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Plant TGN in the stress response: a compartmentalized overview

Michel Ruiz Rosquete and Georgia Drakakaki

The cellular responses to abiotic and biotic stress rely on the regulation of vesicle trafficking to ensure the correct localization of proteins specialized in sensing stress stimuli and effecting the response. Several studies have implicated the plant *trans*-Golgi network (TGN)-mediated trafficking in different types of biotic and abiotic stress responses; however, the underlying molecular mechanisms are poorly understood. Further, the identity, specialization and stress-relevant cargo transported by the TGN subcompartments involved in stress responses await more in depth characterization. This review presents TGN trafficking players implicated in stress and discusses potential avenues to understand the role of this dynamic network under such extreme circumstances.

Address
Department of Plant Sciences, University of California, Davis, CA 95616, United States

Corresponding authors:
Rosquete, Michel Ruiz (mrrruizrosquete@ucdavis.edu),
Drakakaki, Georgia (gdrakakaki@ucdavis.edu)

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The plant *trans*-Golgi network (TGN) is an intriguingly versatile organelle orchestrating the traffic of transport vesicles between Golgi, plasma membrane (PM), late endosomes and vacuoles [1–3]. We stand far from finalizing a map that functionally contextualizes the multiple players of TGN mediated trafficking under resting and stress conditions. TGN's apparent heterogeneity, intense dynamics and internal functional overlaps represent, perhaps, the greatest obstacles.

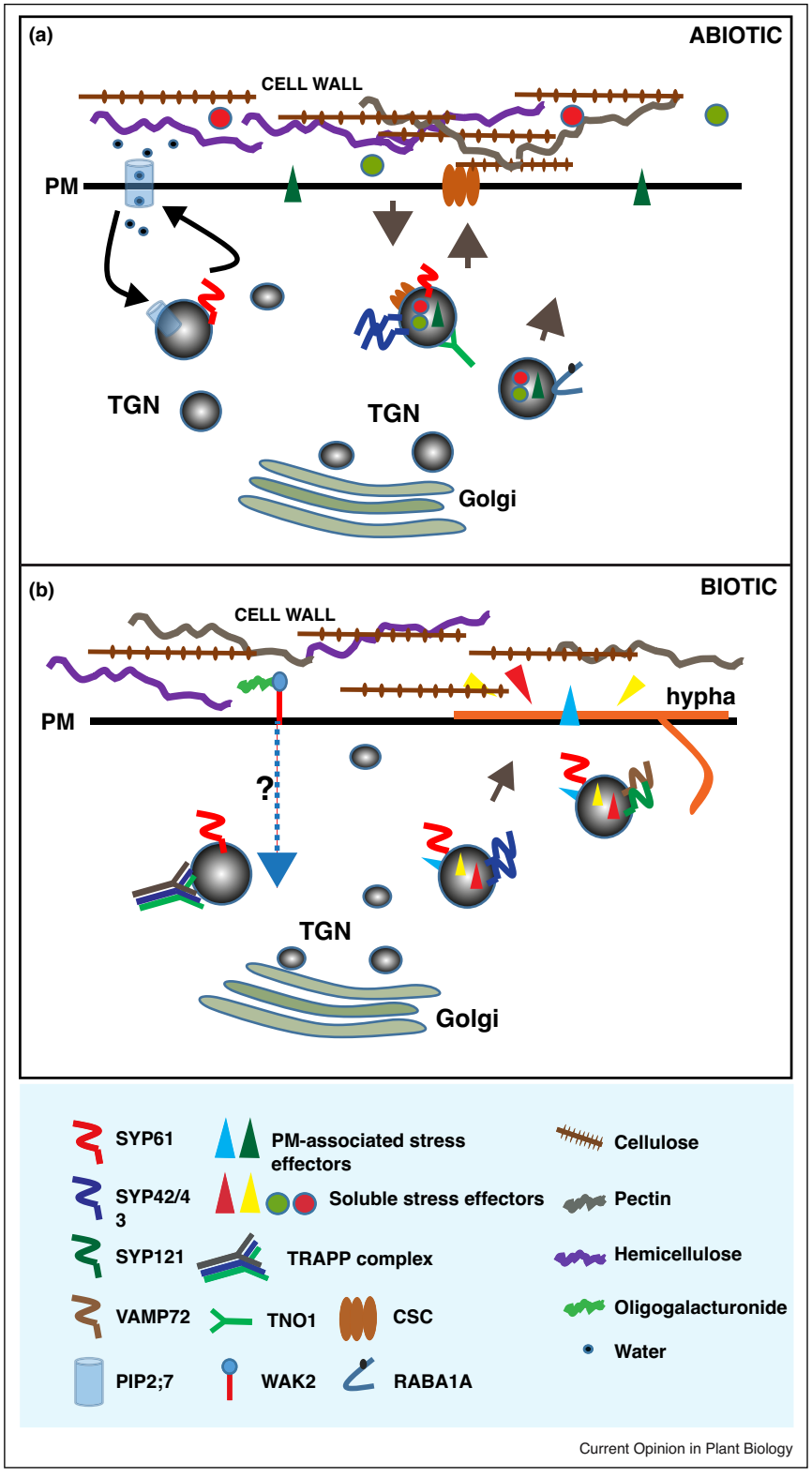
The main molecular players of TGN mediated trafficking can be functionally categorized into SNAREs, small GTPases, tethering factors and various types of regulators, such as guanine nucleotide exchange factors (GEF), GTPase activating proteins (GAP) and guanine dissociation inhibitors (GDI). SNARE proteins assemble into complexes that catalyze the fusion between a donor and a target membrane. Tethering factors, amongst other proposed functions, aid bring the membranes into close proximity for an efficient SNARE activity. Finally, the selective recruitment of tethers during membrane fusion is controlled by different small GTPases, such as those in the RAB family, whose activity is, in turn, regulated by GEFs, GAPs and GDIs. Excellent articles review the biochemistry and trafficking functions of these players [4–8]; here we focus on their involvement in TGN mediated intracellular trafficking associated with plant stress responses (Figure 1, Table 1).

