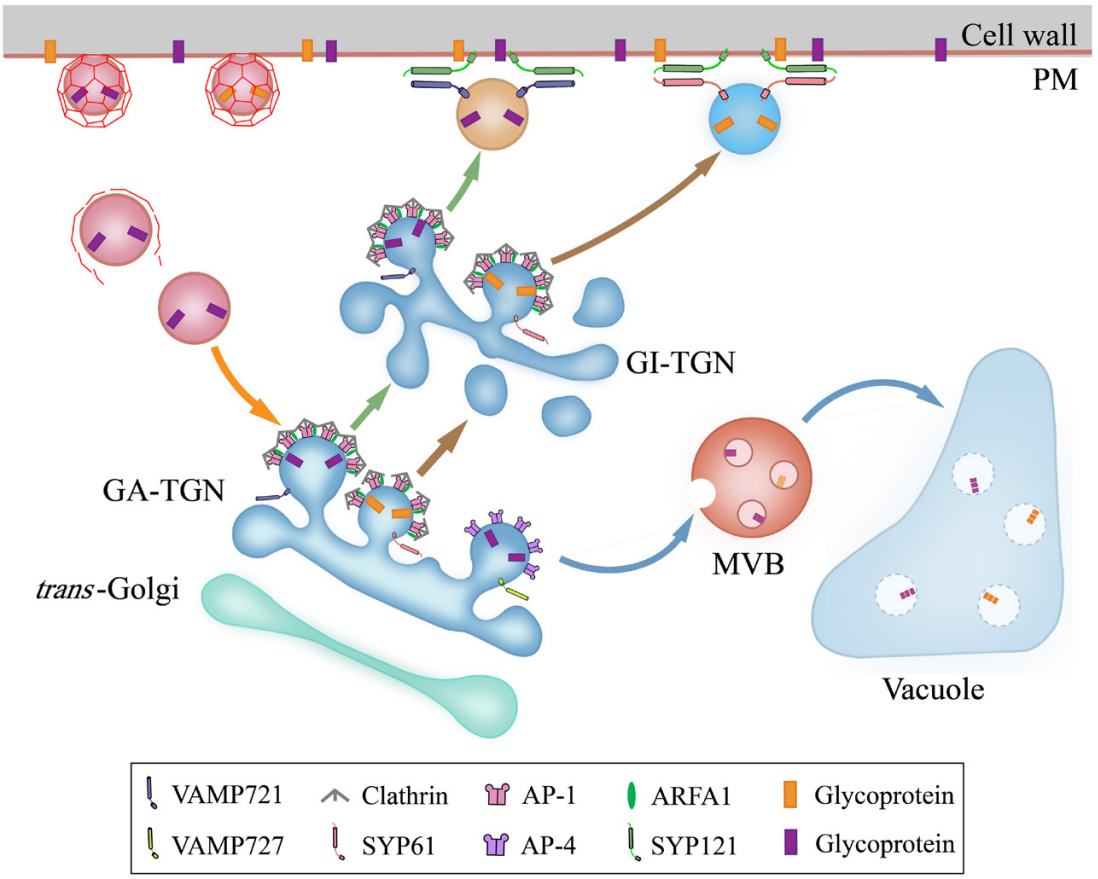

Fig. 2. Hypothetical model of glycoprotein sorting via TGN in plants. Two structural types of plant TGN exist within plant cells: the Golgi-associated TGN (GA-TGN) and the Golgi-released independent TGN (GI-TGN). Mature glycoproteins are packaged into clathrin-coated vesicles (CCVs) which are equipped with adaptor protein complex-1 (AP-1) and the SNARE protein VAMP721 at the secretory trafficking subdomains of the GA-TGN. These vesicles subsequently transit through the GI-TGN towards the plasma membrane (PM). The VAMP721–SYP121 pathway mediates the membrane fusion of secretory vesicles with the PM for cargo release. The SNARE protein SYP61, also localized at both the GA-TGN and GI-TGN, is involved in the PM sorting of glycoproteins, potentially coupling with SYP121 via CCVs (Kang *et al.*, 2011; Hoffmann *et al.*, 2021). The ARFA1 GTPase recruits the AP-1 complex, facilitating CCV assembly for cargo sorting at TGN subdomains. The endocytosed PM proteins, including glycoproteins, that enter through the clathrin-mediated pathway first arrive at the GA-TGN, and are subsequently recycled back to the PM by sharing the secretory machinery (Qi *et al.*, 2024). In addition, AP-4 and VAMP727 define another GA-TGN subdomain involved in cargo sorting to the vacuole.

evidence demonstrates that the VAMP721-dependent pathway is essential for the secretion of membrane proteins and cell wall components to the cell surface (Cermesoni *et al.*, 2024). A large-scale glycomic analysis of TGN-resident vesicles revealed that SNARE protein SYP61-mediated secretory trafficking plays a crucial role in the transport and deposition of cell wall polysaccharides and glycoproteins (Wilkop *et al.*, 2019). Moreover, SYP61 probably forms a SNARE complex with PM-resident SYP121 to deliver the aquaporin PIP2;7 to the PM (Hachez *et al.*, 2014). Therefore, the SYP61–SYP121 pathway is likely to also contribute to the targeting and membrane fusion of vesicles loading with glycoproteins (Fig. 2).

Nagashima *et al.* (2025) found that the novel player ARFA1E regulates the cellular dynamics of the glycoprotein KORRIGAN1 (KOR1) via TGN-mediated post-Golgi trafficking. Aggregated TGN structures marked by TGN-resident proteins, including KOR1, are noticed in the ARFA1E^{G69R} mutant. Time-course analysis revealed that both exocytosis and endocytosis of KOR1 under salt stress are inhibited in the mutant, suggesting that ARFA1E is involved in the endosomal sorting of glycoproteins via the TGN, which contributes to salt sensitivity, at least in Arabidopsis. In mammals, ARF1 recruits the AP-1 complex to facilitate the formation of clathrin-coated vesicles for cargo sorting from the TGN (D'Souza-Schorey and Chavrier, 2006). Deficiency of Arabidopsis AP-1 subunits leads to defects in PM protein sorting at the TGN and in protein endocytosis by reducing the association of clathrin and AP-2/TPC adaptors with the PM (Wang *et al.*, 2013; Yan *et al.*, 2021). Therefore, it is essential to verify the regulatory function of ARFA1E in recruiting AP-1 subunits at the TGN to better characterize the precise functions of this ARF GTPase in post-Golgi trafficking.

Perspectives

Glycan decoration on glycoproteins plays numerous important roles and has long been receiving a great deal of attention. However, the mechanisms underlying the compartmental organization of Golgi-resident glycan-processing enzymes and post-Golgi sorting of glycoproteins for cellular functions are still obscure in plants. In light of the mounting evidence in animal systems, deep mutant phenotyping of plant Golgi matrix proteins, such as COG and golgins, will shed new light on the spatial separation of *N*-glycan-modifying enzymes by Golgi trafficking.

A variety of trafficking regulators, including ARFA1 GTPase, localize at the TGN and regulate distinct transport pathways for protein sorting in plants. However, dissecting the TGN subdomains defined by the key regulators and their interacting partners is a major challenge. Now, recent advances in super-resolution microscopy, cryo-electron microscopy, and proximity labeling methods for analyzing the protein interaction networks in the endomembrane compartment will undoubtedly lead to breakthroughs in understanding the function of ARFA1 and other regulators in post-Golgi trafficking in plants.

Conflict of interest

The authors declare no conflicts of interest.

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Keywords: Glycoprotein, glycosylation, post-Golgi trafficking, protein sorting, *trans*-Golgi network.

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