Postdoc Fellowships for non-EU researchers

Final Report

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Selection	2012
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Period covered by this report	from ..7../..9.../..2013.... to ..8../..6.../..2015....
Title	Brassinosteroid signaling requires a functional Trans-Golgi Network/Early endosome compartment

This work was accepted by Nature Plants:

Luo, Y. *, Doering, A. *, Scholl, S. *, Zhang, Y., Irani, N. G., Di Rubbo, S., Neumetzler, L., Van Houtte, I., Mylle, E., Bischoff, V., Vernhettes, S., Friml, J., Schumacher, K, Russinova, E., and Persson, S. V-ATPase mediated acidification of the TGN/EE is required for exocytosis and recycling in *Arabidopsis*. Nature Plants.

1. Objectives of the Fellowship (1/2 page)

Brassinosteroids (BRs) play diverse roles in plant growth and development, including vegetative and reproductive development, germination, senescence, and responses to various biotic and abiotic stresses. The perception of BRs at the plasma memabrane (PM) by their receptor BR INSENSITIVE1 (BRI1) and consequent downstream signaling pathway have been studied in detail during the past decade (Wang et al., 2012). It was recently shown that the majority of BR signaling is initiated by the PM pool of BRI1 (Irani et al., 2012). In plant cells, the trans-Golgi network (TGN) and the early endosome (EE) are functional subdomains of the same compartment which serves as the first compartment along the endocytic pathway. BRI1 constantly cycles between the PM and TGN/EE, but the role of TGN/EE in BR signaling is still elusive. The TGN/EE compartment represents an intracellular trafficking hub in the plant endomembrane systems, receiving and sorting proteins as well as membrane material from secretory and endocytic pathway. Unlike some plant PM receptors which require TGN/EE for signaling (Beck et al., 2012; Sharfman et al., 2011), the increased TGN/EE accumulation of the ligand-bound BRI1 caused by treatment with the vacuolar ATPase (V-ATPase) inhibitor concanamycin A (ConcA) did not influence its signaling output (Irani et al., 2012). On the other hand, the *Arabidopsis det3* mutant which is defective in the function of V-ATPase at the TGN/EE and the tonoplast, was reported to display a reduced sensitivity to BRs (Schumacher et al., 1999). In *Arabidopsis*, the V-ATPase consists of several subunits including the VHA-C subunit which is the protein product of the *DET3* gene. It had been shown that in *Arabidopsis* the V-ATPase in the TGN is essential for endocytic and secretory trafficking (Dettmer et al., 2006). Thus we speculated that BRI1-GFP membrane trafficking might play a role in the *det3* BR insensitive phenotype. The aim of this research is to elucidate the mechanisms of impaired BR signaling in *det3*mutant by focusing on TGN/EE regulated recycling and secretion. Results from this study will provide clues for how plasma mem**b**rane BR signaling is regulated through functional V-ATPase.

2. Methodology in a nutshell (1/2/ page)

Protein extraction and western blot analysis. For BES1 dephosphorylation analysis and BRI1-GFP detection, total proteins were extracted as described previoulsy. Five-day-old *Arabidopsis* seedlings were

homogenized in liquid nitrogen and extraction buffer consisting of 20 mM Tris-HCl, 150 mM NaCl, 1% SDS, 100 mM DTT, and protease inhibitor cocktail (Complete Mini EDTA Free, Roche). BES1 was detected with rabbit polyclonal anti-BES1 antibodies 10 (1:1000) and horseradish peroxidase (HRP)-conjugated anti-rabbit antibodies (1:10000; NA934, GE-Healthcare). GFP was detected with HRP-conjugated anti-GFP antibodies (1:40000, Miltenyi Biotech) and tubulin with mouse monoclonal anti-tubulin antibodies (1:20000, Sigma-Aldrich) and HRP-conjugated sheep anti-mouse antibodies (1:10000, GE-Healthcare). Positive signals were visualized with ECL plus (GE-Healthcare). Western blots were scanned and the percentage of dephosphorylated BES1 was calculated with the ImageJ Gel analyze feature. BRI1-GFP protein levels were quantified with ImageJ and normalized to the detected levels of tubulin.

Live-cell imaging. The laser-scanning confocal images (i.e. BRI1-GFP, BRI1-YFP, FM4-64 and AFCS) were acquired with a ×60 water immersion lens (NA1.2) mounted on a laser scanning confocal microscopy (Olympus Fluo View 1000), at digital zoom 3. The imaging position of etiolated hypocotyls was close to the apical hook and that of roots was kept consistently in the same region of the meristematic zone of the root tip, 10 to 15 cells above the quiescent center. For BFA body size and BFA body number analyses, four images of root epidermal cells each with a 2.5-µm Z axis distance, were taken and stacked together before quantification. GFP fluorescence was excited with a 488 nm laser line and detected between 500 nm and 550 nm. For the heat shock induction assay, pHS:BRI1-YFP/Col-0 and pHS:BRI1-YFP/*det3* were incubated at 37°C on 1/2 MS agar plates for 1 h before chasing the fluorescence signal at room temperature. Fluorescence of the yellow fluorescence protein (YFP) was excited with a 515 nm laser line and detected between 530 nm and 600 nm. For quantification, fixed ROIs were selected to measure the fluorescent intensity of both plasma membrane and intracellular space or background with ImageJ. The relative plasma membrane fluorescence was calculated by dividing the plasma membrane intensity by the intracellular or background intensity. For FM4-64, fluorescence was excited with a 514 nm laser line and detected between 592-759 nm. AFCS was visualized with 635 nm laser excitation and a spectral detection bandwidth of 655–755 nm.

