Plant Science 207 (2013) 25–36

Contents lists available at SciVerse ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Development and disintegration of tapetum-specific lipid-accumulating organelles, elaioplasts and tapetosomes, in *Arabidopsis thaliana* and *Brassica napus*

Toshiya Suzuki[a], Sonomi Tsunekawa[a], Chie Koizuka[b], Kanta Yamamoto[a], Jun Imamura[b], Kenzo Nakamura[a,1], Sumie Ishiguro[a,*]

[a] *Department of Biological Mechanisms and Functions, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan*
[b] *Faculty of Agriculture, Tamagawa University, Machida 194-8610, Japan*

ARTICLE INFO

Article history:
Received 6 October 2012
Received in revised form 11 January 2013
Accepted 9 February 2013
Available online 1 March 2013

Keywords:
Arabidopsis thaliana
Brassica napus
Elaioplast
Pollen coat
Tapetal cell
Tapetosome

ABSTRACT

The pollen coat covering the surface of pollen grains has many important roles for pollination. In Brassicaceae plants, the pollen coat components are synthesized and temporarily accumulated in two tapetum-specific organelles, the elaioplast and the tapetosome. Although many biochemical and electron microscopic analyses have been attempted, the structure and biogenesis of these organelles have not been fully elucidated. To resolve this problem, we performed live imaging of these organelles using two markers, FIB1a-GFP and GRP17-GFP. FIB1a is an Arabidopsis fibrillin, a structural protein of elaioplast plastoglobules. In transgenic Arabidopsis, fluorescence of FIB1a-GFP appeared in young elaioplasts, in which small plastoglobules were developing. However, the fluorescence disappeared in later stages, while enlargement of plastoglobules continued. GRP17 is an Arabidopsis oleopollenin, an oleosin-like protein in tapetosomes. Fluorescence microscopy of GRP17-GFP expressed in Arabidopsis and *Brassica napus* revealed that tapetosomes do not contain oleopollenin-coated vesicles but have an outer envelope, indicating that the tapetosome structure is distinct from seed oil bodies. Visualization of GRP17-GFP also demonstrated that the tapetal cells become protoplasts and migrate into locules before pollen coat formation, and provided live imaging of the foot formation between pollen grains and stigmatic papilla cells.

1. Introduction

In many species of flowering plants, the surface of pollen grains is covered with adhesive compounds called pollenkit, tryphine, or the more general term, pollen coat. The pollen coat is rich in lipids and proteins and has various proposed functions such as adhesion to pollinators and protection from environmental stresses such as desiccation and ultraviolet irradiation [1]. The pollen coat also plays important roles in pollen–stigma interaction. In crucifer plants, for example, it contains signaling molecules involved in self-incompatibility. Furthermore, the pollen coat plays an essential role in supplying water from papilla cells to pollen grains at pollen germination [2–5].

The composition of pollen coats in *Brassica napus* and other *Brassica* species has been biochemically analyzed and reported. They contain many classes of lipids such as sterol esters, alkanes, and free fatty acids, flavonoids, and a limited number of proteins such as pollenins (see below), protein kinases, and caleosins [6–12]. These components are synthesized in tapetal cells, which surround anther locules harboring developing microspores (Supplementary Fig. S1A).

The tapetum, consisting of tapetal cells, is a specialized tissue that supplies various nutrients and structural components to developing microspores. At a later stage, the tapetal cells begin to accumulate the components of the pollen coat. In cruciferous plants, two specific organelles called elaioplasts and tapetosomes play a central role in accumulating these components [7,9,13,14]. When the microspores are almost fully developed, the tapetal cells collapse and release their contents into the locules. The released materials are then partially degraded and deposited onto the pollen surface to become the pollen coat.