SNARE proteins and RAB GTPases function in compartmentalized TGN mediated trafficking of stress response cargo

The SNARE Syntaxin of Plants 61 (SYP61) defines a TGN compartment transporting essential cargo along both the secretory and endocytic pathways [9•,10]. An Arabidopsis mutant of SYP61, *osm1* (for osmotic stress-sensitive mutant), is hypersensitive to salt and osmotic stress, demonstrating a role for the syntaxin or the SYP61 vesicle cargo in abiotic stress responses [11•] (Figure 1a). The SYP61 vesicle's proteome revealed the presence of cell wall components, such as Cellulose Synthase Complexes (CSC) [9•]. Examples of cellulose deficient mutants displaying enhanced sensitivity to salt stress include the recently characterized *she1* and *she2*, affecting Cellulose Synthase 6 and Cellulose Synthase-Interactive Protein CSI1, respectively [12]. Whether the salt phenotype of *osm1* is caused, at least partly, by an altered CSCs transport to/recycling from the PM or it is the result of altered deposition of cell wall components is yet to be determined. More direct, cargo-centered evidence for a potential, specialized role of the SYP61 compartment in post-Golgi trafficking during responses to salinity and other stresses thus warrants verification.

The TGN resident syntaxins SYP42 and SYP43 were also identified in the SYP61 proteome [9•] and the *syp42 syp43* double mutants are hypersensitive to salt and osmotic stress [13]. Interestingly, SYP42 and SYP43 have also been implicated in plant responses to both non-adapted and host-adapted fungi (Figure 1b). When inoculated with non-adapted *Erysiphe pisi*, leaves of the mutant display more secondary hyphae formation, compared to wild type plants, suggesting a role for SYP42 and SYP43 in secretion of cargo relevant to disease resistance responses. Intriguing observations were made upon inoculation of *syp42 syp43* mutants with the host-adapted *Golovinomyces*

