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Intracellular Transport of Phosphatidic Acid and Phosphatidylcholine into Lipid Bodies in an Oleaginous Fungus, *Mortierella ramanniana* var. *angulispora*[1]

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Exogenous fluorescent phosphatidic acid (PA) and phosphatidylcholine (PC) were transported into lipid bodies in an oleaginous fungus, *Mortierella ramanniana* var. *angulispora* [Kamisaka *et al.* (1999) *Biochim. Biophys. Acta* 1438, 185–198]. We further investigated the processes of fluorescent PA and PC transport into lipid bodies in this fungus by changing culture conditions. Lowering incubation temperature decreased lipid body labeling by 1-palmitoyl, 2-[5-(5,7-dimethyl boron dipyrromethene difluoride)-1-pentanoyl]-PA (C_5-DMB-PA), but fluorescence did not accumulate in organelles other than lipid bodies. C_5-DMB-PC transport into lipid bodies was blocked at temperatures below 15°C and fluorescence accumulated in intracellular membranes, presumably endoplasmic reticulum membranes. The low-temperature block of C_5-DMB-PC transport enabled us to do pulse-chase experiments in which fungal cells were pulse-labeled at 15°C with C_5-DMB-PC and chased at 30°C. The results clearly depicted transport of C_5-DMB-PC and its derivatives from intracellular membranes to lipid bodies. Transport was temperature-dependent and ATP-dependent, although microtubules and actin filaments were not substantially involved. Experiments using ^{14}C-labeled fatty acids and glycerol instead of C_5-DMB-PC under the same conditions suggested that transport depicted by fluorescence agreed with metabolism and transport of PC containing native fatty acids. Furthermore, the transport mechanism preferred PC containing unsaturated fatty acids such as linoleic acid. This study dissect lipid transport of PA and PC into lipid bodies and reveal regulatory steps for lipid body formation in this fungus.

Key words: fluorescent lipids, lipid bodies, lipid transport, oleaginous fungi, triacylglycerol biosynthesis.

Mortierella ramanniana var. *angulispora*, an oleaginous fungus, accumulates large amounts of triacylglycerol (TG) and lipid bodies (*1*, *2*), making it suitable for studying triacylgycerol biosynthesis and lipid body formation. Lipid bodies consist of a hydrophobic neutral lipid core, phospholipids, and structural proteins such as oleosins (*3*, *4*). Their basic structure appears to be common among eukaryotic organisms (*5*), although their formation mechanisms are not well understood. In particular, it remains unclear how neutral lipids are preferentially synthesized and transported into lipid bodies. To address this question, we conducted subcellular fractionation of TG biosynthetic enzymes and visualization of lipid transport with fluorescent lipid analogs containing boron dipyrromethene difluoride (BODIPY) (*1*, *2*, *6*). Results indicated that TG biosynthetic enzymes were gradually enriched from the membrane fraction to the lipid body fraction along the *de novo* TG biosynthetic pathway and that fluorescent phosphatidic acid (PA) and phosphatidylcholine (PC) probed two different routes for lipid body formation. The next questions are, what are the driving forces of this lipid transport for lipid body formation and how are they regulated? In mammalian cells, a variety of intracellular lipid transport systems have been documented, such as vesicular transport, protein-facilitated transport, lateral diffusion on membrane contact sites, and spontaneous diffusion (*7*, *8*). Their roles in membrane biogenesis, protein traffic, and other cellular functions have received much attention, although the molecular mechanisms of lipid traffic are still poorly understood. In the present study, we dissected intracellular PA and PC transport for lipid body formation and found regulatory factors for transport processes in this fungus.

MATERIALS AND METHODS

Materials—[1-^{14}C]Oleic acid (52 mCi/mmol), [1-^{14}C]stearic acid (59 mCi/mmol), [1-^{14}C]linoleic acid (53 mCi/mmol),

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Abbreviations: BODIPY, boron dipyrromethene difluoride; C_5-DMB-PA, 1-palmitoyl, 2-[5-(5,7-dimethyl BODIPY)-1-pentanoyl]-phosphatidic acid; C_5-DMB-PC, 1-palmitoyl, 2-[5-(5,7-dimethyl BODIPY)-1-pentanoyl]-phosphatidylcholine; DG, diacylglycerol; ER, endoplasmic reticulum; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TLC, thin-layer chromatography; TG, triacylglycerol.

and [^{14}C(*U*)]glycerol (166 mCi/mmol) were obtained from NEN Life Science Products (Boston, MA, USA). 1-Palmitoyl, 2-[5-(5,7-dimethyl BODIPY)-1-pentanoyl]-PA (C_5-DMB-PA) and 1-palmitoyl, 2-[5-(5,7-dimethyl BODIPY)-1-pentanoyl]-PC (C_5-DMB-PC) were purchased from Molecular Probes (Eugene, OR, USA). Colchicine and cytochalasin B were purchased from Sigma (St. Louis, MO, USA). Sodium azide and 2-deoxy-D-glucose were purchased from Nacalai Tesque (Kyoto). Silica gel 60 thin-layer chromatography (TLC) plates were obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

Strains and Cultures—*M. ramanniana* var. *angulispora* (IFO 8187) was obtained from the culture collection of the Institute for Fermentation (Osaka). The liquid culture was conducted as described elsewhere (*2*, *9*). Aliquots of cultures (1.5 ml of 42-h culture, about 10 mg dry cell weight) were incubated with a C_5-DMB-PA or C_5-DMB-PC/BSA complex in a rotary shaker for microtiter plates (MBSS-500, Marubishi Bioengineering, Tokyo) as described elsewhere (*2*). After incubation, fungal cells were washed and used for microscopic observation and lipid analysis.

