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Plant exocytosis: Weaving distinct pathways to the plant plasma membrane

During plant growth and development newly synthesized proteins and other cargo molecules must be selectively targeted to a variety of cellular compartments. Perhaps the most important targeting involves the trafficking of proteins to the plasma membrane, where they control interactions with the environment and neighboring cells. During this process, proteins entering the secretory pathway are imported into the endoplasmic reticulum where they are folded and processed. They are then sorted and delivered to the Golgi complex, where they are further processed and modified. At this point, the proteins and cargo are delivered to the *trans*-Golgi network (TGN), where they are finally sorted into distinct vesicle populations that are then targeted to distinct late endosomes, vacuolar compartments, and the plasma membrane (Nielsen, 2020). In polarized cells, membrane trafficking to the plasma membrane may also be split into additional, discrete membrane trafficking pathways potentially to distinct plasma membrane domains.

In yeast and animals, the molecular machinery that functions to transport protein and cargo between these secretory compartments is evolutionarily conserved (Figure 1). Cargo sorting and vesicle budding events are regulated by ADP-ribosylation factor GTPases (ARF GTPases), while vesicle transport and fusion are regulated by Rab GTPases (Anders and Jurgens, 2008; Nielsen, 2020). Rab GTPases have been shown to regulate aspects of membrane fusion through the recruitment of tethering factors, which are either long coiled-coil proteins or conserved multisubunit complexes that hold the vesicle in close association with their target membrane until SNAP receptor (SNARE) protein complexes form and initiate vesicle fusion (Stenmark, 2009). One of these multi-subunit tethering complexes, called the exocyst complex, contains eight evolutionarily conserved subunits (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84; Lepore et al., 2018; Polgar and Fogelgren, 2018) and is thought to assist in tethering of secretory vesicles to the plasma membrane prior to the assembly of SNARE protein fusion complexes (Saeed et al., 2019) during polarized secretion in yeast. The Rab GTPase Ypt31 (and its homolog, Ypt32) recruits Sec2 to newly formed secretory vesicles as they emerge from the TGN (Figure 1). Sec2 recruits and activates a second Rab GTPase, Sec4, to these vesicles. Sec4, in turn, recruits the exocyst complex through interaction with Sec15. The exocyst complex assists in tethering the secretory vesicle to the plasma membrane through the interaction of the Exo70 and Sec3 subunits with phosphoinositide PI-4,5P_2. Similarly, in animals, Rab8 (Sec4-like) and Rab11 (Ypt31/32-like) also recruit the exocyst through Sec15 interactions, and Sec3 and Exo70 subunits also bind PI-4,5P_2 (Polgar and Fogelgren, 2018). Furthermore, in yeast, the exocyst complex likely participates in the assembly of SNARE protein fusion complexes through interactions between the Sec6 subunit of the exocyst and Sec9, a plasma membrane–localized SNARE protein (Sivaram et al., 2005). While some aspects by which secretory vesicles are recruited to plasma membranes for fusion are conserved, there are likely unique aspects that plants have discovered in order to carry out these processes.

One of the intriguing aspects of the roles of Rab GTPases and exocyst complexes during secretion in plants is the presence of greatly expanded gene families for both the Rab8-like and the Rab11-like GTPases, as well as some of the subunits of the exocyst complex in plants. While yeast and animals typically have one or two copies each of Rab8 and Rab11 GTPases, *Arabidopsis* has 5 RabE (Rab8-like) and 26 RabA (Rab11-like) GTPases (Nielsen, 2020). Similarly, while yeast and animal exocyst subunits are generally present as single-copy genes, *Arabidopsis* contains up to 23 distinct Exo70 subunits (Zarsky et al., 2013). Why precisely plants maintain such large gene families of proteins that regulate aspects of membrane trafficking between the TGN and the plasma membrane remains largely unknown, although it is interesting to speculate that this may reflect diversification of distinct plant secretory pathways between these two compartments. In this issue of *Molecular Plant*, Pang and colleagues (Pang et al., 2021) now provide evidence that one member of this greatly expanded plant RabA GTPase family, RabA2a, selectively associates with and regulates a previously uncharacterized membrane trafficking pathway between the TGN and the plasma membrane.

