Article

The *tgd5* Mutation Affects Plastid Structure and Causes Giant Lipid Droplet Formation in Trichomes of *Arabidopsis*

Kanae Matsuoka [1], Hiroko Kubotera [2], Rina Miyazaki [1], Shota Moriyama [1], Makoto T. Fujiwara [1]
and Ryuuichi D. Itoh [2],*

[1] Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, Kioicho, Tokyo 102-8554, Japan; mtf1@mac.com (M.T.F.)

[2] Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Senbaru 1, Nishihara 903-0213, Japan

* Correspondence: ryuitoh@sci.u-ryukyu.ac.jp

Abstract: Trichomes, epidermal protrusions in terrestrial plants, play diverse roles in plant defense, metabolism, and development. *Arabidopsis thaliana*, a model plant with single-celled and non-glandular trichomes, is a valuable system for studying cell differentiation in plants. However, organelle biology in *Arabidopsis* trichomes is relatively underexplored. Using light and transmission electron microscopy, we investigated the phenotypes of intracellular structures in *Arabidopsis* trichomes caused by *tgd5* mutations, which are known to disrupt lipid transfer from the endoplasmic reticulum to plastids and have a large impact on chloroplast morphology in pavement and guard cells. Significant phenotypic changes in the plastid structure were observed in *tgd5* trichome cells, including the absence of plastoglobuli, the emergence of clusters of electron-dense particles in the stroma, and the possibly cup-shaped morphology of plastids. Additionally, the *tgd5* mutations triggered the formation of giant, up to 15 μm in diameter, neutral lipid-containing droplets in the trichome cells, as revealed using histochemical staining with lipophilic dyes. These lipid droplets were substantially larger and more frequent in trichome cells than in other types of cells in *tgd5*. These findings highlight the role of TGD5 in maintaining plastid structure and implicate the unique activity of lipid metabolism in *Arabidopsis* trichomes.

Keywords: chloroplast; leaf epidermis; oil body; plastid ultrastructure; plastoglobule

1. Introduction

Trichomes are epidermal protrusions comprising a single cell or multiple cells found on the shoots of many terrestrial plants [1]. Various trichome functions have been postulated, including protecting plants against abiotic and biotic stresses, the synthesis and emission of specialized metabolites, developmental regulation, and facilitating interaction with insects [1]. Trichomes have attracted attention not only for their functional aspects but also as a model system for cell differentiation in plants. This is partly because the trichome assumes a predictable form on the plant surface; hence, any morphological changes can be easily detected, and because mutants defective in trichome development can be obtained owing to the trichomes' dispensability for plant growth under ambient laboratory conditions [2]. In particular, the molecular mechanisms underlying trichome development have been systematically investigated in the model plant *Arabidopsis thaliana*, which has single-celled trichomes with two to three branches, using various developmental mutants [3]. A detailed molecular network model that mainly focuses on the interplay of transcription factors has been proposed to explain trichome formation in *Arabidopsis*. Compared to this progress in the study of trichome cell differentiation, there has not been much research on the intracellular structures of trichome cells despite their possible importance in understanding the roles of trichomes. While intracellular structures of "glandular" trichome cells, which are the accumulation and

excretion sites of large quantities of specialized metabolites, have been well documented in relation to the accumulated products and their secretions (see [4] for a recent example), studies on the intracellular structures of "non-glandular" trichome cells that do not have a secretory mechanism but are involved in protective and defensive roles are limited [5]. This situation is similar in *Arabidopsis*, which has non-glandular trichomes, despite its widespread use as a research material for trichome differentiation.