In pulse-chase experiments, fungal cells (1.5 ml of 42-h culture, about 10 mg dry cell weight) were initially incubated with 0.5 ml of 10 μM C_5-DMB-PC/BSA complex at 15°C for 10 h, washed twice, and chased at 30°C for the indicated time with the supernatant of fungal cultures without the C_5-DMB-PC/BSA complex. In some experiments, several drugs or 50 mM 2-deoxy-D-glucose instead of glucose were added to the chase medium.

Fungal cells were pulse-labeled with 0.2 μCi [1-^{14}C]oleic acid (2.6 μM), [1-^{14}C]stearic acid (2.3 μM), [1-^{14}C]linoleic acid (2.5 μM), and [^{14}C(*U*)]glycerol (0.8 μM) at 30°C under the same culture conditions. [1-^{14}C]Oleic acid, [1-^{14}C]stearic acid, and [1-^{14}C]linoleic acid were added for the last 2 h of pulse incubation, while [^{14}C(*U*)]glycerol was incubated for 10 h. Radiolabeled fungal cells were washed and chased under the same conditions as for fluorescent labeling.

Microscopy—A laser scanning confocal microscope (LSM-410, Zeiss) with a 63× oil plan-apochromat objective lens (N.A. 1.4, Zeiss) was used for fluorescence and differential interference contrast images.

Subcellular Fractionation—Homogenization of radiolabeled fungal cells with a Braun Homogenizer (Melsungen, Germany) and subcellular fractionation were conducted as described elsewhere (*1*).

Lipid Analysis—Radiolabeled lipids extracted from fungal cells were separated by TLC on silica gel 60 plates with the following solvents (*2*). TLC plates were developed first with chloroform/acetone/methanol/acetic acid/H_2O (50:20:10:10:5, by volume) to a height of about 8 cm above the origin. After drying, plates were developed with hexane/diethylether/acetic acid (80:40:1, by volume) to the top. Radioactivity of each spot on TLC plates was determined using a liquid scintillation counter (Beckman, LS 1701) with automatic quenching correction. Separation of fluorescent lipids was slightly modified as follows: TLC plates were first developed with hexane/diethylether/acetic acid (80:40:1, by volume) to the top, and then the above two solvents were used as the second and third solvents. This improved separation and quantification of fluorescent TG, whose spot was affected by large amounts of endogenous TG in the previous solvent system. The modified system developed endogenous TG faster than fluorescent TG, which allowed the fluorescent spot to be measured more quantitatively. Fluorescent labeled spots were quantified by a fluorescent image analyzer (AE6905, ATTO, Tokyo) as described elsewhere (*2*).

RESULTS

Effect of Temperature on Fluorescent Lipid Incorporation into Lipid Bodies—Our previous studies indicated that incubation with C_5-DMB-PA and C_5-DMB-PC at 4°C decreased fluorescence uptake into fungal cells and fluorescence incorporation into lipid bodies compared with those at 30°C (*2*). Here, we examined incubation temperatures between 30 and 4°C to determine the critical temperature for the transport of fluorescent lipids. Lowering incubation temperature decreased C_5-DMB-PA incorporation into lipid bodies, although no organelles other than lipid bodies were prominently labeled by fluorescence (Fig. 1). Longer incuba-

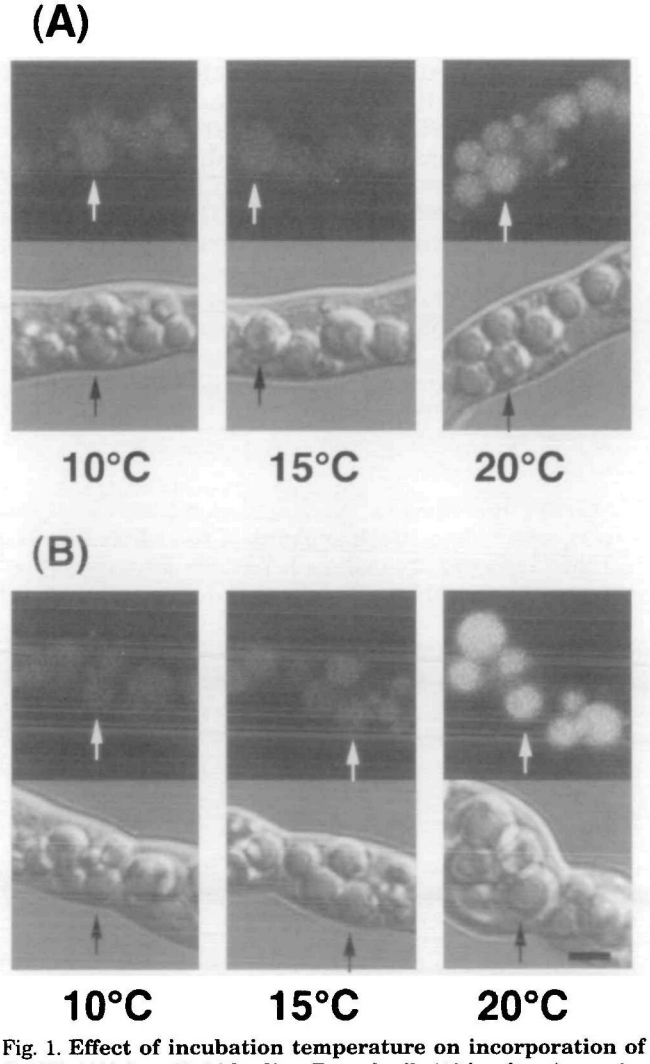

Fig. 1. **Effect of incubation temperature on incorporation of C_5-DMB-PA into lipid bodies.** Fungal cells (42-h culture) were incubated with C_5-DMB-PA/BSA complex for (A) 5 h or (B) 10 h at 10, 15, or 20°C. Details are given in "MATERIALS AND METHODS." Above in each panel is a BODIPY fluorescence image and below is a differential interference contrast image. Arrows indicate lipid bodies. Bar=3 μm.