Figure 1



Simplified illustration of potential roles for selected SYP61-associated TGN/EE players in plant abiotic **(a)** and biotic **(b)** stress responses. (a) Roles for the TGN resident syntaxins SYP61, SYP42 and SYP43, the small GTPase RABA1A and the putative tethering factor TNO1, in trafficking of salt stress response effectors have been suggested. Cellulose Synthase Complexes (CSC) are SYP61 compartment cargo and their activity at the PM is necessary not only for growth under normal conditions but also for stress tolerance. Aquaporins regulate water flow across

Table 1

TGN trafficking players involved in plant stress responses

Protein	Category	Function	Localization	Abiotic	Biotic
SYP61	Q-SNARE	membrane fusion	TGN/EE, PM [9•,10]	salt, mannitol, drought [11•]	–
SYP42/43	Q-SNARE	membrane fusion	TGN/EE [9•,13]	salt, osmotic stress [13]	*Erysiphe pisi, Golovinomyces orontii* [14]
SYP121	Q-SNARE	membrane fusion	TGN/EE, PM [9•,16]	drought [15]	*Blumeria graminis f.sp. hordei* [16]
RABA1A-D	small GTPase	regulation of vesicle trafficking	TGN/EE [20]	salt [20]	–
TNO1	long-coiled coil tethering factor	membrane tethering	TGN/EE [9•,43•]	salt, ionic, mannitol [43•]	–
ROG2	MTC (putative TRAPP complex subunit)	membrane tethering	TGN/EE [9•][a]	salt[a]	oligogalacturonide response [48]
HIT1/ AtVPS53	MTC (subunit of GARP and EARP)	membrane tethering	TGN/EE, Golgi [49]	heat, mannitol [49,50]	–
VHA-a1	V-ATPase	H^+ pump	TGN/EE [26•]	salt [38]	–
NHX5/6	antiporter	exchange H^+/Na^+ or K^+	TGN/EE, Golgi, PVC [40,41•]	salt [40,41•]	–

PVC: Prevacuolar Compartment.
[a] Rosquete, Worden, Drakakaki, unpublished results.

orontii. The striking leaf chlorosis, compared to wild type plants, hinted at a function of SYP42 and SYP43 in a pathogen-inducible and SA-dependent pathway required for chloroplast function during biotic stress [14].

SYP121 is a PM SNARE protein that also partially colocalizes with SYP61 and is present in the SYP61 proteome [9•]. Nicotiana tabacum SYP121 (SYR1) was identified in a screen for signaling factors associated with response to abscisic acid and drought [15]. Further, Arabidopsis mutants of SYP121 (PEN1) showed increased penetration by barley powdery mildew *Blumeria graminis f.sp. hordei* [16]. The specific interaction of SYP121 with TGN/EE-associated VAMP721/722 in plant cells implies that VAMP721/722 vesicles carry defense cargo [17] (Figure 1b). More recently, the interaction of SYP121 with SYP61, likely within a SNARE complex, was shown to be required for the delivery of the aquaporin PIP2;7 to the PM [18•]. Aquaporins, major regulators of water flux through the PM, play a key role in maintaining water homeostasis and balance under different environmental stress conditions (reviewed in [19]). An involvement of SYP121 in salt stress responses, through its interaction with SYP61 or a SYP61 mediated trafficking pathway, seems possible.

TGN associated Arabidopsis GTPases in the RABA1 subfamily are involved in secretory trafficking [20]. Quadruple mutants of the RABA1A–D isoforms as well as those expressing the dominant-negative mutant of RABA1B are hypersensitive to salinity [20]. A role in the salt stress response has also been demonstrated for endosomal RABF1 (ARA6) [21,22]. ARA6 has not been localized to TGN in Arabidopsis [21]; however, its homologue in *Chara australis*, CaARA6, displays TGN, PM and multivesicular endosomes localization, suggesting an involvement of the small GTPase in TGN/EE-mediated transport [23]. Although several GTPases have been identified in TGN proteomes [9•,24•,25•], more studies are necessary to verify whether they define different TGN subcompartments and how they may regulate stress responses.

