

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Strigolactones promote the localisation of the ABA exporter ABCG25 at the plasma membrane in root epidermal cells of *Arabidopsis thaliana*

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1926130> since 2023-09-15T09:20:01Z

Published version:

DOI:10.1093/jxb/erad298

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

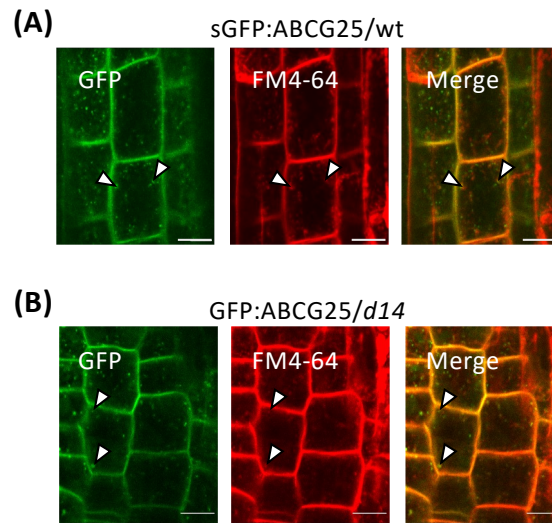
(Article begins on next page)

Supplementary Table S1. List of relevant loci, primers and their sources

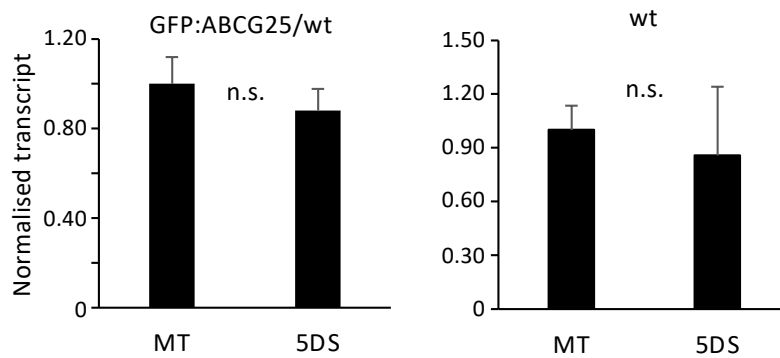
Primers for genotyping			
Primer name	Sequence 5'→3'	Arabidopsis gene ID	Source
Atd14-1-LP	AAGAATATGGCAAGTGAAC	AT3G03990	This work
Atd14-1-RP	GATGATTCCGATCATAGCG		
Atd14-1-T-DNA	TGATCCATGTAGATTTCCCGACATGAAG		
Atmax3-11-LP	TTAGGCGACACCAAAATGAAG	AT2G44990	This work
Atmax3-11-RP	TTATGAATCTAAACCGTGCG		
At-SALK-BP1	ATTTTGCCGATTTCCGGAAC		
Atabcg25-5-LP	AAGAACACGATTGGCTGATTC	AT1G71960	This work
Atabcg25-5-RP	TCGTGGAAACGTATTCATCC		
At-SALK-BP1	ATTTTGCCGATTTCCGGAAC		
GFP-F (also for qRT-PCR)	CACATGAAGCAGCAGCACTT	-	This work
GFP-R (also for qRT-PCR)	TCCTTGAAGTCGATGCCCTT		
Primers for qRT-PCR			
Primer name	Sequence 5'→3'	Arabidopsis gene ID	Source
AtTUA4-qRT-F	AACCTACACCAACCTCAACC	AT1G04820	(Chan, 2012)
AtTUA4-qRT-R	GTGGATTCTTGGGTATGGGAC		
AtUBQ10-qRT-F	GGCCTTGATAATCCCTGATGAATAAG	AT4G05320	(Brotman <i>et al.</i> , 2009)
AtUBQ10-qRT-R	AAAGAGATAACAGGAACGGAAACATAGT		
AtMAX3-qRT-F	CAACCGAGTCAAGCTTAATCCA	AT2G44990	(Booker <i>et al.</i> , 2004)
AtMAX3-qRT-R	AACGCTGATACCATTGGTGACA		
AtMAX4-qRT-F	GAAAGATACCCACTTGGCTGAATG	AT4G32810	(Hayward <i>et al.</i> , 2009)
AtMAX4-qRT-R	TGTGGAGTAGCCGTCGAAGAG		
AtRD29b-qRT-F	AAGGGGAAGAGAAAGGTGTG	AT5G52300	This work
AtRD29b-qRT-R	TCTCTCCTCCTCCAAAA		
AtNCED3-qRT-F	CCATCAAAGGAGTGTATGTGC	AT3G14440	This work
AtNCED3-qRT-R	TTAGTCTGAGTAAACCGGCAA		
AtABCG25-qRT-F	GAGACGCCATGGCTTACTTTGA	AT1G71960	(Kang <i>et al.</i> , 2010)
AtABCG35-qRT-R	AATACATGTTGTTATTCCACCGCC		