tion did not change fluorescence distribution, indicating that C_5-DMB-PA did not accumulate at sites other than lipid bodies after uptake into fungal cells at lower temperatures. Lowering incubation temperature below 15°C completely blocked C_5-DMB-PC incorporation into lipid bodies, and fluorescence accumulated in intracellular membranes, presumably endoplasmic reticulum (ER) (Fig. 2). Longer incubation did not change fluorescence distribution. At 20°C, slow but steady transport into lipid bodies was observed as at 30°C, indicating that 15°C was the critical temperature for C_5-DMB-PC transport from intracellular membranes into lipid bodies.

Transport of C_5-DMB-PC and Its Derivatives from Intracellular Membranes to Lipid Bodies—Taking advantage of the low-temperature block of C_5-DMB-PC from intracellular membranes into lipid bodies, we set up pulse-chase experiments in which fungal cells were pulse-incubated with C_5-DMB-PC at 15°C, washed, and chased at 15 or 30°C (Fig. 3). After pulse incubation at 15°C, fluorescence was incorporated into intracellular membranes but not into lipid bodies. After a chase at 30°C, lipid bodies were gradually labeled, whereas lipid bodies remained unlabeled after a chase at 15°C. This clearly indicated temperature-dependent transport of C_5-DMB-PC and its derivatives from intracellular membranes into lipid bodies. After chase incubation at 30°C, C_5-DMB-PC may also be transported to plasma membranes, although this could not be verified from our images.

Metabolism of C_5-DMB-PC during the pulse-chase conditions was then investigated (Fig. 4). Incubation at 30°C as a positive control caused conversion of C_5-DMB-PC to diacylglycerol (DG), PA, and TG as described elsewhere (2). Conversion was higher than previously measured, probably because quantification of fluorescent TG was improved. In contrast, pulse incubation at 15°C decreased C_5-DMB-PC metabolism, especially into TG, and accumulated intracellular C_5-DMB-PC. Chase incubation at 30°C metabolized accumulated C_5-DMB-PC into DG, PA, and TG, whereas chase incubation at 15°C did not cause further metabolism of C_5-DMB-PC. These results indicate that C_5-DMB-PC was

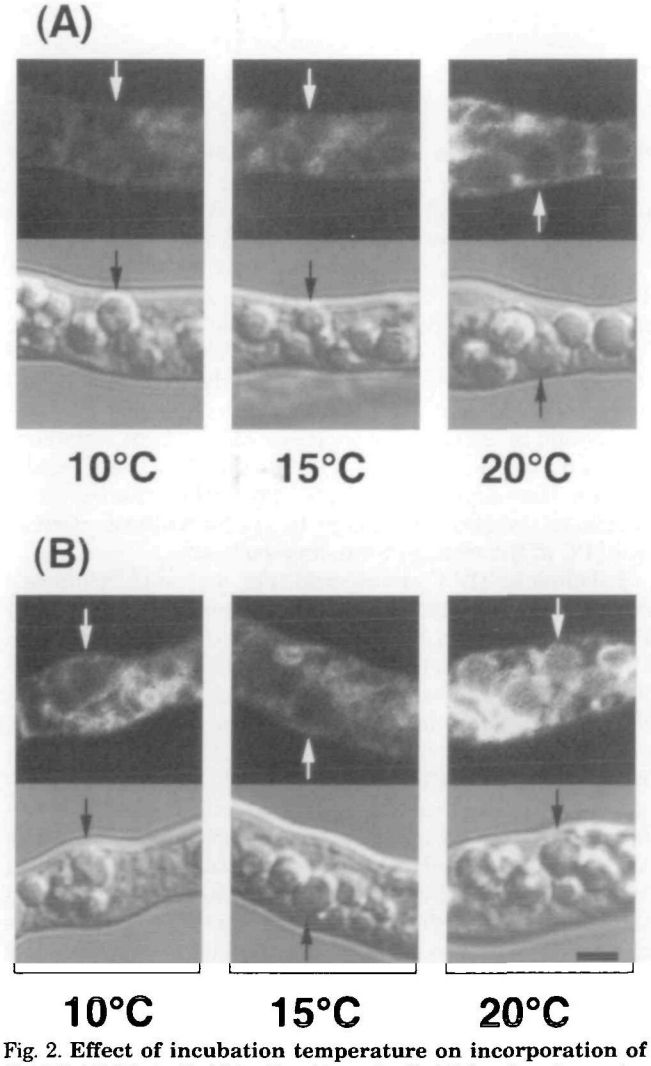

Fig. 2. **Effect of incubation temperature on incorporation of C_5-DMB-PC into lipid bodies.** Fungal cells (42-h culture) were incubated with C_5-DMB-PC/BSA complex for (A) 5 h or (B) 10 h at 10, 15, or 20°C. Details are the same as for Fig. 1.