Plastids are key to plant life, serving as the hubs for crucial processes like photosynthesis and nutrient storage. Their multifaceted functions in energy production and metabolic synthesis make them essential for plant growth and adaptation to environmental changes. We examined plastid morphology in trichome cells of the *Arabidopsis parc6* mutant using a transgene encoded, plastid-targeted cyan fluorescent protein (CFP) used in our previous study [6] because trichome plastids in *Arabidopsis* are otherwise colorless (non-pigmented). *PARC6* was originally identified as a regulator gene of division site placement in mesophyll chloroplasts [7–9]. Later, we identified *PARC6* as the causal gene for one of our plastid morphology mutants (*suba* mutants), in which chloroplasts in the pavement cells of the leaf epidermis frequently emanated one or more extremely long extension(s), known as stromules (stroma-filled tubules) [10]. We found grape-like aggregations of plastids in both the trichome and pavement cells of *parc6* (*suba2*) mutants but not in their mesophyll cells [6], implying a similarity between the regulatory mechanisms of plastid division in both trichome and pavement cells. This was expected, considering that both cell types share the same developmental origin. We also examined plastid morphology in pavement and stomatal guard cells of the other *suba* mutant, *suba1*, using plastid-targeted CFPs and transmission electron microscopy (TEM), and noticed some unique and distinctive phenotypes, including stromule hyperformation in pavement cells and the dismantling of chloroplasts in guard cells [11]. The causally defective gene in *suba1* was identified as *TGD5* [11], which encodes a subunit protein of the plastid envelope localized TGD complex [12]. It has been suggested that the TGD complex is involved in the endoplasmic reticulum (ER)-to-plastid lipid transfer, that is required to assemble thylakoid lipids [13]. These results indicate that ER-to-plastid lipid trafficking plays a critical role in maintaining plastid morphology in epidermal cells. In this context, we were interested in the morphology of trichome plastids in *tgd5*. In the present study, we observed these plastids using fluorescence microscopy and TEM. We also report on the formation and staining properties of giant spherical structures in *tgd5* trichomes, which were unexpectedly found in our observations, and discuss their possible implications.

2. Materials and Methods

2.1. Plant Materials and Cultivation

Arabidopsis thaliana (L.) Heynh. accession Columbia (Col) was used in this study. The transgenic Col line FL4-4, which expresses stroma-targeted CFPs, was used to visualize plastids using fluorescence microscopy, as previously described [11]. Hereafter, the non-mutant lines are referred to as wild-type (WT) plants. The *TGD5* knockout mutant *suba1* was obtained from an ethyl methanesulfonate-mutagenized population of FL4-4 seeds as previously described [10,11]. The T-DNA insertional *tgd5* mutant *tgd5-3* (SAIL_254_A11; Col background) [12] was obtained from the Arabidopsis Biological Resource Center (ABRC; Ohio State University, Columbus, OH, USA). For the electron microscopy examination of *suba1*, it was crossed with WT Col plants, and non-fluorescent *tgd5$^{-/-}$* offspring plants (denoted as *suba1**) which were obtained at the F_3 generation to exclude the possibility of artifacts due to the CFP expression. Seeds were germinated and grown on soil (Jiffy-7 peat pellets; Jiffy Products International BV, Zwijndrecht, The Netherlands) or Murashige–Skoog agar medium as described previously [11].

2.2. Microscopy and Lipid Staining

CFP fluorescence from plastids was observed using an inverted epifluorescence microscope IX71 (Olympus, Tokyo, Japan), equipped with a CMOS camera ORCA-flash2.8

(Hamamatsu Photonics, Hamamatsu, Japan) with previously described settings [6]. Differential interference contrast images were obtained using an IX71. Aqueous solutions of Sudan Black B (FUJIFILM Wako Pure Chemical, Osaka, Japan) and Sudan III (FUJIFILM Wako) dyes were prepared to a final concentration of 0.1% [w/v] for the lipid staining of trichome cells. Excised leaves were submerged in one of the dye solutions in a 1.5-mL microtube and incubated at room temperature (22–24 °C) for 10 min. Microscopic examinations of non-stained and stained lipid bodies were performed under bright-field optics using a BX51 microscope (Olympus, Tokyo, Japan) equipped with a CCD digital camera DP71 (Olympus) and a 40× UPlanApo objective lens (NA 0.85; Olympus). The ultrastructural analysis of the trichome plastids was performed using TEM (JEM-1400Plus; JEOL, Tokyo, Japan) at 100 kV. The leaf samples used for TEM were prepared as previously described [10].