AFCS and FM4-64 uptake analyses. Five 5-day-old seedlings were dipped in a 200-µl droplet of ligand. Seedlings were pulsed for 20 min and chased for 3 min or 40 min in 1/2 MS medium after three washes. Pictures were taken with an Olympus Fluo View 1000. The BRI1-GFP and AFCS signal colocalizations were quantified with the Olympus Fluo View software. FM4-64 uptake was done on 5-day-old light grown seedlings as described previously8. Plants were incubated in liquid 1/2 MS medium with 2 µM FM4-64 for 5 min, 10min , 30 min and 1 h, 2 h, 4 h. Seedlings were washed six times in 1/2 MS medium before imaging. Quantification of relative intracellular/PM fluorescence intensity was carried out on unsaturated images with ImageJ using similar method for heat shock induction assay mentioned above.

3. Results (6-8 pages)

BR signalling is impaired in *det3*.

det3 was identified based on its deetiolated seedling phenotype and shown to have reduced responses to BRs (Schumacher et al., 1999; Cabrera y Poch et al., 1993). To verify this we evaluated hypocotyl and primary root growth of dark- and light-grown *det3* seedlings, respectively, in the presence of increasing concentrations of brassinolide (BL) (Fig. 1). To avoid the previously reported growth inhibitory effect of nitrate (Brüx et al., 2008), the *det3* seedlings were grown on solid medium without salts. The hypocotyl length of the wild type plants was significantly reduced at 10 nM BL, whereas that of the *det3* mutant only at a concentration of 1 µM BL. Similar trends were observed for the root growth of the *det3* mutant. We also monitored the phosphorylation status of the transcription factor BRI1 EMS-SUPPRESSOR1 (BES1), routinely used as readout for BR signalling activation (Yin et al., 2002). In agreement with the growth assays, the dephosphorylated BES1 was significantly reduced in *det3* mutant when compared to the wild type (Fig. 1). In contrast, the double *vha-a2 vha-a3* mutant showed a slight hypersensitivity to BL and only a minor increase in dephosphorylated BES1 (Fig. 1), thus excluding the contribution of tonoplast-localized V-ATPases to the BR insensitive phenotype of *det3*. Reduced sensitivity to BR was also observed when wild type seedlings were grown in the presence of ConcA (Fig. 1). These data suggest that the decreased responses to BR in *det3* results from the impaired V-ATPase function at the TGN/EE compartment.

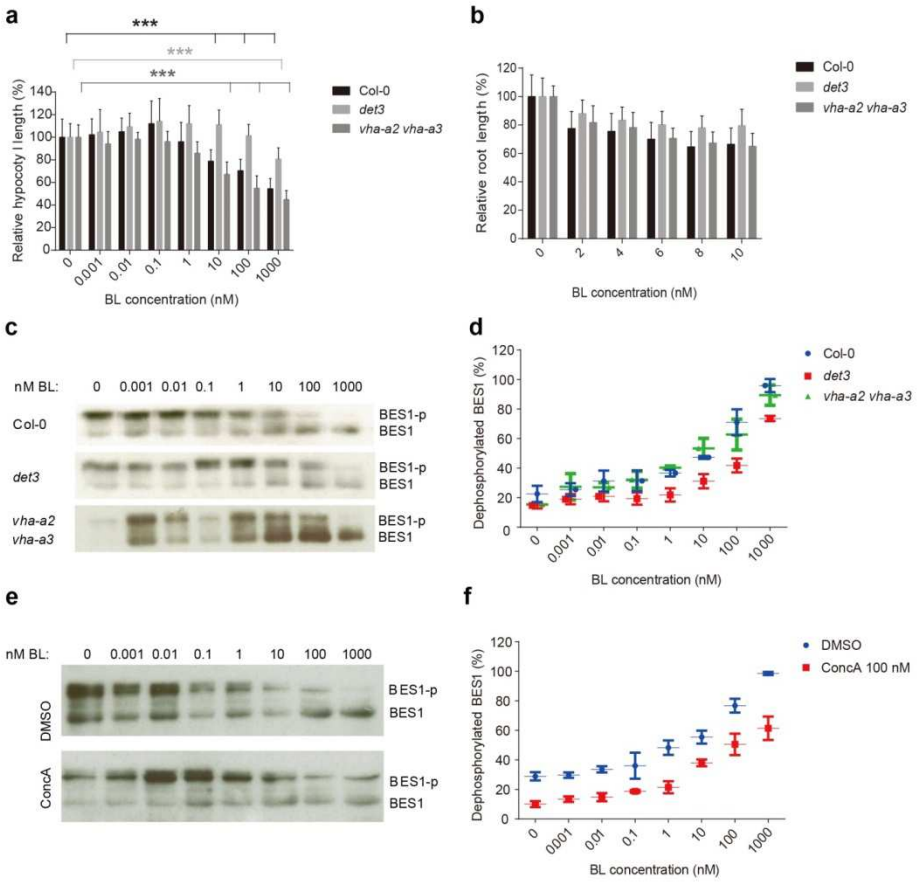

Figure 1. *det3* mutant is insensitive to BRs. a, Relative hypocotyl length of 5-day-old seedlings ($n > 25$) of the wild type (Col-0), *det3*, and *vha-a2 vha-a3* mutants grown in the dark on agar medium supplemented with different concentration of brassinolide (BL). *P*values (*t* -test), *** $P < 0.001$ relative to the respective control. Error bars indicate S.D. **b**, Relative root length of 7-day-old light grown seedlings ($n > 25$) of Col-0, *det3*, and *vha-a2 vhaa3*on agar medium supplemented with different concentration of BL. **c**, Western blot analysis of Col-0, *det3* and *vha-a2 vha-a3* seedlings grown as described in **b** using an anti-BES1antibody. **d**, Percentage of dephosphorylated BES1 relative to the total BES1 detected in **c**. **e**, Western blot analysis of Col-0 seedlings grown either on DMSO or ConcanamycinA (ConcA) (100 nM) for 5 days with and anti-BES1 antibody. **f**, Percentage of dephosphorylated BES1relative to the total BES1 detected in **e**.