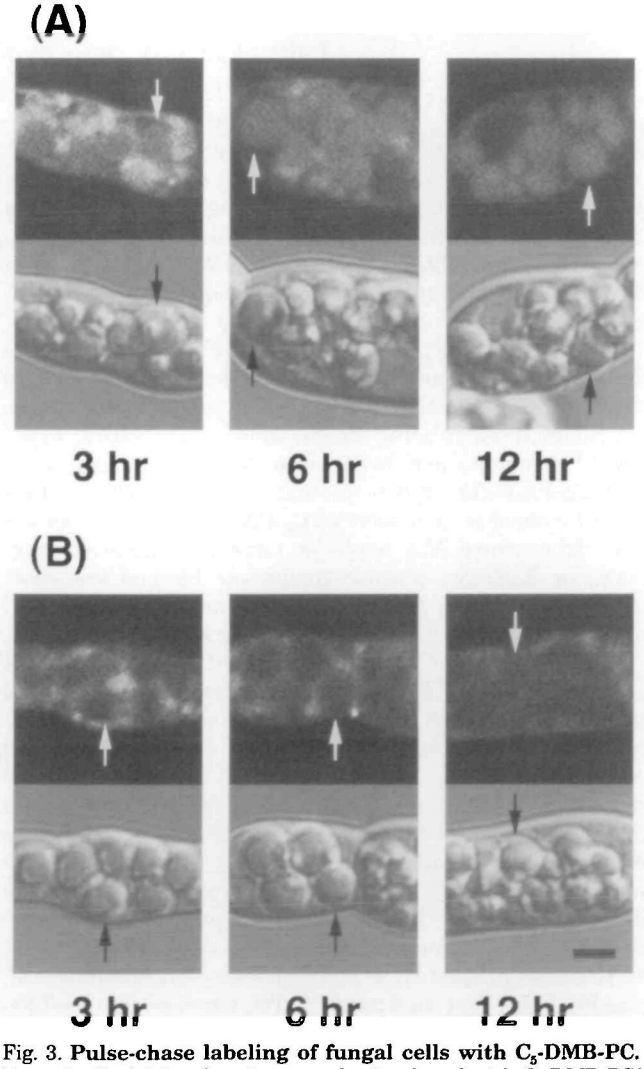

Fig. 3. **Pulse-chase labeling of fungal cells with C_5-DMB-PC.** Fungal cells (42-h culture) were pulse-incubated with C_5-DMB-PC/BSA complex for 10 h at 15°C, washed twice, and chased at (A) 30°C or (B) 15°C for 3, 6, or 9 h with the supernatant of the fungal cultures without C_5-DMB-PC/BSA complex. Details are the same as for Fig. 1.

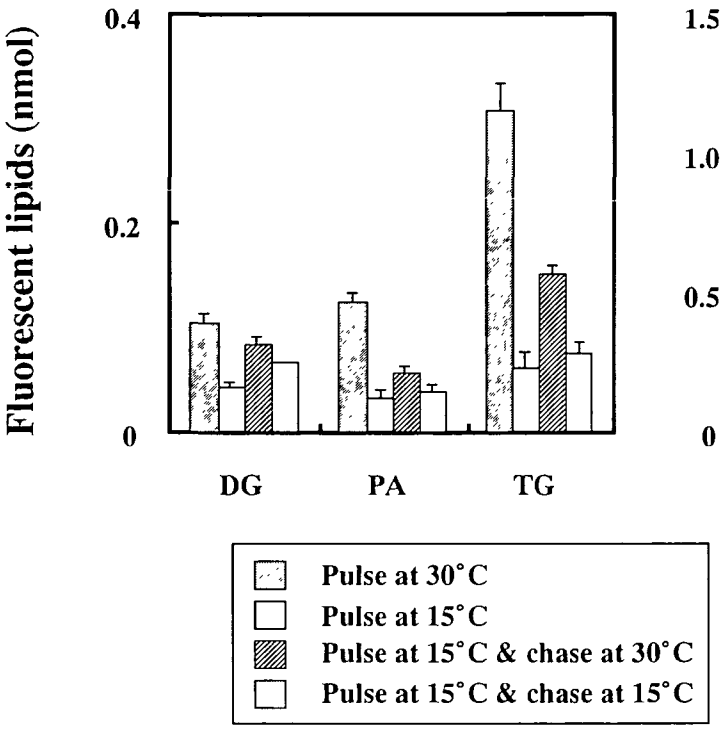

Fig. 4. **Metabolism of C_5-DMB-PC in fungal cells under temperature shift conditions.** Fungal cells (42-h culture) were pulse-incubated with C_5-DMB-PC/BSA complex for 10 h at 30 or 15°C. Fungal cells pulse-labeled with C_5-DMB-PC/BSA complex for 10 h at 15°C were washed twice and chased at 30 or 15°C for 6 h with the supernatant of fungal cultures without C_5-DMB-PC/BSA complex. Fluorescent lipids were extracted, separated by TLC, and fluorescence was measured from their spots on TLC plates by a fluorescent image analyzer. Details are given in "MATERIALS AND METHODS." Data are presented as means of triplicates.

metabolized into DG, PA, and TG during its transport from intracellular membranes into lipid bodies depicted by temperature shift conditions, and that C_5-DMB-PC localized in intracellular membranes was not readily metabolized unless it was involved in transport process.