3. Results

3.1. Morphology and Ultrastructure of Trichome Plastids in the Arabidopsis tgd5 Mutants

First, we examined the morphology of colorless plastids (or leucoplasts) in trichome cells under epifluorescence microscopy using stroma-targeted CFPs (Figure 1). In the trichome cells of *Arabidopsis* WT plants, plastids generally assumed round or ovoid shapes. However, some plastids exhibited one or more constrictions (Figure 1b), as demonstrated in our previous report [6].
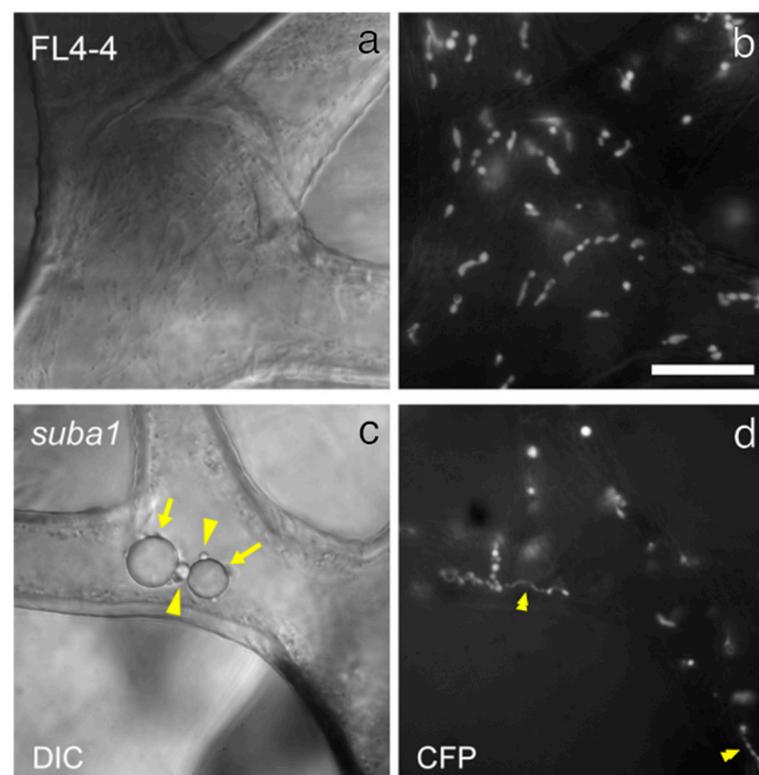

Figure 1. Morphology of plastids at the branch junction of leaf trichome cells of the *Arabidopsis suba1* mutant. Differential interference contrast (DIC) images (left column) and corresponding images of stroma-targeted cyan fluorescent protein (CFP; right column) are shown for trichome cells in the adaxial side of foliage leaves from two-week-old wild type (FL4-4) (**a**,**b**) and *suba1* (**c**,**d**) seedlings. Arrows and arrowheads in (**c**) indicate giant and small spherical structures, respectively. Double arrowheads in (**d**) indicate thin tubular plastids. Note that the image data for wild types (**a**,**b**) were retrieved from our previous study [6]. The scale bar in (**b**) indicates 20 μm and applies to all panels.

Using TEM, we found that plastoglobuli had developed and were abundant in the trichome plastids of the WT, and that the endomembrane system had also developed to some extent, although no grana-like stacks were observed (Figure 2a,b). In the trichome