Booker J, Auldridge ME, Wills S, McCarty D, Klee H, Leyser O. 2004. MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. *Current Biology* **14**, 1232-1238.

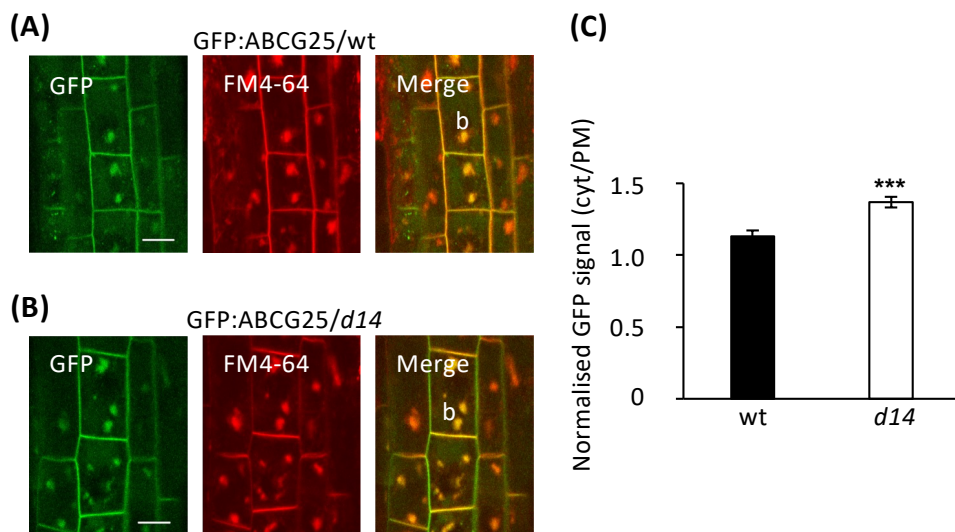
- Brotman Y, Makovitzki A, Shai Y, Chet I, Viterbo A.** 2009. Synthetic ultrashort cationic lipopeptides induce systemic plant defense responses against bacterial and fungal pathogens. *Applied and Environmental Microbiology* **75**, 5373–5379.
- Chan Z.** 2012. Expression profiling of ABA pathway transcripts indicates crosstalk between abiotic and biotic stress responses in *Arabidopsis*. *Genomics* **100**, 110-115.
- Hayward A, Stirnberg P, Beveridge C, Leyser O.** 2009. Interactions between auxin and strigolactone in shoot branching control *Plant Physiology* **151**, 400-412.
- Kang J, Hwang J-U, Lee M, Kim Y-Y, Assmann SM, Martinoia E, Lee Y.** 2010. PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proceedings of the National Academy of Sciences, USA* **107**, 2355-2360.