Defective endocytic trafficking in *det3* does not cause BR insensitivity.

Next, we investigated if the endocytic trafficking in *det3* is impaired. BRI1-ligand complexes undergo constitutive endocytosis (Dettmer et al., 2006; Viotti et al., 2010; Geldner et al., 2007; Russinova et al., 2004), which can be visualized with the bioactive, fluorescent BR, Alexa Fluor 647-catasterone (AFCS) (Irani et al., 2012). In root epidermal cells of *det3*, AFCS strikingly accumulated in the TGN/EEs, marked by the VHA-a1-RFP (Dettmer et al., 2006), together with BRI1-GFP (Friedrichsen et al., 2000) (Fig. 2a,b), similarly to when internalized in the presence of ConcA (Irani et al., 2012). In contrast to the wild type and the *vha-a2 vha-a3* double mutant, where AFCS largely accumulated in the vacuole (data not show here), in *det3* AFCS was mainly observed in the TGN/EEs, with only a minor accumulation in the vacuole even after a chase of 40 min. Altogether these data indicate that the endocytic trafficking from TGN/EE to the vacuole is delayed in *det3*. To address the question whether internalization of plasma membrane material in general is affected in the *det3* mutant, we quantified the uptake of the fluorescent styryl dye FM4-64, routinely used as endocytic tracer in plants (Jelniková et al., 2010) in root epidermal cells. No significant differences between wild type and *det3* were observed when the FM4-64 uptake was quantified at early time points (Fig. 2c,d). Whereas incubation with FM4-64 for 4 h resulted in a clear tonoplast labelling in the wild type, only a faint tonoplast staining was observed in *det3* (Fig. 2c,d), indicating that the endocytic trafficking from TGN/EE to

the vacuole in *det3* mutant is delayed, contrary to the previously reported complete block of FM4-64 internalization in TGN/EE by ConcA (Dettmer et al., 2006).

In contrast to *det3* and to plants grown of ConcA (Fig. 1), short ConcA treatment (2 µM, 2 h) had no effect on BR responses in roots, as evaluated by the BES1 dephosphorylation assay (data not show). These data show that, although the mutation in *DET3* affects endocytic trafficking between the TGN/EE and the vacuole, this phenotype is not accountable for the reduced BR sensitivity of the mutant.

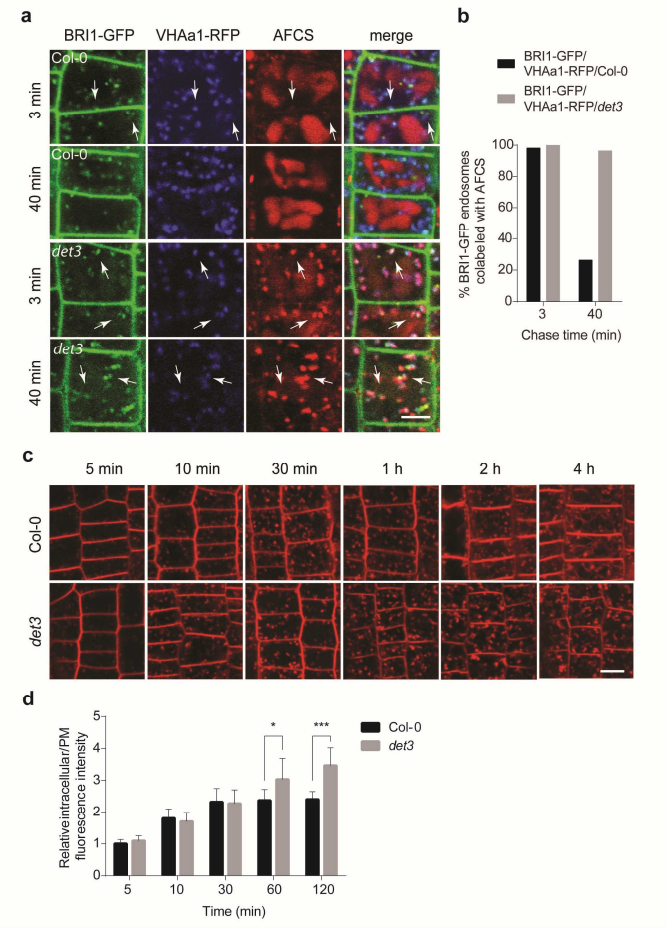

Figure 2. Endocytic trafficking from TGN/EE to the vacuole is delayed in *det3*. a, Pulse-chase AFCS uptake experiments in BRI1-GFP/VHAa1-RFP/Col-0 and BRI1-GFP/VHAa1-RFP/*det3* Arabidopsis lines. Seedlings were incubated in AFCS (20 µM) for 20 min followed by either a f 3-min or 40-min chase as indicated. **b**, Quantification of the percentage of VHAa1-RFP positive endosomes labelled by both BRI1-GFP and AFCS in **a**. **c,** FM4-64 (5 µM) uptake for 5 min, 10 min, 30 min, 1 h, 2 h, and 4 h. *Arabidopsis* root epidermal cells of 5-day-old wild type (Col-0) and *det3* seedlings were imaged immediately after washing. **d**, Maximal intracellular fluorescence intensity normalized to the maximal fluorescence intensity at the plasma membrane (PM) in root epidermal cells of wild type (Col-0) and *det3* after incubation with FM4-64 as in **c**. At least 15 cells from three roots were calculated for each time point. No significant differences were observed for 5 min, 10 min, and 30 min (*t*-test). *P* values (*t* -test), * P < 0.05, *** P < 0.001. Error bars indicate S.D. Scale bars, 5 µm.