The elaioplasts are a type of plastid bounded by a double lipid bilayer and filled with numerous globules, or plastoglobules. Generally, the plastoglobules are lipoprotein particles distributed in various plastids such as chloroplasts and chromoplasts and have a structure closely resembling endoplasmic reticulum

Abbreviations: CLSM, confocal laser scanning microscopy; ER, endoplasmic reticulum; GFP, green fluorescent protein; GRP, glycine-rich protein; TEM, transmission electron microscopy.

* Corresponding author. Tel.: +81 52 789 4097; fax: +81 52 789 4094.
E-mail address: guronyan@agr.nagoya-u.ac.jp (S. Ishiguro).
[1] Present address: College of Bioscience and Biotechnology, Chubu University, Kasugai 487-8501, Japan.

0168-9452/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.plantsci.2013.02.008

(ER)-derived oil bodies [15]. Their lipid contents vary with the type of plastid, but typically include carotenoids, plastoquinone, and prenylquinones [16]. However, the plastoglobules in tapetal elaioplasts are unusual and contain mainly sterol esters [7,10,12]. Recent proteomic analysis revealed that the plastoglobules in chloroplasts contain numerous enzymes of various metabolic pathways, but the most abundant is a class of structural protein, fibrillin or plastid lipid-associated protein [17,18]. The fibrillins surround globular plastoglobules and carotenoid fibrils (a specialized form of plastoglobules in chromoplasts) to stabilize them in hydrophilic moieties [15,19]. The existence of fibrillins in tapetal elaioplasts was shown by immunoblotting experiments using *B. napus* [7,10], and the gene encoding the corresponding protein in *Brassica campestris* was identified as *Bcp32* [20]. Interestingly, however, fibrillins disappear by the completion of tapetum degradation and are never detected in the pollen coat, although the sterol esters derived from the plastoglobules of elaioplasts are one of the major components of the pollen coat [7,20,21]. Among 13 genes encoding fibrillins in Arabidopsis, *FIB1a* (At4g04020; also known as *PGL35*) is the most highly homologous to *Bcp32* [15,17,18,22].

The tapetosomes are another type of lipid-accumulating organelles in tapetal cells. Biochemically isolated tapetosomes contain several classes of lipids such as triacylglycerols and alkanes, and a family of oleosin-like proteins or oleopollenins [7,10–12]. Oleosin is a structural protein covering the surface of oil bodies, and the oleosin domain works as an anchor cast into the interior lipid moiety. Thus, a structural similarity is expected between tapetosomes and oil bodies. According to the current model, tapetosomes have a complex structure composed of numerous low-density small oil droplets surrounded by oleopollenins and high-density vesicles derived from the ER cisternae [23,24].

The most abundant proteins, which account for 50–80% of the pollen coat protein in *Brassica*, are pollenins, which are derived from oleopollenins by removing their N-terminal oleosin domains. The pollenins are released into anther locules at tapetum collapse [8,11,20,24]. The best characterized oleopollenin and pollenin are *B. campestris* 45 kDa oleosin and its truncated 37 kDa fragment, respectively [20]. In amphidiploid *B. napus*, counterparts to the 45 kDa oleopollenin are 48 and 45 kDa proteins, which are encoded by the *OlnB;3*/*Bns41-2* and *OlnB;4*/*Bns41-9* genes, respectively [8,25]. Pollenins derived from these *B. napus* proteins (32–38 kDa fragments) and *B. campestris* 37 kDa fragment share the same N-terminal sequence [8,20]. Interestingly, the sizes of these proteins as estimated by SDS-PAGE are unexpectedly larger than their calculated molecular weights.

Proteomic analysis in Arabidopsis revealed that a class of glycine-rich proteins (GRPs) is a major component of the pollen coat. Because the precursors of GRP contain an oleosin-like domain at their N-terminus, they should be considered Arabidopsis oleopollenins, although no significant sequence similarities are observed in their C-terminal domains [21]. *GRP17* (At5g07530), which was originally reported as *atgrp-7* [26], *OlnB;3* [6] and *T3* [27], provide the largest and the most abundant pollenin in Arabidopsis pollen coat. A mutation in *GRP17* impairs pollen hydration and competitive fertilization, suggesting that the pollenins have important roles in pollen function [28]. It should be noted that we use the words oleopollenin and pollenin as general terms to describe the families of proteins (i.e. oleosin-like proteins and pollen coat proteins derived from the oleosin-like proteins, respectively) in both *Brassica* and Arabidopsis, though the words meant only *Brassica* proteins in previous papers.

