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Authors

Rosquete, Michel Ruiz
Worden, Natasha
Drakakaki, Georgia

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SHORT COMMUNICATION

AtTRAPPC11 is involved in TRAPPIII mediated control of post-Golgi protein trafficking

Michel Ruiz Rosquete *, Natasha Worden *, and Georgia Drakakaki

Department of Plant Sciences, University of California, Davis, CA, USA

ABSTRACT

The plant *trans*-Golgi Network/Early Endosome (TGN/EE), as an organizer of vesicle trafficking, fulfills a crucial role for plant development and adaptation. Because it coordinates the transport of cell material along different routes, it is expected that a number of TGN/EE associated factors function in the rapid organization of post-Golgi trafficking to ensure that proteins reach their destination. The roles of Transport Protein Particle (TRAPP) complexes in the regulation of plant post-Golgi trafficking start to emerge. We previously demonstrated that the plant TRAPPIII complex is involved in maintenance of TGN organization and function and has a role in endocytic trafficking mediated by the SYP61 TGN/EE compartment. Here we show that *attrappc11* mutants display accumulation of the plasma membrane resident proteins CESA6, BRI1 and PIP1;4 in aberrant intracellular compartments. This adds further insights into the functions of TRAPPIII as a regulators of post-Golgi/endosomal traffic.

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The plant SYP61 *trans*-Golgi Network/Early Endosome (TGN/EE) compartment is involved in trafficking of protein cargo between the Golgi and the plasma membrane (PM) along both the endocytic and exocytic routes.[1–3] Plant post-Golgi trafficking is tightly controlled by a myriad of factors, including multisubunit tethering complexes such as the EXOCYST, HOPS/CORVET and TRAPPs.[2,4–9] Only recently, the composition of TRAPP complexes and their roles in intracellular trafficking have been explored. So far, two TRAPP complexes, TRAPPII and TRAPPIII, have been found in plant cells.[4,5] Both associate with the TGN/EE where they likely display compartmentalized functions.[4,5,10–12] Previously, we showed that the plant TRAPPIII associates with the SYP61 TGN/EE population and that it has roles in the maintenance of the TGN/EE organization and function and the SYP61-mediated trafficking of the endocytic tracer FM4-64.[4] Fluorescently-tagged SYP61 is normally observed at TGN/EE and at the PM; however, in mutants of the TRAPPIII subunit AtTRAPPC11, a population of CFP-SYP61 displays aberrant localization to the tonoplast as confirmed using SNARF-1 vacuolar staining.[4] The latter raises the question whether an alternative pathway is activated in cells where the activity of TRAPPIII is inhibited. To investigate this, we examined the localization of three PM protein markers: Cellulose Synthase 6 (CESA6), Brassinosteroid Insensitive 1 (BRI1) and PM Intrinsic Protein 1;4 (PIP1;4) in root cells of *attrappc11* mutants. CESAs are known cargoes of the SYP61 vesicles[1] and evidence exists for SYP61-mediated trafficking of both BRI1 and PIP1;4.[3,13] In the wild type background, fluorescently-tagged versions of all three proteins displayed the expected, previously reported subcellular localization pattern. Both YFP-CESA6 and BRI1-GFP localized to the PM and TGN/EE punctae,[14,15] (Figure 1(a,c)) while mCherry-PIP1;4 localized almost exclusively to the PM[16] (Figure 1(e)). Interestingly, when expressed in the *attrappc11* background, all three proteins frequently showed aberrant localization in intracellular compartments that resemble vacuoles (Figure 1(b,d,f,g)). This is consistent with the aberrant localization of SYP61 into the tonoplast in the mutant.[4] Such findings suggest the existence of a trafficking pathway that is activated, favored or released in the absence of a functional TRAPPIII complex, resulting in the accumulation of, at least, a set of PM proteins in aberrant intracellular "vacuole-like" compartments. Future analysis is necessary to identify the nature of these aberrant compartments using endomembrane markers. Interestingly, defective delivery of PM proteins has been reported also for mutants of the plant TRAPPII.[10,11,17] The latter, together with the observed intracellular accumulation of BRI1, PIP1;4 and CESA6 in *attrappc11* mutants, indicates that both plant TRAPPs regulate the transport of PM proteins. Rerouting of PM proteins in *attrappc11* mutants may point to the existence of trafficking regulation within the TGN/EE, ensuring that PM resident proteins reach their destination, with the involvement of TRAPPIII. A role for AtTRAPPC11 and TRAPPIII in such an intriguing mechanism will be the subject of future studies.