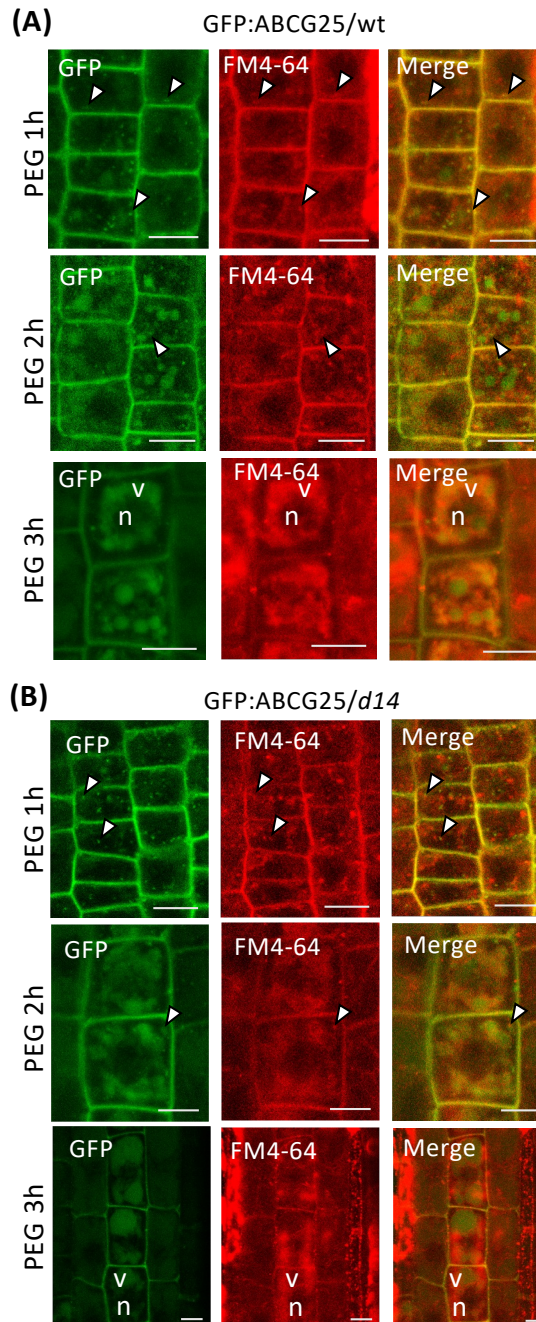
Supplementary Figure S1: Localisation of sGFP:ABCG25 and FM4-64 fluorescent signals. Five-day-old Arabidopsis seedlings expressing GFP:ABCG25 (green) in the wild-type (wt) **(A)** and *d14-1* background **(B)** were counterstained with 4 μ M FM4-64 (red) on ice and then observed at the confocal microscope 5 min after endocytosis was restarted by shifting to room temperature. The imaging of root tip epidermal cells showed GFP:ABCG25 labelling of the plasma membrane and vesicle-like compartments in the cytosol. FM4-64 colocalization at the membrane and (partially) at the vesicle structures (arrowheads) was in line with literature data (Park *et al.*, 2016) and confirmed the endosomal nature of the vesicles. Bars = 10 μ m.



Supplementary Figure S2: Effect of GR24^{5DS} treatment on *ABCG25* transcripts in the roots of the GFP:ABCG25/wt line **(A)** and in the wild type **(B)**. No significant changes were recorded 4 h after treatment with GR24^{5DS} 10 μM (5DS) with respect to mock-treated controls (MT) in 16-day-old seedlings. Relative expression levels were calculated using the geometric means of *AtTUA4* and *AtUBQ10* transcript concentrations as reference (**Supplementary Table S1**). Data represent the mean ± SE of 4 biological replicates for each condition (each replicate the pool of 20 rootlets) and time point using Student's t-test; P value < 0.05.



Supplementary Figure S3: Localization of sGFP:ABCG25 and FM4-64 fluorescent signals upon BFA incubation. Five-day-old Arabidopsis seedlings expressing GFP:ABCG25 (green) in the wild-type (wt) **(A)** and *d14-1* background **(B)** were stained on ice with 4 μ M FM4-64 (red) after 30 min incubation in the BFA solution, and then observed at the confocal microscope 5 min after endocytosis was restarted by shifting to room temperature. The localization of GFP:ABCG25 was examined in epidermal root cells, where both the GFP construct (green) and FM4-64 mark BFA bodies (b) are visible. **(C)** Quantification of the cytosol/plasma membrane (cyt/PM) fluorescence ratio after 30 min BFA incubation. Values were normalised over the corresponding mock-treated samples. GFP:ABCG25 accumulated at BFA bodies more intensely in the *d14-1* mutant than in the wild type. Bars = 10 μ m.



Supplementary figure S4: Localization of sGFP:ABCG25 and FM4-64 fluorescent signals upon PEG treatment. Five-day-old Arabidopsis seedlings expressing GFP:ABCG25 (green) in the wild-type (wt) **(A)** and *d14-1* (*d14*) **(B)** background were stained on ice with 4 μ M FM4-64 (red) after 1, 2 and 3 h incubation in a 20% PEG solution, and then observed at the confocal microscope 5 min after endocytosis was restarted by shifting to room temperature. PEG treatment caused the endocytosis of GFP:ABCG25, visible at endosomes (arrowheads) at early stages and at vacuole (v) at late stages. n = nucleus; bars = 10 μ m.