Building on previous published work (Li et al., 2017) that showed that expression of a dominant-negative mutant of the RabA2a Rab GTPase resulted in selective reduction in trafficking of a subset of plasma membrane–localized proteins, Pang et al. (2021) examined which cellular proteins coimmunoprecipitated with RabA2a, and discovered that two SNARE proteins, VAMP721 and SYP121, were greatly enriched in their coimmunoprecipitated fractions. These interactions were validated using a number of *in vitro* and *in vivo* methods, which showed that RabA2a colocalized on subcellular compartments with SYP121 and VAMP721 fluorescent fusions. They further demonstrated that only the active, GTP-bound form of RabA2a (and the related RabA2b, A2c, and A2d proteins) was able to interact with these two SNARE proteins, and that some other members of the larger RabA GTPase family (e.g., RabA4b) did not associate with these SNARE proteins. Therefore, while VAMP722 and

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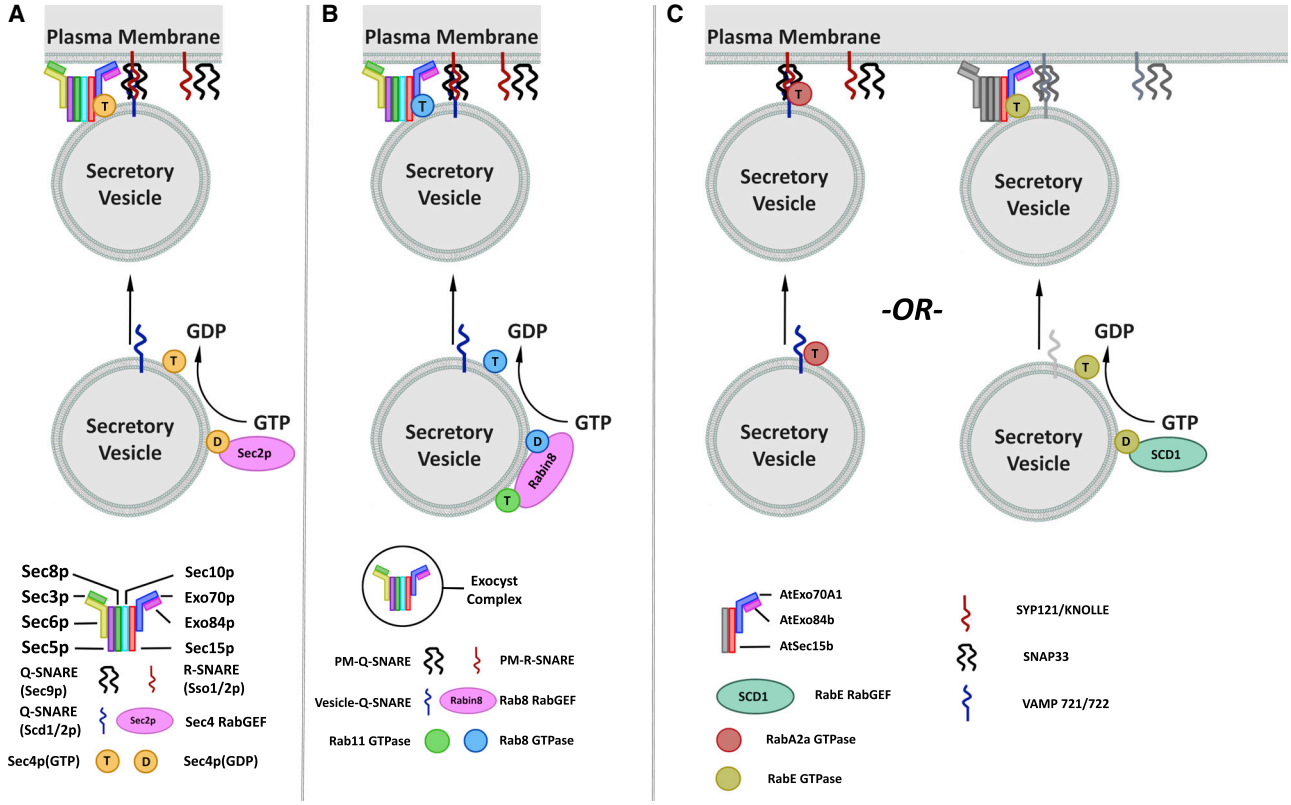

Figure 1. A schematic diagram illustrating the roles of Rab GTPases and exocyst complexes in secretion events to the plasma membrane.

(A) In yeast the Rab GTPase Sec4p associates with post-Golgi secretory vesicles and is activated by the Rab guanine nucleotide exchange factor (GEF), Sec2p. GTP-bound Sec4p interacts with the exocyst complex through the Sec15p subunit. Lipid interactions between Sec3p and Exo70p with the phosphoinositide PI-4,5P2 allow stable tethering of secretory vesicles to the plasma membrane until the vesicle-associated Q-SNARE, Scd1/2p, can assemble into *trans*-SNARE fusion complexes with the plasma membrane–localized Sec9p (Q-SNARE) and Sso1/2p (R-SNARE).

(B) In mammals, post-Golgi secretory vesicles are associated with Rab11-like GTPases, which recruit the Rab8 GEF, Rabin8. Rabin8 activates Rab8, allowing stable association with secretory vesicles. Both Rab11 and Rab8 GTPases recruit exocyst complexes through association with the Sec15 subunit, and PI-4,5P2 binding by Sec3 and Exo70 subunits results in stable association with plasma membranes, while *trans*-SNARE fusion complexes form between vesicle-associated and plasma membrane R- and Q-SNAREs.