cells of *suba1*, a *TGD5*-knockout mutant [11], the plastid morphology appeared relatively heterogeneous compared to that of the WT under a fluorescence microscope (Figure 1d). Some of these plastids were elongated and tube- or worm-like in shape (double arrowheads in Figure 1d). Unlike the trichome cells of *parc6/suba2* [6], no grape-like clusters of plastids were found in those cells of *suba1*. With TEM, we also found that the trichome plastids in *suba1* (*suba1**) were slightly enlarged and contained a less-developed endomembrane system compared to those in the WT (Figure 2c,d). In addition, clusters of electron-dense particles were locally present in the stroma of *suba1* trichome plastids instead of the mature plastoglobuli seen in WT trichome plastids. Interestingly, we frequently observed doughnut-shaped plastids in the TEM analysis of *suba1* trichome cells (Figure 2d), which were presumed to be cross-sections of cup-shaped plastids.
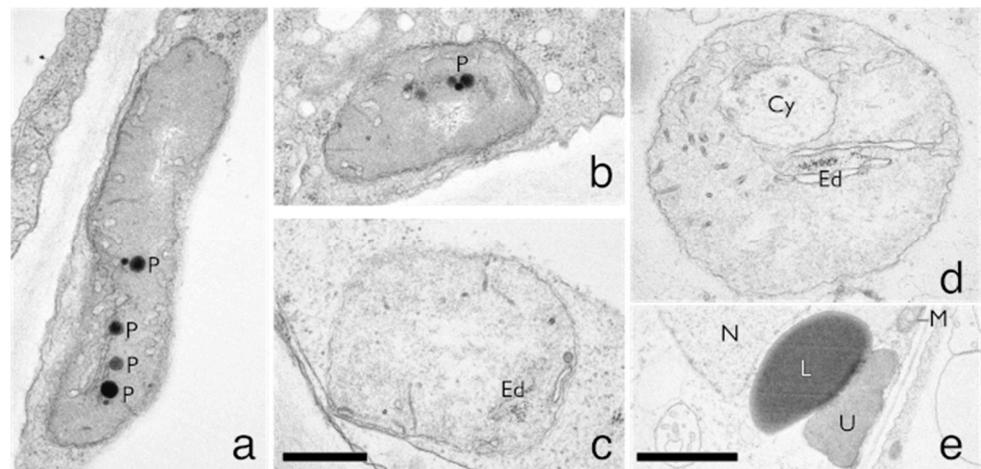

Figure 2. Ultrastructure of plastids and lipid droplets in leaf trichomes of the *Arabidopsis suba1* mutant. Trichome cells in the adaxial side of leaves from 14-day-old wild type (Columbia) (**a**,**b**) and *suba1** (the non-fluorescent line of *suba1*) (**c–e**) seedlings were observed. (**a–d**) Plastids. (**e**) Lipid droplet in an epidermal cell at the base of a trichome. P, plastoglobule; Ed, cluster of electron-dense particles; L, lipid droplet; N, cell nucleus; M, mitochondrion; U, unidentified organelle; Cy, cytosolic region surrounded by a doughnut-shaped plastid, representing the cross-section of the cup-shaped plastid. The scale bar in (**c**) depicts 500 nm for panels (**a–d**). The scale bar in (**e**) depicts 2 μm.

3.2. Formation of Giant Lipid Droplets in Trichomes of the Arabidopsis tgd5 Mutants

During the above observation of plastids, we discovered giant spherical structures in the *suba1* trichome cells (arrows in Figure 1c). These giant spheres were approximately 5–15 μm in diameter and often accompanied by tiny spheres (arrowheads in Figure 1c). Given the emphasis in this study on qualitative observations and exploratory findings, a statistical analysis of sphere sizes was not conducted, which can be considered a limitation. They are usually located at or near the branch junction and occasionally in the middle of the branches. While the exact number of spheres per cell could not be obtained, due to the three-dimensional extended structure of the trichome, one to several spheres were typically observed within one trichome cell. These spheres usually aggregated when cells contained more than one. They did not exhibit the rapid cytoplasmic streaming, or the second-by-second morphological changes as observed for trichome plastids, as reported in our previous paper [6]. We examined and compared the frequency of sphere formation in the trichome cells of the WT, *suba1*, and another *tgd5* mutant, *tgd5-3* [12] (Figure 3), and confirmed that both *tgd5* mutants had a statistically significant higher frequency of sphere formation than the WT (54% in *suba1* and 57% in *tgd5-3 versus* 0% in WT; *p* < 0.001 using Fisher's exact test; Table 1).
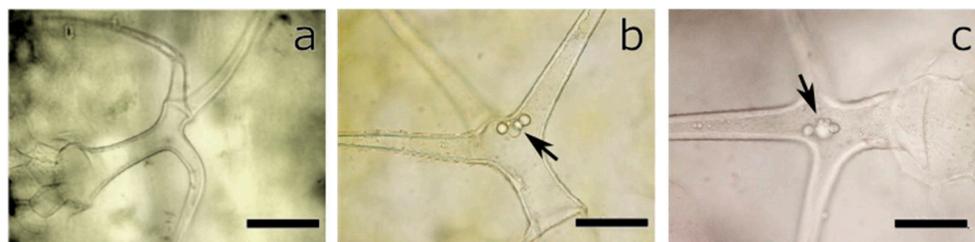
Figure 3. Bright-field images of giant spherical structures in non-stained trichome cells of *Arabidopsis tgd5* mutants. Trichome cells in the adaxial side of leaves from three-week-old wild type (Columbia) (**a**), *suba1* (**b**), and *tgd5-3* (**c**) plants were observed. Arrows in (**b**,**c**) indicate spherical structures. Scale bars depict 100 μm in (**a**) and 50 μm in (**b**,**c**).