Secretion defects of BRI1 contribute to the impaired BR signalling in *det3*.

Plasma membrane-localized BRI1 accounts for most of the BR signalling (Irani et al., 2012). To unravel the BR insensitive phenotype of the *det3* mutant, we introduced the *pBRI1::BRI1-GFP* construct (Geldner et al., 2007) into *det3*. No significant differences in *BRI1* expression and protein levels were observed between the wild type and the *det3* (data not show). However, the relative plasma membrane BRI1-GFP fluorescence in root epidermal cells was approximately 20% reduced in *det3* (Fig. 3a,b), suggesting that the BR insensitivity of the mutant might be caused by decreased BRI1 levels in the plasma membrane.

To assess whether BRI1 trafficking to the plasma membrane is affected in *det3*, we employed fluorescence recovery after photobleaching (FRAP) in root epidermal cells. The recovery of the plasma membrane BRI1-GFP fluorescence in *det3* was remarkably slower than that of the wild type (Fig. 3c,d), suggesting that the BRI1 exocytosis was impaired. As a further validation, we introduced the heat-shock inducible BRI1-YFP (Geldner et al., 2007) into *det3*, ensuring that the expression levels of *BRI1-YFP* after the heat shock were the same in wild type and *det3* (data not show) and monitored the YFP fluorescence in a time course manner after the heat shock. After a 30-min recovery, most of the BRI1-YFP signal was intracellular in *det3*, whereas a considerable amount of BRI1-YFP was already located in the plasma membrane of the wild type (Fig. 3e). Quantitative analyses after 90 min revealed that the plasma membrane BRI1-YFP was significantly lower in *det3* than that in the wild type (Fig. 3f), indicating that BRI1 secretion was partially inhibited in *det3*.

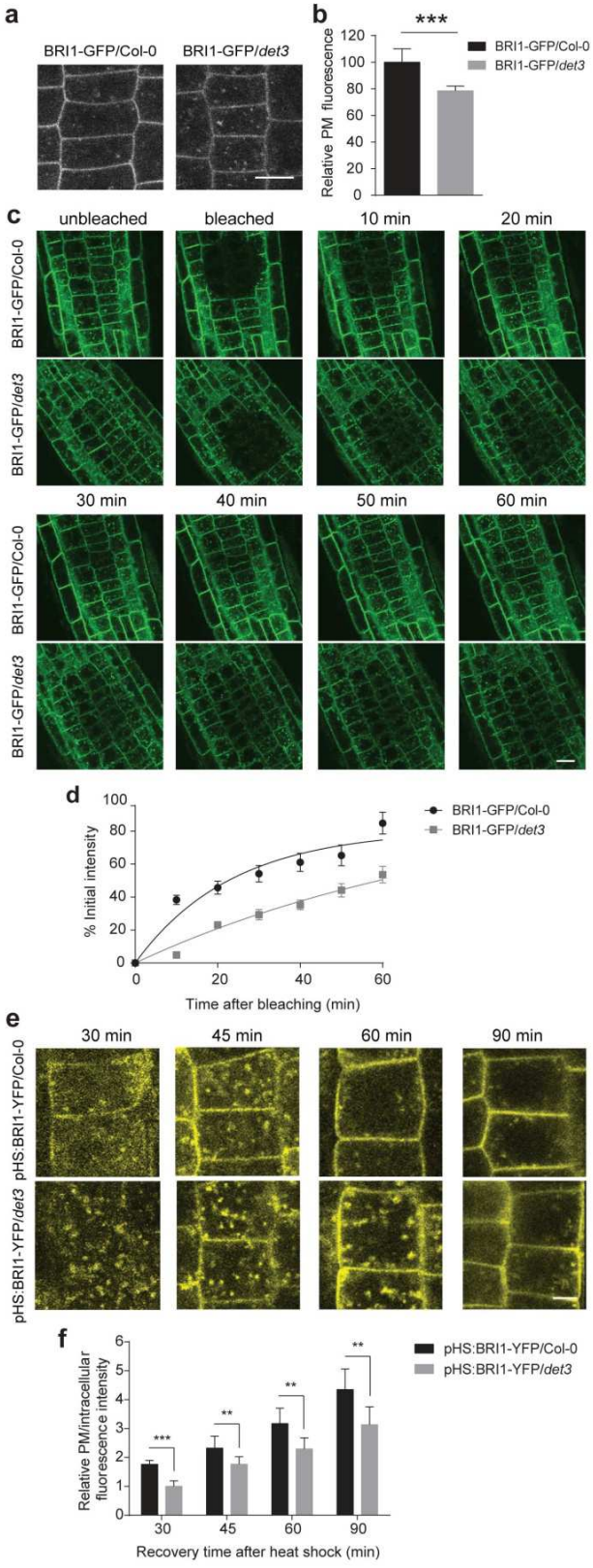

Figure 3. Secretion of BRI1 in *det3* is reduced. **a**, Confocal images of 5-day-old BRI1-GFP/Col-0 and BRI1-GFP*/det3* root epidermal cells. Scale bar, 5 μm. **b**, Quantification of relative plasma membrane (PM) fluorescence intensity of BRI1-GFP/Col-0 and BRI1-GFP*/det3* as shown in **a** (30 cells from five roots were measured). Plasma membrane fluorescence was normalized to background fluorescence for each measurement. *P* values (*t* -test), *** *P*< 0.001 relative to the respective control. **c**, FRAP analysis of BRI1-GFP/Col-0 and BRI1-GFP*/det3* plasma membrane BRI1 recovery rate. Consistent ROI was selected for individual roots, followed by photobleaching to significantly reduce the target area fluorescence. Root epidermal cell fluorescence was recorded before bleaching and at different