To further characterize transport of C_5-DMB-PC and its derivatives into lipid bodies, several drugs were added to the chase medium (Fig. 5). Colchicine and cytochalasin B, cytoskeleton modulating drugs, allowed transport of C_5-DMB-PC and its derivatives into lipid bodies, suggesting that microtubules and actin filaments were not substantially involved in transport of C_5-DMB-PC and its derivatives. In contrast, ATP depletion caused by the addition of NaN_3 or 2-deoxy-D-glucose completely blocked transport into lipid bodies, indicating that transport of C_5-DMB-PC and its derivatives into lipid bodies was ATP-dependent.

Metabolism and Transport of Fungal Lipids Prelabeled with Radiolabeled Fatty Acids and Glycerol—We verified the existence of PC transport from intracellular membranes into lipid bodies depicted by C_5-DMB-PC using radiolabeled lipids. Fungal cells were pulse-labeled with radiolabeled fatty acids and glycerol, washed, and chased under the same conditions as C_5-DMB-PC incorporation. Fungal cells incubated with [1-^{14}C]oleic acid at 15°C accumulated more ^{14}C-PC and less ^{14}C-TG compared with those incubated at 30°C (Fig. 6). Other ^{14}C-phospholipids, especially phosphatidylethanolamine (PE), also accumulated at 15°C. Chase incubation at 30°C decreased accumulated ^{14}C-phospholipids and increased ^{14}C-TG, whereas that at 15°C only partly decreased ^{14}C-phospholipids and did not increase ^{14}C-TG. The decrease in ^{14}C-phospholipids and increase in ^{14}C-TG during chase incubation at 30°C were inhibited by ATP depletion caused by NaN_3 (Fig. 7). ATP depletion increased ^{14}C-phospholipids and decreased ^{14}C-TG during pulse incubation at either 15 or 30°C, suggesting that ATP depletion affected more diverse sites than low temperature. These results indicate that metabolism of ^{14}C-phospholipids and TG was compatible with the metabolism of C_5-DMB-PC under the same culture conditions.

Pulse-incubation of fungal cells with [^{14}C(*U*)]glycerol at 15°C accumulated ^{14}C-phospholipids such as PC and PE and decreased ^{14}C-TG compared with 30°C incubation (Fig. 8). Chase incubation at 30°C decreased accumulated ^{14}C-PC and PE, whereas that at 15°C only partly decreased ^{14}C-PC and PE. In contrast, ^{14}C-TG did not increase during chase incubation at 30°C, although there was a slight difference between ^{14}C-TG after a chase at 30 and 15°C. These results suggest that acyl transfer from PC rather than glycerol backbone transfer contributed to TG biosynthesis derived from PC in the transport into lipid bodies.

Labeling by [1-^{14}C]stearic acid (Fig. 9A) or [1-^{14}C]linoleic acid (Fig. 9B) similarly exhibited decrease in ^{14}C-PC and PE and increase in ^{14}C-TG after 30°C chase incubation to [1-^{14}C]oleic acid labeling, but several differences in metabolism could be due to fatty acid specificity of the transport machinery. PC labeled by [1-^{14}C]linoleic acid, for example, accumulated much more after 15°C pulse incubation than PC labeled by [1-^{14}C]oleic acid, while PC labeled by [1-^{14}C]-stearic acid did not change compared with 30°C pulse incubation. PE labeled by [1-^{14}C]linoleic acid or [1-^{14}C]stearic acid did not change between 15 and 30°C pulse incubation, while PE labeled by [1-^{14}C]oleic acid did. These observations indicate that the mechanism operating under temperature shift conditions preferentially metabolized particular molecular species of phospholipids such as PC containing linoleic acid and oleic acid.

The phospholipid pool and TG pool that were changed following the temperature shift were assumed to be in intracellular membranes and lipid bodies, respectively. This was confirmed by the distribution of radiolabeled lipids in

