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研究課題名（和文）The molecular mechanism and cellular function of interaction between oil bodies and peroxisomes

研究課題名（英文）The molecular mechanism and cellular function of interaction between oil bodies and peroxisomes

研究代表者

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研究成果の概要（和文）：真核生物の細胞では、貯蔵脂肪を代謝して、成長に必要なエネルギーとなるスクロースを合成するため、オイルボディとペルオキシソームが協調的に機能すると考えられているが、その生物学的な意義は明らかでなかった。本研究では、オイルボディとペルオキシソーム間の膜付着を制御する遺伝子を発見し、脂肪分解とこれら細胞小器官どうしの相互作用について生物学的なリンクを初めて明らかにした。私たちの結果から、植物細胞は最終産物であるスクロースをシグナルとして、オイルボディとペルオキシソームの物理的相互作用を制御することにより脂肪分解ひいては脂肪酸の移動を微調整する新規のメカニズムを明らかにした。

研究成果の概要（英文）：Oil bodies and peroxisomes are the central players for lipolysis and sucrose production. Their physical interaction is expected to play an important role during lipid metabolism in three eukaryotic kingdoms. In this study, we found the genes that control membrane attachment between oil bodies and peroxisomes and provide the biological link between lipolysis and oil body-peroxisome interaction. We found that when the sucrose is limited in the seedlings, oil bodies and peroxisomes enhance the physical interaction through a membrane anchor protein PED3 on peroxisomal membrane that encodes a peroxisomal ABC transporter. During seed germination the genes involved in lipolysis in oil bodies and peroxisomes facilitate sucrose production to suppress their physical interaction. The finding reveal a novel mechanism where the cells use their downstream product, sucrose, as a signal to fine tune lipolysis by controlling oil bodies and peroxisomes interaction.

研究分野：　生物学

キーワード：　oil body　peroxisome　organelle interaction　lipoysis　sucrose

１．研究開始当初の背景

(1) Organelle interaction is occasionally observed in the cells of different organisms including yeast, mammals and plants. It involves the interaction of endomembrane system between different organelles, including ER-mitochondria, ER-peroxisome and peroxisome-oil body, and is required for various aspects of cellular functions. A great example is the interaction between mitochondria and ER through which they facilitate material transfer such as Ca2+ across membrane association site to enhance the cellular response to environmental signal in animal cells. Therefore, such organelle interaction studies have prompted the importance of understanding molecular mechanisms on organelle-organelle communication.
Even if the physiological interaction between oil bodies and peroxisomes were occasionally observed in the cells, its regulatory mechanism as well as its physiological function remains largely uncharacterized.

(2) Oil bodies and peroxisomes are essential organisms and play central roles in lipid degradation to produce sucrose during seed germination. Seed storage lipids known as triacylglycerol accumulated inside seed oil bodies is degraded to fatty acids through lipases on the oil body membrane. Fatty acid products are then translocated into peroxisomes and further degraded by peroxisomal matrix enzymes involved in the peroxisomal fatty acid beta-oxidation and glyoxylate cycle to produce sucrose. Sucrose serves as energy and carbon sources to support post-germinative growth. Defects in the biogenesis of either oil bodies or peroxisomes results in germination problem in plants, which is due to the inability of sucrose production in the tissue. Based on such an intimate relationship between oil bodies and peroxisomes, fundamental questions raised how their interactions are regulated during lipid degradation and whether their physical interactions are associated with lipid metabolism.

２．研究の目的

(1) With the purpose of understanding the molecular mechanism of oil body-peroxisome interaction, I aimed to find out the factors that regulate the organelle communication between OBs and peroxisomes. I strongly believe that this study will provide fundamental knowledge to understand regulatory mechanism and physiological function of oil body-peroxisome interaction.

(2) On the basis of identification of related components for oil body-peroxisome interaction, we aimed to characterize the physical interaction between oil bodies and peroxisome through imaging experiments and incorporate of such behavior to lipolysis throughout plant germination where these organelles are supposed to play extremely important roles. Recently, manipulation of storage oil degradation was shown to have a great impact on increasing storage oils inside seeds. With the combination of analysis of our oil body-peroxisome interaction mutants, I believe this study will provide a novel mechanism and targets for practical attempts on increasing lipid contents in crops.

３．研究の方法

(1) Oil bodies and peroxisomes were visualized in *Arabidopsis thaliana* by introducing fluorescent protein markers, OleGFP and ThiRFP that targets to oil bodies and peroxisomes, respectively, in the wild type plants. By using forward genetic screening through the microscopic observation, a mutant that showed enhanced interaction between oil bodies and peroxisomes was isolated. Genetic mapping was carried out and the gene locus that is responsible for the mutant phenotype was identified.