Table 1. Occurrence of spherical structures in non-stained trichome cells of *Arabidopsis tgd5* mutants. Trichome cells in the adaxial side of foliage leaves from three-week-old plants were examined. Frequencies of sphere occurrence were compared between the wild type (Columbia) and each mutant using Fisher's exact test with Benjamini–Hochberg correction for multiple testing.

Plant Line	Number of Examined Trichome Cells (Number of Plants)	Number of Trichome Cells with at Least One Sphere	Frequency of Sphere Occurrence (%)	Significant Difference from Wild Type ($p < 0.001$)
Wild type	191 (5)	0	0	–
suba1	171 (6)	93	54	yes
tgd5-3	168 (6)	96	57	yes

We conducted histochemical assays to identify the main components of *tgd5*-specific spherical structures. We focused on lipid-staining dyes for three reasons. First, the appearance of the spherical structures resembles that of lipid droplets (LDs, also referred to as oil bodies) in various organisms. Second, TGD5 is involved in lipid synthesis and transfer [12]. Last, LD-like structures were also observed in the pavement and stomatal guard cells of *suba1* using TEM in our previous study [11], although those LD-like structures were approximately 1–2 μm in diameter and much smaller than the spheres seen in the trichome cells of *suba1* and *tgd5-3*. As a result, the spheres were stained with Sudan Black B (Figure 4) and Sudan III (Figure 5), and the staining was statistically specific to *tgd5* mutants ($p < 0.001$, Fisher's exact test; Table 2).
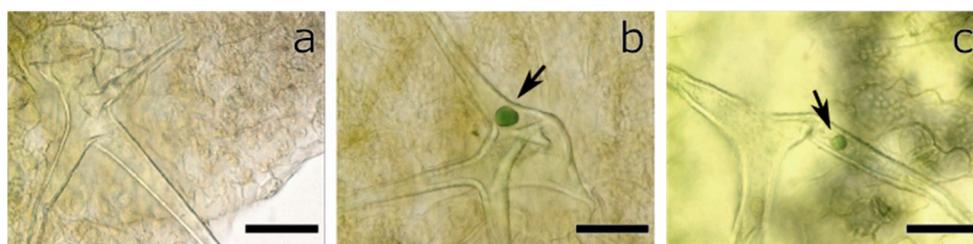

Figure 4. Sudan Black B-stained images of giant spherical structures in trichome cells of *Arabidopsis tgd5* mutants. Trichome cells in the adaxial side of leaves from four-week-old wild type (Columbia) (**a**), *suba1* (**b**), and *tgd5-3* (**c**) plants were stained and observed. Arrows in (**b**,**c**) indicate Sudan Black B-stained spherical structures. Scale bars depict 50 μm.

As both dyes have the common property of staining neutral lipids, the spheres were identified as LDs containing neutral lipids. We did not find giant LDs larger than 5 μm in diameter, corresponding to those observed with light microscopy, and in TEM images of the *suba1** trichome cells. This might be because these giant LDs were usually located at or near the branch junction of trichomes, and it is difficult to include the junction site, which is usually displaced from the axis of the main stalk, in the longitudinal ultrathin

sections (80 nm thickness) of trichomes. Nonetheless, we found enhanced development of middle-sized LDs (approximately 2–3 μm in diameter) in epidermal cells around the base of trichomes [14] in *suba1** (Figure 2e).
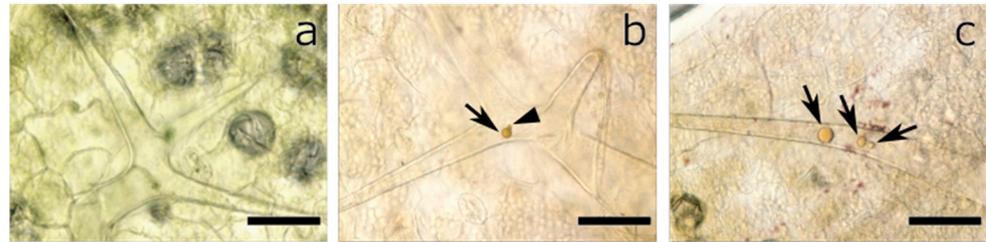