time points after bleaching. Scale bar, 10 µm. **d**, The fluorescence recovery was calculated on cells with totally bleached whole plasma membrane fluorescence. Plasma membrane fluorescence before and immediately after bleaching was set as 100 % and 0 %, respectively. Each recovery value was normalized to fluorescence value of the unbleached region (at least 15 cells from three roots were measured). **e**, *In vivo* analysis of heat shock-induced BRI1-YFP exocytosis. YFP signal in 5-day-old pHS:BRI1-YFP/Col-0 and pHS:BRI1-YFP/*det3* root epidermal cells chased at 30 min, 45 min, 60 min, and 90 min after 1-h 37°C induction. Scale bar, 5 µm. **f**, Relative plasma membrane to intracellular fluorescence intensity values calculated by fluorescence intensities measured using ImageJ. ROI was kept constant for each measurement (at least 15 cells from three roots were measured). Error bars indicate S.D.

BRI1 recycling is defective in *det3.*

To assess whether recycling contributes to the exocytosis defects of *det3* we performed washout experiments after BFA (Geldner et al., 2001) application (50 µM, 30 min) in the presence of cycloheximide (CHX) (50 µM) in *det3*/BRI1-GFP and wild type roots (Fig. 4a). The number of BFA body-containing cells and the BFA body sizes were scored at different time points after washing. Almost all BRI1-GFP was relocated from the BFA body after 90 min of BFA washout in wild type cells but not in *det3* cells even after 150 min (Fig. 4a,b). Quantitative analyses also revealed that the BFA compartment size decreased more slowly after BFA removal in the *det3* mutant than in the wild type (Fig. 4c).

To evaluate the contribution of recycling to the plasma membrane pool of BRI1 in *det3*, we measured the plasma membrane levels of BRI1-GFP in the presence of CHX and after a combined BFA and CHX treatment (Fig. 4d-f) and calculated the recycling ratio as the relative reduction in plasma membrane fluorescence intensity after BFA application (Fig. 4g). The CHX treatment decreased the plasma membrane BRI1 levels in both wild type and *det3* epidermal root cells with approximately 10% due to inhibited *de novo* protein synthesis. Application of BFA in the presence of CHX additionally reduced the plasma membrane BRI1 pool, thus reflecting the recycling contribution (Fig. 4d-f). Interestingly, this reduction was lower in *det3* than the wild type (recycling ratios of 4 % and 13 %, respectively; Fig. 4g).

Previously BRI1 endocytosis and degradation had been shown to be independent of its ligand (Geldner et al., 2007; Russinova et al., 2004). Consistently, application of BL in the presence of CHX had no impact on BRI1-GFP fluorescence intensity at the plasma membrane in root epidermal cells of wild type and *det3* (data not show), germinated with or without brassinazole (BRZ), a BR biosynthesis inhibitor (Asami et al., 2000). The recycling ratio was also not affected by the depletion of BRs in either genotypes (data not show). Interestingly, the BL application was able to release some constrained BRI1 from BFA bodies to the plasma membrane in wild type cells only when grown in the presence of BRZ, indicating the existence of a BFA-insensitive BRI1 recycling route (data not show). Hence, we conclude that BRI1 recycling is impaired in *det3*, thus contributing to its reduced plasma membrane pool.