To date, the developmental process of elaioplasts and tapetosomes has mainly been analyzed by transmission electron microscopy (TEM), and live imaging of these organelles has not previously been reported. In this study, we labeled Arabidopsis FIB1a and GRP17 with green fluorescent protein (GFP), and visualized the process of development of elaioplasts and tapetosomes in living tapetal cells. Subsequent organelle disintegration and pollen coat formation in anther locules were also observed. Furthermore, we obtained new knowledge about the structure of tapetosomes.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana (L.) Heynh. accession Col-0 and *B. napus* L. cv. Westar were used unless otherwise indicated. The Arabidopsis *grp17-1* mutant (Wassilewskija or Ws-2 background) was derived from CS11664 seed obtained from the Arabidopsis Biological Resource Center [28]. Arabidopsis seeds were sown on agar plates of Gamborg's B5 medium (Wako Pure Chemical) supplemented with 1% sucrose and grown for 2 weeks under continuous illumination at 22 °C. The plants were transplanted to vermiculite and grown under the same conditions. *B. napus* was sown on commercial nursery soil and grown under long day (16 h light/8 h dark) conditions at 20 °C.

2.2. Construction of transgenic plants

2.2.1. Plasmid construction

Sequences of the primers used in this study are summarized in Supplementary Table S1. The promoter region of *GRP17* (*GRP17p*, up to −1716 bp from the A of the initiation codon) was amplified by PCR of Arabidopsis genomic DNA using primers GRP17p-attB4-F and GRP17p-attB1-R. The promoter region of *FIB1a* (*FIB1ap*, up to −1764 bp from the A of the initiation codon) was amplified using primers Fibp-attB4-F and Fibp-attB1-R. These amplified fragments were respectively cloned into vector pDONR P4-P1R by a Gateway BP reaction (Invitrogen) to make promoter entry clones pDONR-GRP17p and pDONR-FBP1p. The coding sequences of *GRP17* and *FIB1a* were respectively amplified by RT-PCR of Arabidopsis inflorescence RNA, using primers GRP17-attB1-F and GRP17-attB2-R or Fib-attB1-F and Fib-attB2-R. These amplified fragments were respectively cloned into vector pDONR201 by a Gateway BP reaction to make cDNA entry clones pDONR-GRP17 (for *GRP17*) and pDONR-FBP1 (for *FIB1a*). Corresponding promoter and cDNA fragments were connected and transferred to the binary vector R4pGWB450 [29] by a Gateway LR reaction to generate binary plasmids pGWB-GRP17p:GRP17-GFP (for making GRP17-GFP plants) and pGWB-FBP1p:FBP1-GFP (for making FIB1a-GFP plants).

2.2.2. Plant transformation

Arabidopsis plants were transformed using a modified floral dip method [30]. Harvested seed (T1 seed) from dip-treated plants was sown on agar plates of Gamborg B5 medium supplemented with 1% sucrose, 30 mg/l kanamycin and 100 mg/l carbenicillin. Antibiotic-resistant seedlings were selected as transgenic plants. Transformation of *B. napus* plants was carried out as described previously [31].

2.3. Microscopy

2.3.1. Fluorescence microscopy

Arabidopsis anthers isolated from developing flower buds were mounted on a slide glass with a drop of water. Confocal laser scanning microscopy (CLSM) was performed using FV500 confocal laser scanning microscope (Olympus). An argon laser (488 nm) was used for the excitation of fluorescence, and light emission was detected in the range of 505–525 nm for GFP and above 560 nm for chlorophyll autofluorescence.