Mutants of SYP61 are salt hypersensitive,[18] reflecting a role of the SYP61-mediated trafficking in the timely and selective delivery of PM proteins necessary for the stress response. Interestingly, a similar defect is observed in mutants of AtTRAPPC11.[4] We previously showed that AtTRAPPC11, and plausibly TRAPPIII, regulates SYP61-mediated post-Golgi trafficking.[4] Thus, it is tempting to speculate that defective

CONTACT Georgia Drakakaki gdrakakaki@ucdavis.edu Department of Plant Sciences, University of California, Davis, CA 95616, USA
*These authors have equally contributed

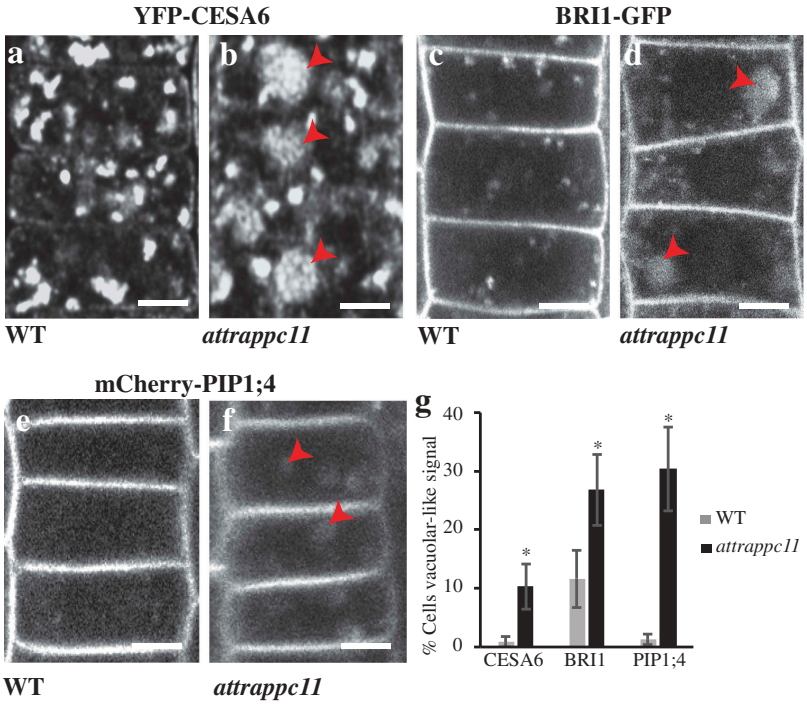

Figure 1. Localization of YFP-CESA6, BRI1-GFP and mCherry-PIP1;4 in root cells of *attrappc11* mutants. (a, c and e) In the wild type background, the plasma membrane (PM) proteins CESA6 (a), BRI1 (c) and PIP1;4 (e) are observed at the PM and Golgi/TGN in root cells. (b, d and f) In the *attrappc11* mutant background, aberrant localization (arrowheads) is frequently observed for the three proteins (b, d and f, respectively), in addition to PM and TGN/Golgi localization. (g) The graph shows the percentage of root cells where aberrant localization of YFP-CESA6, BRI1-GFP and mCherry-PIP1;4 is observed in seedlings of the WT and *attrapc11* backgrounds. n > 10 seedlings, n > 20 cells per seedling. (*) $P < .05$, Student's T test. Error bars represent standard errors. Scale bar = 5 μm.

trafficking of CESA6, BRI1, PIP1; and other PM residents critical the stress response,[19–24] evidenced by their abnormal intracellular accumulation, is responsible for the salt hypersensitivity of *attrappc11*.