(2) Time course observation of oil body-peroxisome interaction was carried out in the wild type and mutant using confocal microscope during seed germination. High resolution membrane interaction between oil bodies and peroxisomes was also analyzed by electron microscope. Quantitative analysis of their interaction and storage lipid degradation assay during seed germination showed that there is a positive correlation between oil body-peroxisome interaction and storage lipid degradation. The effect of sugar was tested on the physical interaction by applying different sugars including metabolizable and unmetabolizable sugars.
In vitro isolation of oil bodies from the mutant seedlings was carried out followed by immuno blotting assay, which confirmed the existence of physical tethering between oil bodies and peroxisomes.

(3) Oil body-peroxisome interaction was analyzed using confocal microscope in other mutants that was shown to be defective in sugar production. Such reverse genetic screening led us to identify a protein that is necessary for physical interaction between oil bodies and peroxisomes in sucrose limited condition. Further characterization of this protein localization was carried out using immuno-electron microscope.

４．研究成果

(1) Identification of *sdp1* mutant with increased oil body number

The morphology of oil bodies was shown to be associated with the oil body biogenesis, lipolysis, or environmental responses of the plant. With this significance, we established transgenic Arabidopsis that expresses a chimeric gene of OLE1, which encodes oleosin the most abundantly found on oil body membrane, fused with GFP under 35S promoter (OleG) to visualized oil bodies in the living cells (Hayashi et al., 2012). We conducted a forward genetic screening from the ethyl methanesulfonate (EMS)- treated seeds to isolate mutants with altered morphology of oil bodies, and identified 2 mutants that accumulate increased number of oil bodies in the seedlings. High-resolusion mapping of these mutants revealed that the mutation in both mutants was located on At5g04040, which encodes SDP1 that is a triacylglycerol lipase located on the oil body membrane. The post germinative growth of *sdp1* mutant was severely affected due to its inability of sucrose production and exogenous sucrose supplementation to *sdp1* restored the seedling growth to the wild type. Quantification of triacylglycerol, a main storage lipid form in the seeds, during germination indicated that *sdp1* was indeed defective in storage lipid degradation.

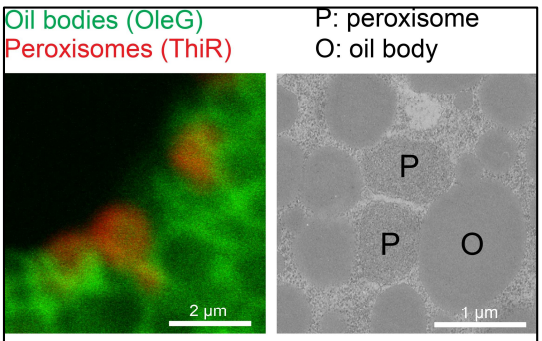

Figure 1. Interaction of oil bodies and peroxisome in Arabidopsis.

(2) Positive correlation between storage lipid degradation and oil body-peroxisome interaction

To understand the correlation between lipid degradation and dynamic behavior of oil bodies and peroxisomes, we observed both organelles in the wild type and *sdp1*. Peroxisomes were visualized in the wild type and *sdp1-6*, in which the oil body marker OleG was expressed, by a stable expression of a chimeric gene of RFP fused with N-terminus of peroxisomal targeting signal 2 (ThiR) that is derived from 3-ketoacyl-CoA thiolase in Arabidopsis. In wild type cotyledon cells, 100% of peroxisomes were attached with oil bodies at 2-day-post germination (DPG) when seedlings were grown without sucrose (Figure 1). Membrane attachment was observed with electron microscope. Peroxisomes started to separate from oil bodies at 3DPG and more peroxisomes were separated from oil bodies following germination. We tested the triacylglycerol degradation and found that the degradation happened rapidly during 2-3DPG.

Interestingly, the sucrose application on the medium of wild type seedlings during germination significantly reduced oil body-peroxisomes to less than 70%. Comparison analysis showed that the interaction in the presence of sucrose was reduced throughout germination compared to the one in the absence of sucrose. To our surprise, the interaction in the presence of sucrose jumped to 80% at 3DPG, from 70% at 2DPG, and gradually dropped afterward. The storage lipid degradation assay showed that the degradation of triacylglycerol in the presence of sucrose was significantly delayed compared to that in the absence of sucrose. Interestingly, the rapid oil body degradation started between 2 to 3 days, during which the increasing interaction with between oil bodies and peroxisomes were observed. This quantitative observation of oil body and peroxisome indicated that positive correlation between oil body-peroxisome interaction and lipid degradation during seed germination.