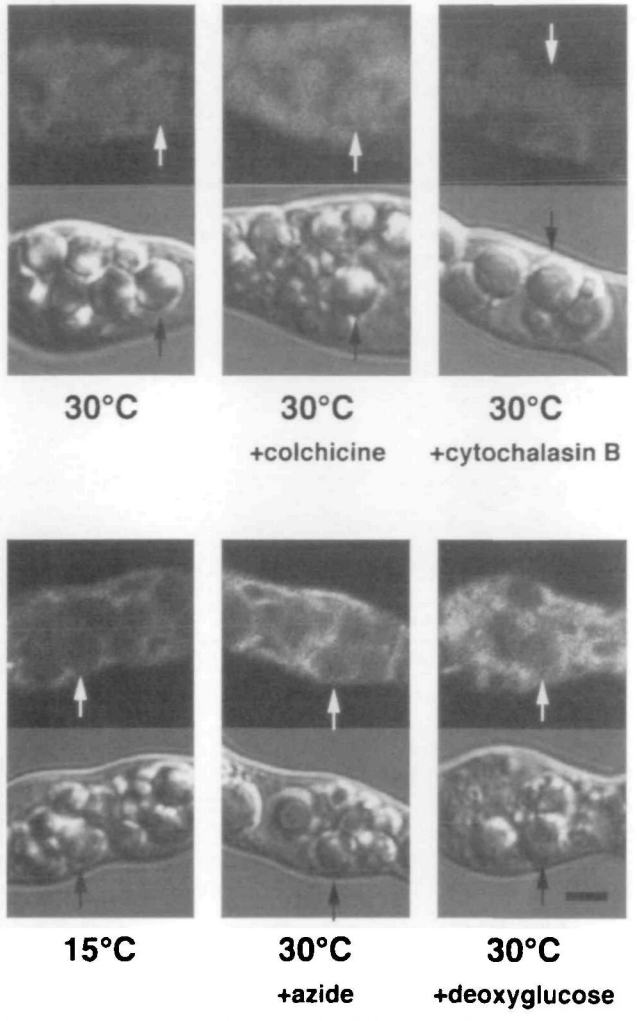

Fig. 5. **Effects of cytoskeleton inhibitors and ATP depletion on transport of C_5-DMB-PC and its derivatives into lipid bodies.** Fungal cells (42-h culture) were pulse-incubated with C_5-DMB-PC/BSA complex for 10 h at 15°C, washed twice, and chased at 30 or 15°C for 6 h with the supernatant of fungal cultures without C_5-DMB-PC/BSA complex. The chase medium was supplemented with 5 μM colchicine, 5 μM cytochalasin B, or 0.01% sodium azide. Chase medium containing 2-D-deoxyglucose was prepared by including 50 mM 2-D-deoxyglucose instead of glucose in the glucose medium. Above in each panel is a BODIPY fluorescence image and below is a differential interference contrast image. Arrows indicate lipid bodies. Bar=3 μm.

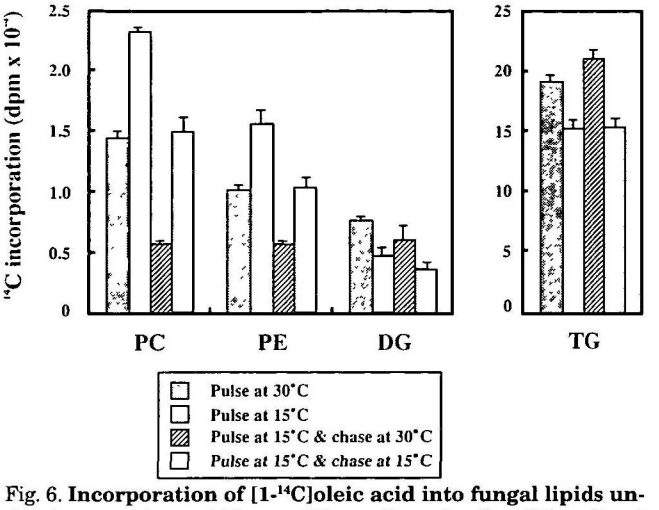

Fig. 6. **Incorporation of [1-^{14}C]oleic acid into fungal lipids under temperature shift conditions.** Fungal cells (42-h culture) were cultured for 10 h at 30 or 15°C. During the last 2 h of incubation, fungal cells were pulse-labeled with 0.2 μCi [1-^{14}C]oleic acid (2.6 μM). Fungal cells pulse-labeled with [1-^{14}C]oleic acid at 15°C were washed twice and chased at 30 or 15°C for 6 h with the supernatant of fungal cultures. Lipids were extracted, separated by TLC, and radioactivity was measured from their spots on TLC plates using a liquid scintillation counter. Data are presented as means of triplicates.

membrane and lipid body fractions from fungal cells labeled by [1-^{14}C]oleic acid (Fig. 10). Radioactivity in the membrane and lipid body fractions was lower than that in whole fungal cells (Fig. 6), and significant amounts of radiolabeled lipids were recovered in the low-speed centrifuge pellet after homogenization (data not shown). Radiolabeled lipids in the low-speed pellet may represent unbroken cells, nuclei, and parts of ER membranes attached to nuclear membranes. Nevertheless, radiolabel incorporation into lipids in the membrane and lipid body fractions was reproducible, and reflected distribution between intracellular membranes and lipid bodies. Results indicate that the ^{14}C-PC and PE that accumulated after 15°C pulse incubation were present in the membrane fraction, and ^{14}C-TG concomitantly decreased from the lipid body fraction. After 30°C chase incubation, ^{14}C-PC and PE decreased in the membrane fraction and ^{14}C-TG increased in the lipid body fraction, confirming that these metabolisms involving PC, PE, and TG under the temperature-shift conditions accompanied transport from membranes to lipid bodies. This also suggests that PC and PE that have departed from intracellular membranes are mostly converted to DG and TG before their entry to lipid bodies. ^{14}C-DG was comparable to ^{14}C-PC and PE in subcellular fractions, probably because ^{14}C-DG was less distributed in the low-speed pellet.

DISCUSSION

Our previous studies described two lipid transport pathways for lipid body formation in an oleaginous fungus by C_5-DMB-PA and C_5-DMB-PC. The present study further characterized these transport pathways. C_5-DMB-PA was directly transported to lipid bodies after its uptake into fungal cells, and the transport was basically unchanged by lowering the incubation temperature. No intermediate sites between plasma membranes and lipid bodies for C_5-DMB-PA traffic were detectable at either higher or lower temperature. Since no fluorescence accumulated on plasma membranes, decreased C_5-DMB-PA uptake into fungal cells at low temperature was probably due to its slow insertion into plasma membranes through cell walls. Mechanisms for the specific transport of C_5-DMB-PA into lipid bodies are not clear at present. Transport was not inhibited at temperatures below 15°C, which may exclude the possibility of low-temperature sensitive endocytosis or vesicular transport (*10–12*). In mammalian cells, fluorescent PA was degradated to DG at plasma membranes, and DG was then transported to intracellular membranes (*13*). This is probably not the case in this fungus, since the majority of C_5-DMB-PA taken up into fungal cells was not degradated to