Figure 5. Sudan III-stained images of giant spherical structures in trichome cells of *Arabidopsis tgd5* mutants. Trichome cells in the adaxial side of leaves from four-week-old wild type (Columbia) (**a**), *suba1* (**b**), and *tgd5-3* (**c**) plants were stained and observed. Arrows in (**b**,**c**) indicate Sudan III-stained spherical structures. The arrowhead in (**b**) indicates a tiny spherical structure attached to the giant one. Scale bars depict 50 μm.

Table 2. Occurrence of lipid-stained spherical structures in trichome cells of *Arabidopsis tgd5* mutants. Trichome cells in the adaxial side of foliage leaves from four-week-old plants were stained with the indicated lipophilic dye and examined. Frequencies of occurrence of the positively stained sphere(s) were compared between the wild type (Columbia) and each mutant using Fisher's exact test with Benjamini–Hochberg correction for multiple testing.

Staining Dye	Plant Line	Number of Examined Trichome Cells (Number of Plants)	Number of Trichome Cells Possessing at Least One Dye-Positive Sphere	Frequency of Dye-Positive Sphere Occurrence (%)	Significant Difference from Wild Type ($p < 0.001$)
Sudan Black B	Wild type	209 (6)	0	0	–
	suba1	206 (7)	95	46	yes
	tgd5-3	200 (6)	88	44	yes
Sudan III	Wild type	212 (6)	0	0	–
	suba1	192 (6)	70	36	yes
	tgd5-3	198 (6)	72	36	yes

4. Discussion

In this study, we examined the plastid phenotypes of trichome cells in *Arabidopsis tgd5* mutants. Previously, a lack of functional TGD5 was shown to lead to the occurrence of achlorophyllous or faintly chlorophyllous stomata [15], shrinkage and fragmentation of plastids in the stomatal guard cells, and excessive formation of stromules in leaf pavement cells [11]. In contrast, *tgd5* mutations do not appear to affect mesophyll chloroplasts in terms of size, shape, division, or ultrastructure [11]. Thus, we postulated that TGD5 plays an important role in maintaining plastid morphology in epidermal (and other non-mesophyll) tissue, but not in mesophyll tissues in *Arabidopsis*. The cell type dependent differential effects of *tgd5* mutations in *Arabidopsis* may reflect the differential activity of the ER pathway (or eukaryotic pathway) of plastid lipid assembly, which requires the TGD complex; while mesophyll cells depend on both the ER and plastidial (or prokaryotic) pathways. In contrast, guard cells (and maybe other non-mesophyll cells) exclusively depend on the ER pathway [15]. Our results demonstrate that the loss of functional TGD5 affects the morphology and differentiation (as estimated from the development of the subplastidial membrane system) of plastids in trichome cells (Figures 1 and 2), implying that plastid lipid assembly in *Arabidopsis* trichomes relies heavily on the ER pathway. Nevertheless, the influence of *tgd5* mutations on plastid morphology in trichome cells differed from that in pavement and guard cells, which share the same developmental origins as trichome cells. One of the plastid phenotypes observed in the trichome cells of *tgd5* was the disappearance

of plastoglobuli and the emergence of clusters of electron-dense particles in the stroma (Figure 2b,c). Because plastoglobuli are known to contain various types of lipids, including galactolipids, diacylglycerol, and triacylglycerol [16], this phenotype may represent a lack of certain lipids and the concomitant accumulation of their reactive substrates in the plastid stroma. This is caused by the inability of ER-to-plastid lipid transfer due to the absence of the functional TGD complex. Another plastid phenotype in *tgd5* trichome cells was the appearance of probably cup-shaped plastids (Figure 2c), which has never been observed in other cell types in *tgd5* [11] and is thus considered to be a trichome-specific phenotype. While the meaning of this unique shape is not yet understood, this phenotype indicates the large impact of the TGD5 defect on trichome plastid morphology and, therefore, the crucial role of TGD5 in maintaining plastid morphology.