Cellulose synthase subunits (CESAs) are assembled into complexes (CSCs) that are transported by post-Golgi vesicles to the PM.[25–28] In etiolated wild type hypocotyl cells, YFP-CESA6 particles associated with Golgi have the appearance of ring-shaped structures, while PM associated CESAs appear as smaller punctae[14] (Figure 2(a,c,e)). Interestingly, in *attrappc11*, the CESAs do not form the characteristic ring type but irregularly-shaped structures with a reduced diameter, when compared to the wild type (Figure 2(b,d,e)). The assembly of CSC complexes is thought to be coordinated in the Golgi apparatus by the STELLO proteins.[29] Whether the altered appearance of CESA6 in *attrappc11* mutants reflects a disrupted association of CSCs with Golgi stacks or a feedback mechanism of CSC regulation due to altered TRAPPIII function at the TGN/EE, are relevant questions to be explored.

In conclusion, the accumulation of CESA6, BRI1 and the aquaporin PIP1;4 in intracellular compartments of mutants of the plant TRAPPIII subunit AtTRAPPC11 points toward a regulatory role of the complex in TGN-mediated trafficking of PM resident proteins. Mutants of the two plant TRAPP complexes identified so far, TRAPPII and TRAPPIII, display defects in PM protein localization,[4,12] which opens exciting questions such as how instrumental TGN associated TRAPPs are for the plant endomembrane system's plasticity and to which extent their post-Golgi trafficking functions are compartmentalized.

1. Materials and methods

1.1. Plant material and growth

Arabidopsis seedlings of the Columbia ecotype (Col-0) were used in this study. The T-DNA insertional mutant line of AtTRAPPC11, SALK119008 (*attrappc11-2*) was obtained from the Arabidopsis Biological Resources Center (ABRC) (http://www.arabidopsis.org;[30]) and genetically characterized in a previous study[4]. The following Arabidopsis lines have been described previously: UBQ10pro:mCherry-PIP1;4 (WAVE 138R[16]), CESA6::YFP-CESA6[14] and BRI1::BRI1-GFP.[31] Genetic crosses of the above-mentioned PM marker lines with *attrappc11-2* mutants were established in this study. Seeds were sterilized using 30% (v/v) sodium chlorate in ethanol (absolute) with 0.06% (v/v) of Triton X-100 (Sigma-Aldrich). Seeds were plated on 0.25 Murashige and Skoog medium (1.15 g L^{-1} Murashige and Skoog minimal organics medium, 10 g L^{-1} sucrose, 5 g L^{-1} Phytagel (Sigma-Aldrich), and cold vernalized for 48 h at 4°C in the dark, after which plates were transferred to a plant growth chamber for seedling growth. Plants were grown in temperature- and photoperiod-controlled environments, set to long-day (16-h-light/8-h-dark cycle) conditions, using fluorescent light (at 100 to 150 mmol quanta photosynthetically active radiation [PAR] m–2 s^{-1}) at 22 to 24°C.

1.2. Light microscopy and image analysis

A Leica SP8 confocal microscope was used for localization studies of mCherry-PIP1;4, BRI1-GFP and YFP-CESA6. Fluorescence