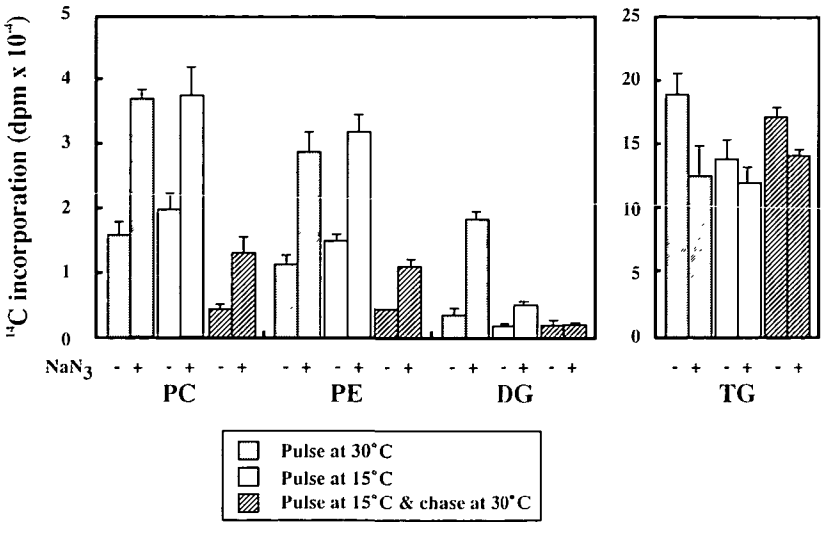

Fig. 7. **Effect of NaN_3 on [1-^{14}C]oleic acid incorporation into fungal lipids under temperature-shift conditions.** [1-^{14}C]Oleic acid incorporation into fungal lipids was measured as in Fig. 6. Fungal cells were pulse-labeled with [1-^{14}C]oleic acid at 30 or 15°C in the presence of 0.01% NaN_3 for the last 2 h, or fungal cells pulse-labeled with [1-^{14}C]oleic acid at 15°C were washed twice and chased at 30 or 15°C in the presence of 0.01% NaN_3 for 6 h. Data are presented as means of triplicates.

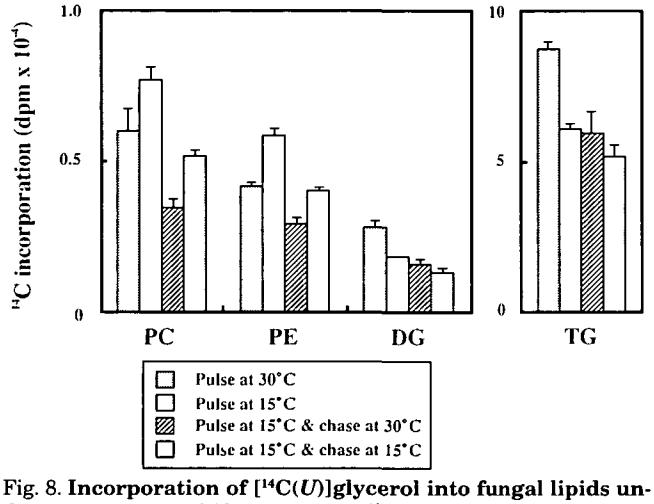

Fig. 8. **Incorporation of [^{14}C(*U*)]glycerol into fungal lipids under temperature shift conditions.** [^{14}C(*U*)]Glycerol incorporation into fungal lipids was measured as [1-^{14}C]oleic acid incorporation in Fig. 6 except that cells were pulse-labeled with 0.2 μCi [^{14}C(*U*)]glycerol (0.8 μM) for 10 h. Data are presented as means of triplicates.

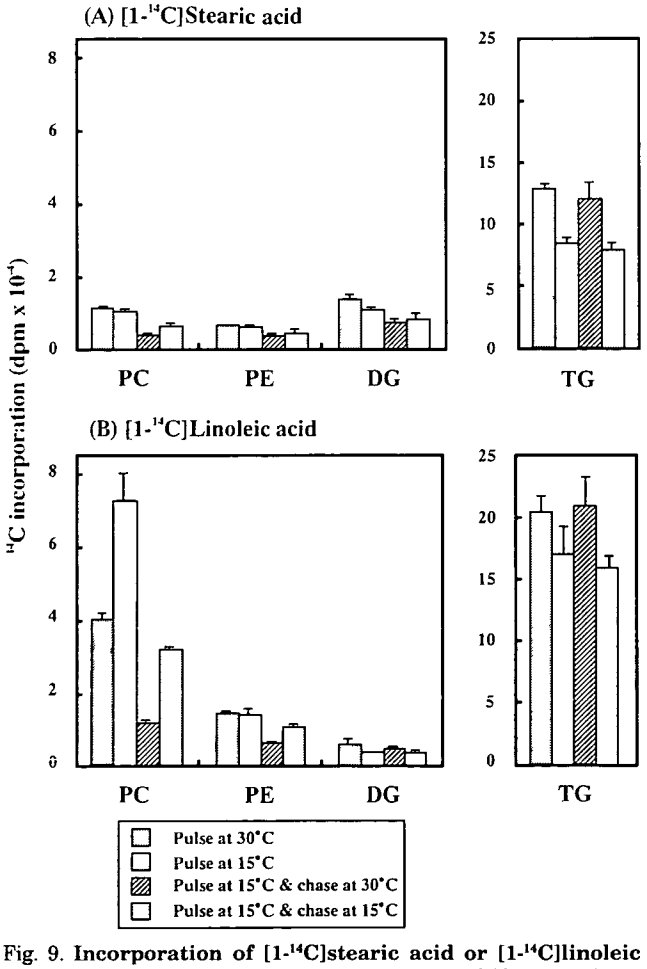

Fig. 9. **Incorporation of [1-^{14}C]stearic acid or [1-^{14}C]linoleic acid into fungal lipids under temperature shift conditions.** (A) [1-^{14}C]stearic acid (0.2 μCi, 2.3 μM) or (B) [1-^{14}C]linoleic acid (0.2 μCi, 2.5 μM) incorporation into fungal lipids was measured as [1-^{14}C]oleic acid incorporation in Fig. 6. Data are presented as means of triplicates.