The plant TGN not only operates along the secretory pathway but it also acts as an early endosome (EE) [26•,27•] and endocytic processes are activated as part of the cellular response to a variety of stresses including salinity, high extracellular boron, ammonium and iron [28–31]. The endosome-PM recycling rates of the aquaporins AtPIP1;2 and AtPIP2;1 but not their overall endosomal localization, experience a boost in root cells under salt stress, as shown with a combination of FRAP and

(Figure 1 Legend Continued) the PM, which is critical during the salt stress response. The SYP61 compartment has been implied in anterograde and retrograde trafficking of the aquaporin PIP2;7.
(b) A role for SYP42 and SYP43 in limiting the formation of fungal hyphae through a secretory function of these syntaxins has been suggested. The putative Arabidopsis TRAPP protein ROG2, depicted as part of an elusive TGN-localized TRAPP tethering complex, regulates the intracellular signaling (dashed blue arrow) triggered by the wall associated kinase receptor WAK2 upon recognition of oligogalacturonides during biotic stress. The SNAREs SYP121 and VAMP72 transiently associate with the SYP61 compartment and are involved in pathogen responses. PM: plasma membrane. TGN: trans-Golgi Network.

trafficking inhibitors [32]. Also, stress-induced, clathrin-mediated endocytosis is involved in the transport of AtPIP2;1 to the vacuole, under salinity [33]. A recent excellent review describes the roles of endomembrane trafficking in biotic stress responses [34].

TGN pH homeostasis is critical for the cellular response to stress

Luminal pH along the endomembrane system is tightly regulated by the coordinated function of vacuole-type H^+-ATPases (V-ATPases), NHX antiporters specialized in the exchange of H^+ for Na^+ or K^+ and counter anion transporters, exemplified by the TGN associated AtCLC-d (reviewed in [35,36]). Interfering with TGN luminal pH leads to secretory and recycling defects, as shown by *det3*, an Arabidopsis mutant of the cytosolic V-ATPase subunit C (VHA-C), required for V-ATPase activity at the TGN/EE. These plants display defective TGN/EE acidification, defective recycling of the brassinosteroid receptor BRI1 and reduced cellulose content [37•]. Mutants of the TGN localized H^+ pump VHA-a1 are sensitive to salt stress [38]. It is likely that the salt sensitivity of *vha-a1* arises from a disturbed trafficking of salt stress protectors, mediated by the VHA-a1 TGN/EE compartment. Interestingly, two recently characterized components of the Cellulose Synthase Complex, CC1 and CC2 (for companion of Cellulose Synthase) were proposed to 'safeguard' CSCs activity during salt stress by promoting the assembly of a 'salt-tolerant microtubules array'. In doing so, CC1/2 counteract the salt-induced depolymerization of cortical microtubules, which are necessary for CSCs presence at the PM. CC1/2 colocalized with the TGN marker VHA-a1 in this study [39•]. It would be thus interesting to explore how salt stress responses alter the dynamics and/or the cargo transported by the VHA-a1 compartment.

NHX5 and NHX6 localize to Golgi, TGN and prevacuolar compartments and are required for cell elongation, growth and vacuolar trafficking [40,41•]. Double *nhx5 nhx6* knockouts contain a more acidic TGN, as indicated by *in vivo* pH measurements, and are salt hypersensitive [40]. In line with these results, overexpression of AtNHX5 leads to enhanced salt tolerance [42]. Interestingly, gene ontology analysis of the Arabidopsis *nhx5 nhx6* transcriptome revealed an enrichment of stress-related factors, such as abscisic acid (ABA) receptors, ABA signal transducers and cell wall modifying enzymes, in comparison with wild type plants [41•], suggesting a role of TGN /EE not only in salt stress response but also in its perception.

Roles of TGN tethering factors in the stress response. Versatile TRAPPs might prove pivotal

Tethering factors facilitate vesicle fusion events upstream of SNAREs. In the subclass of long coiled-coil tethers, only the putative TNO1 (for TGN-localized SYP41-interacting protein) has been so far described in plant TGN, where it is required for efficient vacuolar trafficking [43•]. *tno1* mutants display increased sensitivity to salt and osmotic stress, and SYP61 mislocalization. It is thus plausible that the altered SYP61 trafficking observed in *tno1* results in defective transport to the PM of salt stress response effectors, such as cation transporters, a hypothesis also advanced for *osm1* mutants' salt hypersensitivity [11•,43•].