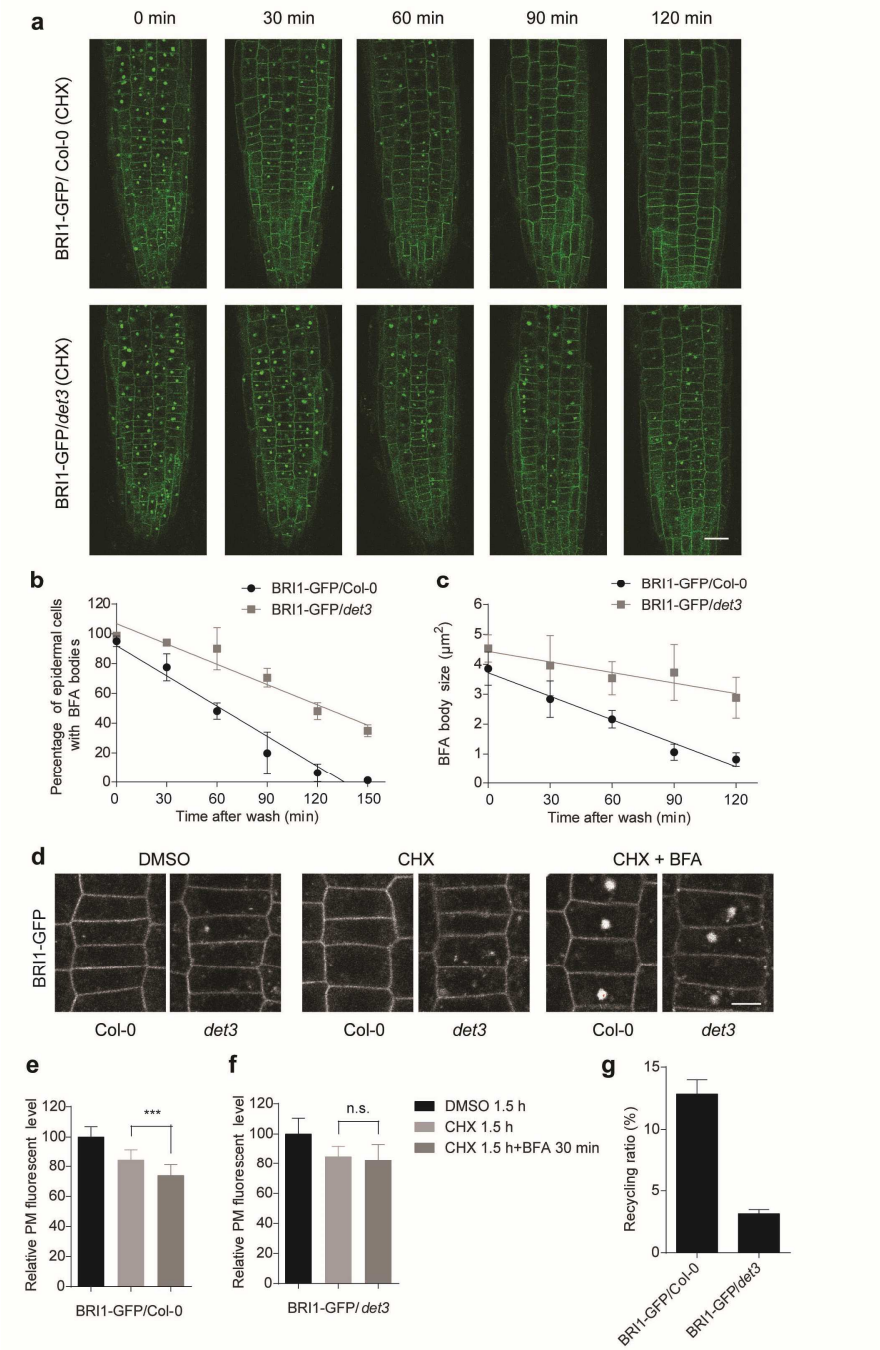

Figure 4. BRI1 recycling from TGN/EE to plasma membrane is reduced in *det3*. a, BRI1-GFP in roots of 5-day-old wild type (Col-0) and *det3* seedlings pretreated with cycloheximide (CHX) (50 μM) for 1 h, followed by treatment for 30 min with CHX (50 μM) plus brefeldinA (BFA) (50 μM). Seedlings were washed in CHX (50 μM) and mounted for imaging at 0 min, 30 min, 60 min, 90 min, and 120 min after BFA washout. Scale bar, 20 μm. **b** and **c**, BRI1-GFP relocalization from the BFA bodies to the plasma membrane was quantified by either as percentage of cells with BFA body in **b** or BFA body size in **c**. **d**, BRI1-GFP fluorescence of 5-day-old wild type (Col-0) and *det3* root epidermal cells treated separately with DMSO (1 %) and CHX (50 μM) for 1.5 h and compared with seedlings treated with BFA (50 μM) for 30 min in the presence of CHX (50 μM) for 1.5 h. Scale bar, 20 μm. **e** and **f**, Quantification of relative plasma membrane fluorescence intensities of BRI1-GFP in **e** and BRI1-GFP/*det3* in **f** of the root epidermal cells represented in **d** (for each treatment, at least 15 cells from three roots were measured). *P* values (*t* -test), ***, *P*< 0.001 relative to the respective control (CHX). **g**, Recycling of BRI1-GFP protein in BRI1-GFP/Col-0 and BRI1-GFP/*det3* (at least 15 cells from three roots were measured). Error bars indicate S.D.

pH is increased in TGN/EE of *det3*

To investigate whether impairment of V-ATPase activity would change the pH of the TGN/EE and the Golgi we fused the ratiometric pH-sensor pHusion (Gjetting et al., 2012), to the TGN/EE marker SYNTAXIN OF PLANTS61 (SYP61) that co-localizes with VHA-a1 in the TGN/EE (Sanderfoot et al., 2001) (Fig. 5a). A C-terminal fusion of ecliptic pHluorin to Syntaxin 1a has been used to determine localization in CHO cells (Yang et al., 2006) and thus, we generated stable transgenic lines expressing SYP61-pHusion and pHusion-SYP61. Fluorescence was detected in root meristem epidermal cells (Fig. 5b) and *in vivo* calibration of SYP61-pHusion, pHusion-SYP61 and free cytosolic pHusion (data not show) resulted in sigmoidal calibration curves. pH values of 5.6 for SYP61-pHusion and pH 7.2 for both pHusion-SYP61 and cytosolic pHusion were determined (Fig. 5c). Based on these values, we concluded that SYP61-pHusion has indeed been targeted to the TGN/EE lumen and was suitable for *in vivo* pH measurements in this compartment. Inhibition of V-ATPase activity by treatment with ConcA for 3 h, raised pH in the TGN/EE from 5.58 to 6.75 (Fig. 5d). In contrast, the pH in the TGN/EE of *det3*, was found to be 6.13 (Fig. 5e). To check whether V-ATPase activity also determines pH in *trans*-Golgi cisternae, we linked pHusion C-terminally to rat Sialyltransferase (ST) (Wee et al. 1998). For both, wild type and *det3*, a pH-value of 6.3 was determined (Fig. 5f). Similarly, no significant change in vacuolar pH was detected in the *det3* mutant (pH 5.82) compared to the wild type (pH 5.92), whereas in *vha-a2 vha-a3* double mutant, that lacks tonoplast V-ATPase, vacuolar pH was increased to 6.45 (Fig.5g).