Unexpectedly, we also found that the loss of functional TGD5 led to the formation of neutral lipid-containing giant LDs in trichome cells (Figures 3–5), as a new phenotype of *Arabidopsis tgd5* mutants. These LDs were large enough to be readily visible without staining under a light microscope (Figure 3). Nile Red-positive LDs have also been reported to exist in the cytosol of leaf cells in *tgd5-3* in a former study [12]. Those LDs in leaf cells were much smaller in size (<0.5 μm in diameter) and less frequent (detected in 6 of 42 cells under TEM) than LDs in trichome cells of *suba1* and *tgd5-3* (approximately 5–15 μm in diameter and >50% frequency of occurrence). Pavement and guard cells in *suba1* were also found to contain LD-like structures in our previous study [11], with a size of approximately 1–2 μm in diameter. Thus, we conclude that trichome cells are a particularly prominent site of LD formation due to *tgd5* mutations. The observation that giant LDs were found only in the trichome cells of *tgd5* plants is intriguing, and the activity of neutral lipid synthesis might be high in *Arabidopsis* trichomes. To further elucidate the properties of those giant LDs, additional research including time-lapse observation and lipidomic profiling will be necessary in the future. In particular, potential dynamic behaviors of lipid droplets in trichome cells, including growth, merging, movement, and morphological changes, are worth further investigation by employing long-term time-lapse microscopy. Although the functions of trichomes in *Arabidopsis* are not well understood, a recent study demonstrated that they act as mechanosensory cells that activate a rapid immune response in plants [17]. The trichome-specific phenotypes observed in the present study provide clues for exploring the functions of *Arabidopsis* trichomes.

Author Contributions: Conceptualization, M.T.F. and R.D.I.; Investigation, K.M., H.K., R.M., S.M. and M.T.F.; Resources, M.T.F. and R.D.I.; Writing, R.D.I.; Visualization, M.T.F. and R.D.I.; Supervision, M.T.F. and R.D.I.; Funding Acquisition, M.T.F. and R.D.I. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Li, C.; Mo, Y.; Wang, N.; Xing, L.; Qu, Y.; Chen, Y.; Yuan, Z.; Ali, A.; Qi, J.; Fernández, V.; et al. The Overlooked Functions of Trichomes: Water Absorption and Metal Detoxication. *Plant Cell Environ.* **2023**, *46*, 669–687. [CrossRef] [PubMed]
2. Hülskamp, M. Trichomes. *Curr. Biol.* **2019**, *29*, R273–R274. [CrossRef] [PubMed]
3. Han, G.; Li, Y.; Yang, Z.; Wang, C.; Zhang, Y.; Wang, B. Molecular Mechanisms of Plant Trichome Development. *Front. Plant Sci.* **2022**, *13*, 910228. [CrossRef] [PubMed]

4. Livingston, S.J.; Rensing, K.H.; Page, J.E.; Samuels, A.L. A Polarized Supercell Produces Specialized Metabolites in Cannabis Trichomes. *Curr. Biol.* **2022**, *32*, 4040–4047.e4. [CrossRef] [PubMed]

5. Karabourniotis, G.; Liakopoulos, G.; Nikolopoulos, D.; Bresta, P. Protective and Defensive Roles of Non-Glandular Trichomes against Multiple Stresses: Structure–Function Coordination. *J. For. Res.* **2020**, *31*, 1–12. [CrossRef]

6. Ishikawa, H.; Yasuzawa, M.; Koike, N.; Sanjaya, A.; Moriyama, S.; Nishizawa, A.; Matsuoka, K.; Sasaki, S.; Kazama, Y.; Hayashi, Y.; et al. *Arabidopsis* PARC6 Is Critical for Plastid Morphogenesis in Pavement, Trichome, and Guard Cells in Leaf Epidermis. *Front. Plant Sci.* **2020**, *10*, 1665. [CrossRef]