(C) In plants, on RabA2a-labeled secretory vesicles, GTP-bound RabA2a directly associates with the vesicle-associated Q-SNAREs VAMP721/722 and promotes formation of *trans*-SNARE fusion complexes with the plasma membrane–localized SNAP33 (Q-SNARE) and SYP121. Strikingly, RabA2a-labeled vesicles do not interact with plant exocyst Sec15b subunits and do not colocalize with Exo70A1 or Exo84b exocyst components. Instead, a distinct population of secretory vesicles is labeled by RabE, which is activated by a plant RabGEF, SCD1. RabE GTPases interact with Sec15b, and presumably additional plant exocyst complex components (Exo70, Exo84) and other uncharacterized subunits (gray subunits), stabilizing the association of these secretory vesicles with the plasma membrane during the formation of *trans*-SNARE fusion complexes with yet uncharacterized vesicle and plasma membrane SNARE proteins.

SYP122 also interacted with RabA2a, other members of these two SNARE families did not, consistent with specific RabA2a–SNARE interactions.

What might the role of these RabA2a–SNARE protein interactions be? To be a fusion-competent SNARE complex, it must include an R-SNARE and two Q-SNARE proteins that generate a fusion complex with four α helices (1 R-SNARE + 3 Q-SNARE helices) assembled into a coiled-coil complex (Yoon and Munson, 2018). Interaction of RabA2a could either promote formation of this SNARE fusion complex or inhibit its formation by sequestering SNARE proteins. To test this, Pang et al. (2021) first showed that, in addition to the Q-SNARE, SYP121, and the R-SNARE, VAMP721, RabA2a also interacts with another Q-SNARE, SNAP-33. In addition, induced expression of the active, GTP-locked RabA2a GTPase promoted interactions between SYP121, VAMP721, and SNAP-33, while an inactive, GDP-locked RabA2a GTPase inhibited these interactions. These results suggest that RabA2a GTPase association promotes formation of SNARE protein fusion complexes.

If the vesicle-associated RabA2a GTPase itself can promote formation of SNARE protein fusion complexes, what role does the exocyst complex play in the tethering and fusion of these RabA2a-labeled vesicles to plant plasma membranes? Surprisingly, Pang et al. (2021) present evidence that Sec15b, Exo84b, and Exo70A1 subunits of plant exocyst complexes do not significantly interact with either RabA2a GTPases or SYP121 and VAMP721. However, it should be noted that these results are in direct contradiction to earlier studies showing

interactions between Exo70A1 and SYP121 and VAMP721 (Larson et al., 2020). Pang et al. (2021) further show that, while fluorescently tagged RabA2a and VAMP721 colocalize on motile subcellular compartments within the cell, these VAMP721-positive compartments do not significantly overlap with either Exo84b- or Exo70A1-labeled compartments. Finally, induced expression of a GDP-locked RabA2a GTPase selectively impaired plasma membrane localization of PIN2, but had no discernable effect on trafficking of two other plasma membrane–localized proteins, PIN1 and PEN3. On the other hand, while PIN2 and PIN1 trafficking to plasma membranes in an *exo84b* mutant was unaffected, trafficking of PEN3 was impaired in the *exo84b* mutant background.

Where do we go from here? The work presented in Pang et al. (2021) provides an excellent starting point for investigating the various membrane trafficking pathways connecting the TGN and the plasma membrane compartments in plants. In this paper they provide compelling evidence for at least three distinct pathways with differing molecular requirements to deliver PIN1, PIN2, and PEN3 proteins to plant plasma membranes. The work raises important questions as well. It will be necessary to unravel the apparent discrepancy between this work and earlier reports that Exo70A1 can interact with SYP121 and VAMP721 SNARE proteins (Larson et al., 2020), although it should be noted that Exo70B2, but not Exo70A1, was shown to interact with SYP121 in a subsequent study (Ortmannova et al., 2022). Also, while RabA2a does not appear to interact with Sec15b, is this a special case? Does RabA2a interact with other exocyst subunits, and do other RabA GTPases interact with Sec15b or other exocyst subunits? Finally, while Rab GTPase interactions with SNARE proteins have been demonstrated previously (Lupashin and Waters, 1997; Grote and Novick, 1999), those studies indicated that Rab GTPase–SNARE interaction was non-specific and occurred only when Rab GTPases were in a nucleotide-free conformation. This appears to be distinct from the results presented by Pang et al. (2021), which are promoted by the GTP-locked RabA2a conformation and which are specific for the SYP121–VAMP721–SNAP33 complex. Understanding how GTP-bound RabA2a promotes SNARE fusion complex formation and whether this requires other yet undetermined components should provide important insight into RabA2a-mediated trafficking of vesicles to plant plasma membranes.

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