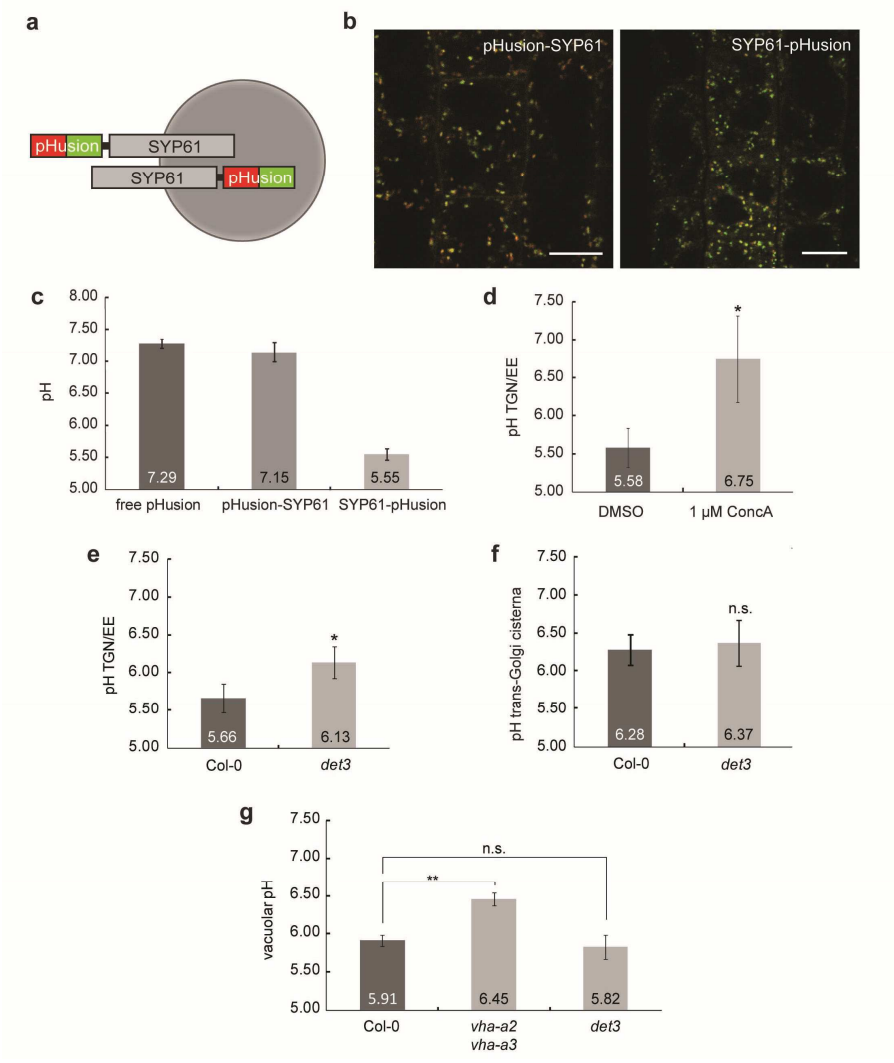

Figure 5. pH in the TGN/EE of *det3* is increased. a, The ratiometric pH sensor pHusion fused N-terminally (pHusion-SYP61) and C-terminally (SYP61-pHusion) to SYP61. **b**, Confocal images of *Arabidopsis* plants stably expressing N- and C-terminal fusions. Scale bars, 10 µm. **c**, *In vivo* pH measurements of transgenic *Arabidopsis* lines expressing pHusion in the cytosol (free pHusion), N-terminally (pHusion-SYP61) or C-terminally linked to SYP61 (SYP61-pHusion). **d**, *In vivo* pH measurements of the TGN/EE in wild type (Col-0) cells treated with 1 µM ConcA for 3 h or equal amounts of DMSO. **e**, *In vivo* pH measurements of the TGN/EE in wild type (Col-0) and *det3*. **d** and **e**, Values represent averages of three individual experiments. *P* values (*t* -test), *, $P < 0.05$. **f**, *In vivo* pH measurements of the *trans*-Golgi cisterna in wild type (Col-0) and *det3*. **c** and **g**, Values represent averages of three individual experiments. *P* values (*t* -test), not significant (n.s.). **g**, Vacuolar pH measurements in wild type (Col-0), *vha-a2 vha-a3* and *det3* epidermal and cortex cells. Values represent averages of three individual experiments *P* values (*t* -test), **, $P < 0.01$, relative to the respective controls, Col-0 for *vha-a2 vha-a3* and *vha-a2 vha-a3* for *det3*. 6-day-old seedlings were used for all measurements. Error bars indicate S.D.

The role of TGN/EE pH in endomembrane BRI1 trafficking

In yeast a functional VMA5 (VHA-C) subunit is required for the pump assembly (Ho et al., 1993). In wild type and the *vha-a2 vha-a3* mutant plants the pH of the TGN/EE is acidic (5.6), and Golgi and TGN/EE are fully functional. In *det3* mutant the pH of the TGN/EE is less acidic (6.1) but the morphology is not affected. In contrast, in plant cells treated with ConcA the pH in the TGN/EE is increased to 6.5 and is causing changes in Golgi and TGN/EE morphology. The change in pH leads to decreased Golgi and TGN/EE motility and respectively reduced secretion of BRI1. These defects result in an impaired ability of the plant to respond to BRs and to produce cellulose. Although the exact process that underpins the secretion defects remains to be determined, our data propose an explanation the observed phenotypic defects in the V-ATPase-related mutant *det3* (Fig. 6).