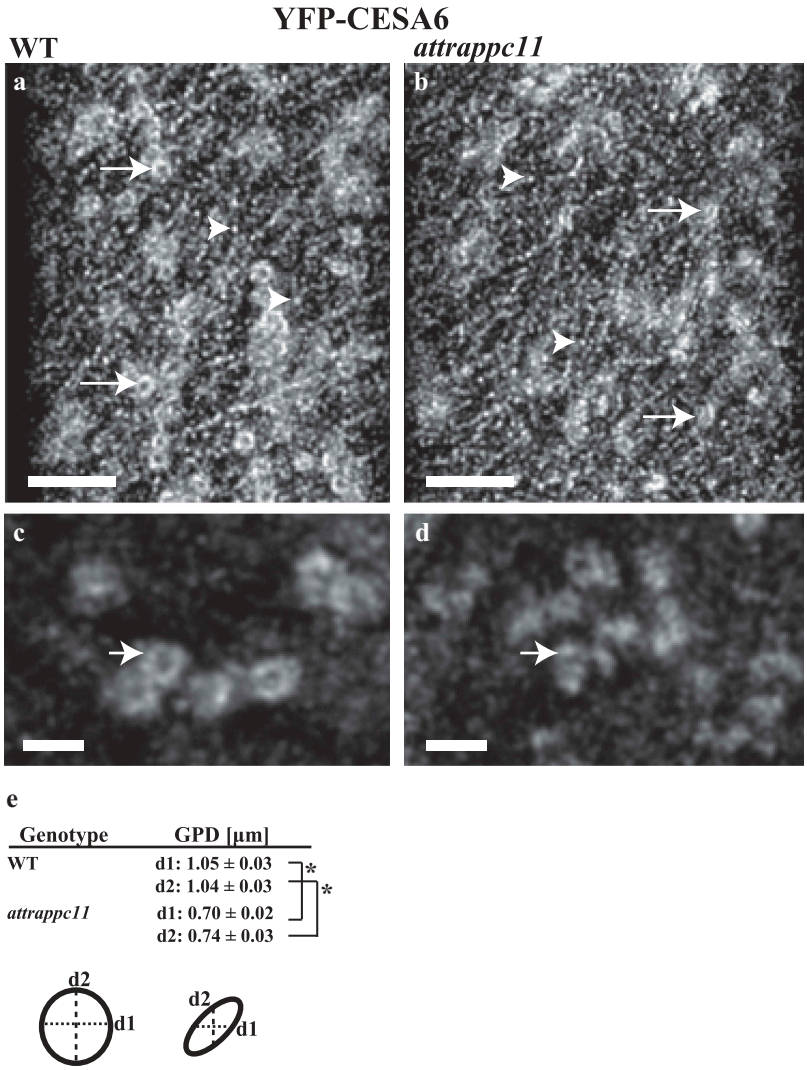

Figure 2. The Golgi/TGN association of CESA6 complexes is affected in *attrappc11* mutants. (a) In Col-0 wild type background (WT), CESA complexes (CSCs) associated with the Golgi appear as ring-like structure (arrows), while the smaller particles (arrowheads) represent CSCs at the PM. Z-projections. Scale bar= 5 µm. (b) The large ring structures are lost in the *attrappc11* mutant background. (c, d) CSCs observed using Stimulated Emission Depletion (STED) super-resolution microscopy show an irregular shape in the *attrappc11* mutant background (d) compared to WT (c). Z-projections. Scale bar= 2 µm. (e) The CSC particles arranged in ring shape structures are significantly reduced in diameter in the *attrappc11* mutants, compared with WT. (*) $P < .001$, Student's T test. GPD: Golgi/TGN particle diameter. d1: horizontal diameter. d2: vertical diameter. Error values represent standard errors. A cartoon with two hypothetical ring structured particles is provided to illustrate d1 and d2.

signals of mCherry (excitation 587 nm, emission 598 to 684 nm), GFP (excitation 488 nm, emission 493 to 549 nm) and YFP (excitation 513 nm, emission 518 to 582 nm) were collected with 63× (oil), and 100× (oil) objectives. Stimulated Emission Depletion (STED) was used for imaging of YFP-CESA6. Image analysis was performed using LAS AF lite and Image J. Data represent images from more than ten independent seedlings. The diameter of CESA6-labeled Golgi/TGN compartments was measured using the line tool of the ImageJ software.[32]

1.3. Statistical analysis

P-values were calculated with a two-tailed Student's t test (R Development Core Team, 2006[33]).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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ORCID

Michel Ruiz Rosquete http://orcid.org/0000-0002-0373-9738
Natasha Worden http://orcid.org/0000-0002-0804-2884
Georgia Drakakaki http://orcid.org/0000-0002-3949-8657

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