7. Glynn, J.M.; Yang, Y.; Vitha, S.; Schmitz, A.J.; Hemmes, M.; Miyagishima, S.; Osteryoung, K.W. PARC6, a Novel Chloroplast Division Factor, Influences FtsZ Assembly and Is Required for Recruitment of PDV1 during Chloroplast Division in Arabidopsis. *Plant J.* **2009**, *59*, 700–711. [CrossRef]

8. Zhang, M.; Hu, Y.; Jia, J.; Li, D.; Zhang, R.; Gao, H.; He, Y. CDP1, a Novel Component of Chloroplast Division Site Positioning System in *Arabidopsis*. *Cell Res.* **2009**, *19*, 877–886. [CrossRef]

9. Ottesen, E.; Zhong, R.; Lamppa, G.K. Identification of a Chloroplast Division Mutant Coding for ARC6H, an ARC6 Homolog That Plays a Nonredundant Role. *Plant Sci.* **2010**, *178*, 114–122. [CrossRef]

10. Itoh, R.D.; Ishikawa, H.; Nakajima, K.P.; Moriyama, S.; Fujiwara, M.T. Isolation and Analysis of a Stromule-Overproducing *Arabidopsis* Mutant Suggest the Role of *PARC6* in Plastid Morphology Maintenance in the Leaf Epidermis. *Physiol. Plant.* **2018**, *162*, 479–494. [CrossRef] [PubMed]

11. Itoh, R.D.; Nakajima, K.P.; Sasaki, S.; Ishikawa, H.; Kazama, Y.; Abe, T.; Fujiwara, M.T. TGD5 Is Required for Normal Morphogenesis of Non-mesophyll Plastids, but Not Mesophyll Chloroplasts, in *Arabidopsis*. *Plant J.* **2021**, *107*, 237–255. [CrossRef] [PubMed]

12. Fan, J.; Zhai, Z.; Yan, C.; Xu, C. Arabidopsis TRIGALACTOSYLDIACYLGLYCEROL5 Interacts with TGD1, TGD2, and TGD4 to Facilitate Lipid Transfer from the Endoplasmic Reticulum to Plastids. *Plant Cell* **2015**, *27*, 2941–2955. [CrossRef] [PubMed]

13. Leterme, S.; Michaud, M. Non-Vesicular Glycerolipids Transport in Plant Cells. *Adv. Bot. Res.* **2021**, *101*, 121–189. [CrossRef]

14. Bowman, J.; Callos, J.D.; Behringer, F.J.; Vasinda, J.; Stewart, D.; Link, B.M.; Medford, J.I.; Griffith, M.; Pyke, K.A.; Marrison, J.L.; et al. Vegetative Development. In *Arabidopsis, an Atlas of Morphology and Development*; Bowman, J., Ed.; Springer: Berlin/Heidelberg, Germany, 1994; pp. 1–89. [CrossRef]

15. Negi, J.; Munemasa, S.; Song, B.; Tadakuma, R.; Fujita, M.; Azoulay-Shemer, T.; Engineer, C.B.; Kusumi, K.; Nishida, I.; Schroeder, J.I.; et al. Eukaryotic Lipid Metabolic Pathway Is Essential for Functional Chloroplasts and CO_2 and Light Responses in *Arabidopsis* Guard Cells. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 9038–9043. [CrossRef] [PubMed]

16. Shanmugabalaji, V.; Zita, W.; Collombat, J.; Kessler, F. Plastoglobules: A Hub of Lipid Metabolism in the Chloroplast. *Adv. Bot. Res.* **2021**, *101*, 91–119. [CrossRef]

17. Matsumura, M.; Nomoto, M.; Itaya, T.; Aratani, Y.; Iwamoto, M.; Matsuura, T.; Hayashi, Y.; Mori, T.; Skelly, M.J.; Yamamoto, Y.Y.; et al. Mechanosensory Trichome Cells Evoke a Mechanical Stimuli–Induced Immune Response in *Arabidopsis thaliana*. *Nat. Commun.* **2022**, *13*, 1216. [CrossRef] [PubMed]