In addition to TNO1, several Arabidopsis orthologues in the TRAPP family of multisubunit tethering complexes (MTC) (reviewed in [7,8]) are enriched in the TGN proteome [9•,24•,25•]. TRAPPs have been well characterized in yeast and mammalian cells, where they mediate processes such as endoplasmic reticulum (ER)-to-Golgi traffic, Golgi-mediated trafficking and autophagy [7,8,44]. Regarding plant TRAPPs, very little is known about their functions and organization into complexes; however, their evolutionary conservation in Arabidopsis suggests their involvement in multiple plant trafficking pathways. The only plant TRAPP subunits characterized to date are AtTRS33, AtTRS120 and AtTRS130, which localize to TGN and whose corresponding mutants exhibit secretion and cytokinesis defects [45–47]. So far, no direct involvement of these plant TRAPPs in stress responses has been reported. At5g65950 is a putative Arabidopsis TRAPP overrepresented in the SYP61 proteome [9•], which was also identified in a response to oligogalacturonide (OG), a pectin oligosaccharide normally elicited as part of pathogen defense [48]. *rog2* (for response to oligogalacturonides), a mutant of this putative TRAPP, suppressed the curly leaf and stunted growth phenotypes caused by a hyperactive dominant allele of the wall associated kinase WAK2, involved in the OG response [48]. Such result implicates ROG2 in signaling during biotic stress (Figure 1b). In addition, the same mutant is hypersentitive to salinity (Rosquete, Worden and Drakakaki, unpublished results), suggesting a role of this putative TRAPP member also in abiotic stress responses. Altogether, this shows the amazing plasticity of the multisubunit tethering complexes in the regulation of plant development and plant stress responses.

TGN associated lipids. Stress response cargo and beyond

Homologues for mammalian genes encoding different subunits of the TGN localized MTC GARP exist in the Arabidopsis genome. The plasma membrane of *hit1/atvps53*, a mutant of the putative Arabidopsis orthologue GARP subunit, is unstable under heat stress, indicating a role for GARP-regulated retrograde trafficking to Golgi, via TGN/EE, in heat tolerance [49,50]. Plant cells readjust the relative abundance of saturated versus unsaturated lipids at the PM to better cope with elevated temperatures [51]. Given that fatty acids synthesis is

confined to the plastids and the endoplasmic reticulum, such readjustments most likely require transport of lipids to the PM [52]. Interestingly, a recent approach combining vesicle isolation and lipidomics revealed that the SYP61 subcompartment, unlike the RABA2A, is enriched in sphingolipids with a-hydroxylated acyl-chains of at least 24 carbon atoms [53••]. Although Wattelet-Boyer *et al.* examined the physiological relevance of the SYP61 population's distinctive lipid composition in the context of PM protein polar sorting (PIN2 auxin carrier), their findings opened avenues for the study of unexplored roles of TGN compartments in trafficking of PM lipids implicated in stress response. Equally exciting, changes in membrane lipid composition are known to trigger signaling events, which in turn are indispensable for the orchestration of stress responses. Currently, lipid-mediated signaling at the plant Golgi/TGN interphase has not been reported; however, phosphatidic acid levels and the activity of Phospholipase D have been shown to play roles in the release of secretory vesicles from the mammalian TGN [54,55]. It is tempting to speculate that the formation and budding of plant TGN vesicles transporting stress cargo are subjected to regulation by stress. It is known, for example, that Golgi-independent TGN subpopulations are highly abundant in the root differentiation zone, in contrast with the meristematic region [56]. It is possible that similarly to developmental cues, environmental factors determine changes in the relative abundance of different TGN subpopulations and these are tuned to meet specific trafficking demands during stress responses.