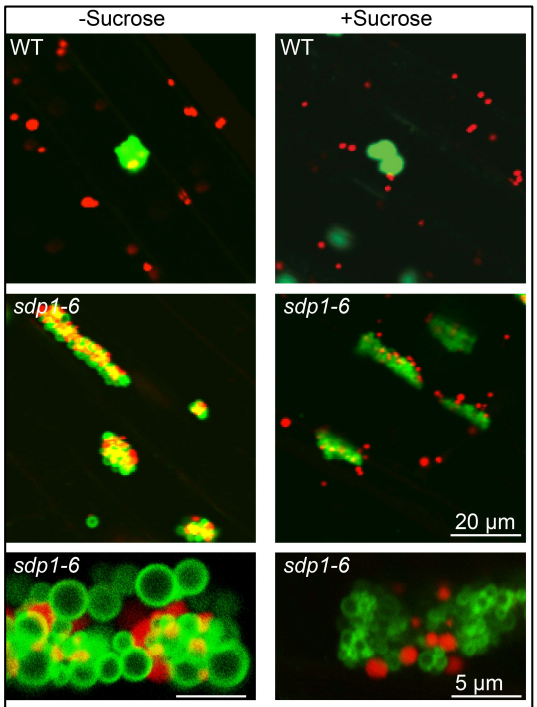

Figure 2. Enhanced physical interaction between oil bodies and peroxisomes in sdp1 and sucrose suppresses the interaction.

(3) Sucrose produced through storage lipid degradation by SDP1 suppresses physical interaction between oil bodies and peroxisomes

In the hypocotyl of the 3-day-old wild type seedlings, most of peroxisomes were located in the cytosol without physical contact with oil bodies (Figure 2). The separation of peroxisomes is almost completed in the hypocotyl at this stage

and the sucrose did not confer significant effect on their interaction (Figure 2). In contrast to the wild type, early 100% peroxisomes were attached to oil bodies forming oil body-peroxisome aggregates in *sdp1* that are grown without sucrose (Figure 2). Interestingly, exogenous supplementation of sucrose greatly reduced the attachment (Figure 2). Sucrose did not affect the morphology of oil bodies in *sdp1*, suggesting its specific role on oil body-peroxisome attachment. Although some peroxisomes were still clustered to oil bodies in *sdp1* grown with sucrose, the membrane interaction between oil bodies and peroxisomes was less observed compared to the seedlings grown without sucrose (Figure 2), indicating that sucrose suppresses membrane attachment between oil bodies and peroxisomes. In vitro isolation of oil bodies from *sdp1* mutant showed that more peroxisomes were co-isolated with oil bodies from the seedlings grown without sucrose than those from seedlings grown with sucrose, indicating that the existence of physical tethering between oil bodies and peroxisome.

Actin filaments drive the movements of various organelles, including peroxisomes in plants. Question raised whether actin filament is involved in the sucrose-induced dissociation of peroxisomes from oil bodies. Based on the inhibitor test for actin filament, I found that sucrose induced separation of peroxisomes from oil bodies occurs in the actin filament dependent manner. Taken together, these results suggest that the sucrose produced through lipolysis by SPD1 generates a negative signal for physical attachment between oil bodies and peroxisomes.

(4) PED3 is required for physical interaction between oil bodies and peroxisomes

Peroxisomal mutants, which are impaired in organelle biogenesis of peroxisomes or oxidation of fatty acids, cause blockage in lipid breakdown in all eukaryotic cells. Therefore, I analyzed two peroxisomal mutants *ped1* and *ped3* to test whether there are similar links between lipolysis and oil body-peroxisome attachment as *sdp1* (Figure 3). PED3, which shows homology to human adrenoleukodystrophy (ALD) proteins, encodes an ATP-binding cassette transporter on the peroxisomal membrane and transfers fatty acids into peroxisomes. PED1 is an enzyme encoding 3-ketoacyl CoA thiolase that catalyzes the last step of peroxisomal fatty acid β-oxidation inside peroxisomes. I found strikingly similar phenomena between *ped1* and *sdp1* mutants in terms of the oil body-peroxisome interaction and the negative regulation of sucrose (Figure 3). On the other hand, the *ped3* showed reduced oil body-peroxisome attachment compared to *sdp1* and *ped1* (Figure 3). Under low sucrose condition, many peroxiosmes remained unattached with oil bodies in *ped3*. In addition, the peroxisomes in proximity to the oil bodies remained largely untouched to oil bodies, similar to those grown in the presence of sufficient sucrose. The reduced attachment between oil bodies and peroxisomes despite of the lack of sucrose production in *ped3* indicates that PED3 is important for the oil body-peroxisome tethering under low sucrose condition.

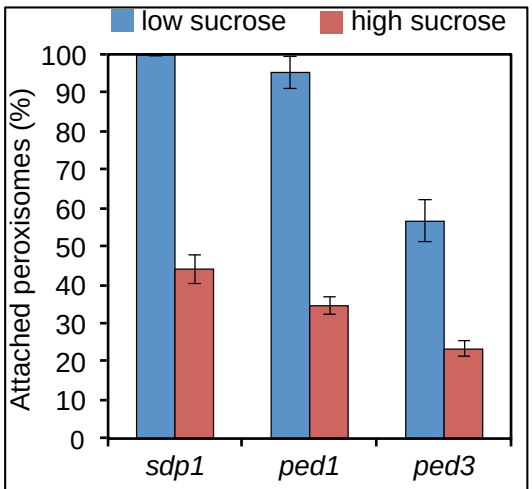

Figure 3. PED3 is required for physical interaction between oil bodies and peroxisomes under low sucrose condition.