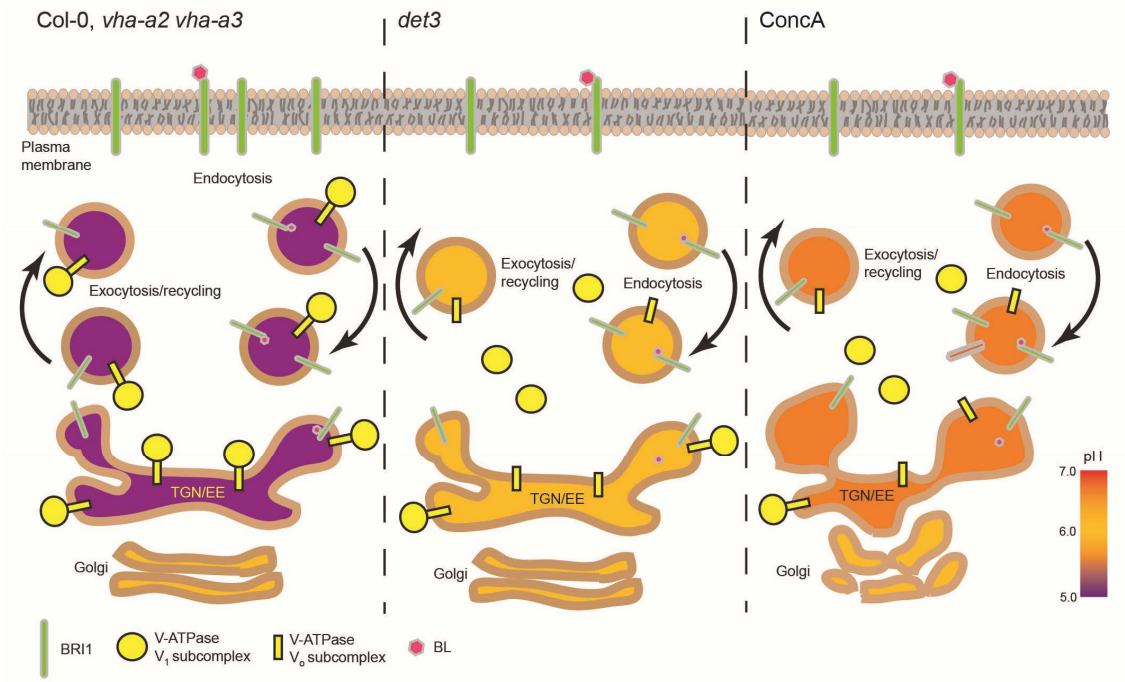

Figure 6 . Schematic model for the impact of the DET3 mutation on endomembranes trafficking in plant cells.

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4. Perspectives for future collaboration between units (1 page)

This research project has been designed to develop lasting cooperation between my former group in China and the host group in Belgium. In recent years, Dr Russinova has made much progress in understanding the molecular mechanisms of endocytosis in plants and its interaction with the signalling outputs of the cell surface receptors such as the main receptor for brassinosteroid hormones, BRI1 in Arabidopsis (Di Rubbo et al., 2013, Plant Cell; Irani et al., 2012, Nature Chemical Biology; Drakakaki et al., 2011, PNAS; Van Damme et al., 2012, PNAS).

My PhD supervisor Prof. Li-Jia Qu is a group leader in State Key Laboratory of Protein and Plant Gene Research, Peking University. His research topics including auxin signaling related endocytosis and developmental biology, and he had made considerable contributions in those field (Liu et al., 2013, Curr. Biol., 23: 993-998; Tao et al., 2012, Plant Cell, 25: 421-437; Luo et al., 2011, Plant Cell, 23: 1352-1372; Wu et al., 2011, Plant Cell, 23: 3392-3411; Guo et al., 2009, Plant Cell, 21: 3518-3534; Liu et al., 2008, Plant Cell, 20: 1538-1554). Recently he had made significant progress to the field of inositol regulated Arabidopsis embryo pattern formation. From our results, we built up the link between inositol production and auxin regulated embryo formation (Luo et al., 2011, Plant Cell, 23: 1352-1372)

Based on these common interests in the link between membrane dynamics, endocytosis and hormone signalling and this project the two laboratories will have a long-term cooperation plan in order to dissect new endosomal components and clarify their roles in regulating plant growth and development.

In the near future, we plan to expand our collaboration topics relating to BR signaling. After the end of this fellowship, we aim to collaborate on elucidating the mechanism of BR-related plant development. The bioactive compounds screened out using chemical genetics in the host lab will serve as powerful tolls for phytohormone studies in China. Meanwhile, the tools and platforms such as membrane-based yeast two hybrid, laser capture microdissection, and spinning disk confocol microscopy that developed in my lab in China will help in facilitate BR research and data accumulation in host lab in Belgium. We also plan to seek funding opportunities available in China and Europe to support the long term bidirectional cooperation activities.

5. Valorisation/Diffusion (including Publications, Conferences, Seminars, Missions abroad...

- European Network for Plant Endomembrane Research Meeting (ENPER). Short talk. 2014. Sep. Lecce, Italy. Luo, Y., et al., Brassinosteroid signaling requires a functional Trans-Golgi Network/Early endosome compartment.
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6. Skills/Added value transferred to home institution abroad (1/2 page)

This project aims to set up a long-term partnership and transfer knowledge bidirectional between the host lab in VIB and my lab in Peking University. Based on the advanced technologies and rich experiences of host lab, we had employed Arabidopsis system to study the role of TGN/EE in recycling/secretion regulated BR signalling. To achieve the goal of this project, I was trained for new techniques and experimental methods in VIB, Department of Plant Systems Biology.

After my return to China, I will train my colleagues in home institution with skills and knowledge that essential but lacked in my home lab in biochemistry, molecular biology, as well as cell biology. Such as the technical skills I learned for quantitative confocol imaging, protein related assays and chemical genetics. Before I came to Belgium, I have RA experience of training two undergraduate students for their thesis, TA experience in evolutionary biology, and one year's overseas collaborative research experience. Thus I have the ability for knowledge transferring related to the methodology, experimental design, and technology of this project.

In addition, Prof. Eugenia Russinova has the experience of collaboration with labs world-wide, and demonstrated the ability for knowledge transferring by those successful joint publications. Although she is renowned for her research in BRI1 endocytosis process, she is always open to new ideas, techniques, and seeking for new collaboration opportunity to bring vitality to her research. After all, one of the most valuable things I could bring back to China is the cooperation and connections with people in Belgium. Thus, Prof. Eugenia Russinova and I will work together to bring knowledge to my home institution after finish of the BELSPO fellowship.