Cell wall modification in response to stress. A plant-specific, specialized trafficking role for TGN

The TGN/EE fulfills a specialized function in plant cells, that is, coordinating the secretion of cell wall components, including structural polysaccharides (pectins, hemicelluloses), cell wall structural proteins, cellulose synthase complexes and enzymes that modify the cell wall [57,58]. The protein cargo of the SYP61 TGN compartment includes Cellulose Synthases, Callose Synthases and cell wall modifying enzymes, and its polysaccharide cargo profile is currently being characterized (Drakakaki lab, unpublished results). Defective xyloglucan and pectin secretion are observed in mutants of the SYP61 associated, trafficking regulators ECHIDNA and the RAB GTPase-interacting proteins YIP4A and YIP4B, demonstrating the involvement of the SYP61 TGN compartment in trafficking of cell wall material [59,60]. In addition, defective recycling of CSCs to the PM and reduced cellulose content were observed in the Arabidopsis mutant *det3* (discussed above), correlating with perturbed TGN motility [37•]. Cell wall polysaccharides are modified during stress [61]. For example, in wheat, increased size of the pectic polymers rhamnogalacturonan I and II correlates with drought tolerance [62] while enhanced secretion of wall modifying beta-glucanases wass observed in a drought-sensitive cultivar [63]. Further, TGN cargo GSL5, a member of the Arabidopsis family of Callose Synthases, is implied in the deposition of callose at sites of fungal infection [9•,24•,25•,64], indicating a prominent role of the TGN in plant stress responses through the sorting and transport of cell wall components.

Dissecting the roles of TGN in the plant stress response

Tools for plant glycomics, including carbohydrate microarrays, have rapidly developed [65,66] and vesicle glycoprotein profiling starts to emerge in the mammalian field, as shown in a recent glycome analysis of extracellular vesicles derived from human stem cells [67]. Plant vesicle glycomes, such as that of the SYP61 compartment, and the availability of new probes for dynamic glycan imaging ([68], reviewed in [69]) could help shed light on a likely differential Golgi/TGN sorting and transport of polysaccharides during stress responses

Several examples of proteomic studies in crop plants, aiming to unravel the contribution of organelle-specific proteins to stress responses and covering stress stimuli as diverse as drought and heavy metals exist [reviewed in Ref. [70]]; however, TGN as an organelle has received scarce attention in those approaches likely due to its complex dynamics and nature. Vesicle isolation and proteomic analyses with focus on the plant endomembrane system and its different compartments have started to illuminate TGN populations' cargo [71]. While still emerging, their potential future use in combination with transcriptomics, lipidomics, glycomics and metabolomics techniques, offers great promise for the elucidation of TGN cargo selectively transported as part of the different plant stress responses as well as stress-related trafficking regulators.

In addition to sorting of specialized cargo, the plant response to stress includes reprogramming of TGN/EE vesicle trafficking dynamics along existing routes, illustrated by the enhanced endosome-PM recycling rates of the aquaporins AtPIP1;2 and AtPIP2;1 under salt stress [32]. Other similar dynamic readjustments likely verify in stressed cells and include fine tuning of the activity of TGN trafficking regulators but are currently uncharacterized. Recent advances, such as the generation of a FRET (Förster resonance energy transfer)-based probe for the *in vivo*, spatiotemporal monitoring of changes in the activity of a rice small GTPase in response to stress [72], are paving the way.

Mathematical and biophysical approaches that exploit high resolution 4D imaging transport data could also reveal very valuable to describe stress-triggered trafficking dynamics readjustments. Computational modelling

has proved useful to study biophysical aspects of intracellular trafficking, such as membrane remodeling [73,74]. Recently, the use of Spatio-Temporal Image Correlation Spectroscopy, a technique that measures the directed transport or flow of proteins inside living cells, in combination with computer simulations enabled the establishment of a time course of cell plate formation that included the spatial and temporal pattern of vesicle incorporation to the forming plate [75]. This, and similar approaches offer great potential for the assessment of global and pathway-specific TGN dynamic fluctuations associated with plant physiological responses, still poorly explored.

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