(5) PED3 localization at the membrane interaction site between peroxisomes and oil bodies

We previously found that the enlarged peroxisomes in *ped1* contain small vesicle-like structures, which are invaginated from membrane interaction site between oil bodies and peroxisomes. Serial sections of microscopy revealed that they are enclosed by membranes that are derived from peroxisomal membranes and connected with each other forming elongated and tubular structures within peroxisomes. Although their roles and identify were not clear yet, these internal membrane structures load either storage lipids or fatty acids from oil bodies. Interestingly, PED3 was more predominantly located on internal membranes than peroxisomal membranes. This is comparable to PEX14, another peroxisomal membrane protein that has a role as a peroxisomal protein import machinery, that was predominantly located on peroxisomal membranes. These results suggest that PED3 is associated with membrane invagination of peroxisomes at oil body-peroxisome attachment site. It was reported that fatty acyl-CoA synthatase (ACS) was also located on the internal membranes of peroxisomes in ped1. ACS encodes a peroxisomal membrane protein, which activates fatty acids into fatty acyl-CoAs and thus provides substrate for peroxisomal β-oxidation.

The presence of PED3 and ACS and the accumulation of their fatty acid substrates cargo in these internal membrane structures of ped1 suggest that they are probably associated with lipid degradation process. Therefore, it may represent at least a part of fatty acids delivery mechanism from oil bodies to peroxisomes.

(6) In summary, our data concluded a model as shown in Figure 4, which involves PED3 as an anchor protein and integrates lipid metabolism pathway. Additional factors that could function as physical anchoring for oil bodies and peroxisomes (i and ii) and sucrose induced signal transduction for suppressing the interaction (iii) are included. We found that SDP1 and PED1/KAT2 and most likely other genes involved in lipolysis suppress physical interaction between oil bodies and peroxisomes by generating sucrose as negative feedback signals. This study reveals a novel mechanism where the plant cells utilize sucrose-dependent signal in fine-tuning lipolysis through physical interaction between oil bodies and peroxisomes to regulate cellular sucrose homeostasis. Modulating the anchor protein of peroxisomes and oil bodies might have a potential contribution for the strategy on improving oil production. Our study thus increases the potential targets on the future application and biological approach on such attempts to increasing plant yield of oils.

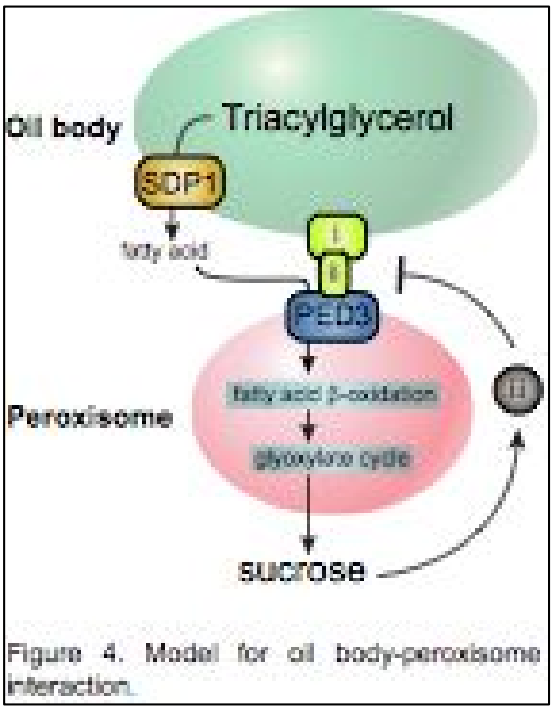

Figure 4. Model for oil body-peroxisome interaction.

Reference

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５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　０件）

〔学会発表〕（計　　２件）

1. Songkui Cui, Juliane K Ishida, Ken Shirasu and Satoko Yoshida. Molecular and genetic approaches for elucidation of host infection mechanisms by parasitic plants *Striga hermonthica* and *Phtheirospermum japonicum*. XVI International Congress on Molecular Plant-Microbe Interactions, 2014.07.07~2014.07.10, Rhodas, Greece

2. Songkui Cui, Makoto Hayashi, Mikio Nishimura. Study of organelle interaction between oil bodies and peroxisomes. Annual Meeting of JSPP, 2014.03.18~2014.03.20, Toyama, Japan

〔図書〕（計　　０件）

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〔その他〕
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