DG (*2*). These observations lead us to speculate that there exists a protein-facilitated mechanism that transports PA but not PC. The intensive transport of C_5-DMB-PA to lipid bodies is probably linked to active TG biosynthesis in this fungus. During *de novo* TG biosynthesis, PA may be transported form ER membranes to lipid bodies by the mechanism depicted by C_5-DMB-PA. Lipid transfer proteins specific to PA in cytosol may explain this transport, although no lipid transfer proteins specific to PA have been reported yet (*11*, *14*).

Fungal cultures with C_5-DMB-PC at temperatures below 15°C showed fluorescence accumulation at intracellular membranes, presumably ER membranes. The mechanism of C_5-DMB-PC transport to the intracellular membranes, which is distinct from C_5-DMB-PA transport into lipid bodies, also remains unclear. In mammalian cells, fluorescent PC inserted into plasma membranes was transported into Golgi membranes (*15*) or other organelles (*16*), depending on cell type, mainly by endocytosis. Newly synthesized PC was transported from ER to plasma membranes by an

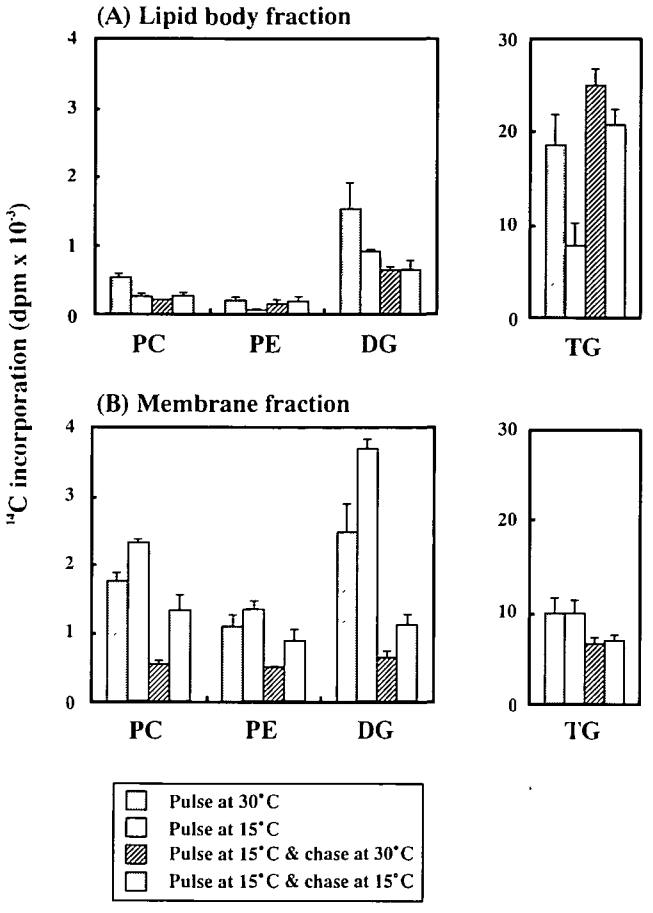

Fig. 10. **Distribution of ^{14}C-lipids labeled by [^{14}C]oleic acid in the lipid body and membrane fractions of fungal cells under temperature-shift conditions.** [1-^{14}C]Oleic acid incorporation into fungal lipids was conducted as in Fig. 6. Fungal cells were then homogenized and the lipid body (A) and membrane fractions (B) were obtained. Lipids were extracted from both fractions, separated by TLC, and radioactivity was measured from their spots on TLC plates using a liquid scintillation counter. Data are presented as means of triplicates.

ATP-independent, non-vesicular mechanism (*17*). In the yeast *Saccharomyces cerevisiae*, fluorescent PC inserted into plasma membranes was transported into vacuoles by endocytosis and into nuclear envelope and mitochondria by a non-endocytic mechanism (*18*). Newly synthesized PC in yeast was transported to plasma membranes through a process independent of the protein secretory pathway (*19*). Since temperatures below 15°C did not block the C_5-DMB-PC transport into intracellular membranes in this fungus, transport appears to be due to a non-endocytic mechanism.

Although C_5-DMB-PC transport from intracellular membranes to lipid bodies had been expected from time-dependent fluorescence distribution (*2*), we confirmed this transport by temperature-shift experiments. The transport was temperature- and ATP-dependent, consistent with vesicular transport. Transport was slower (hours) than secretory transport of proteins (*20*, *21*) or lipids (*8*, *11*, *12*, *22*) (minutes). The slow transport may arise because the driving system for vesicles is insensitive to inhibitors for microtubules and actin filaments, whereas protein secretory transport was essentially sensitive to inhibitors for microtubules in mammalian cells (*20*, *21*) or actin filaments in yeasts (*23*). Since another cytoskeletal component, intermediate filaments, was involved in lipid droplet formation of adipocytes (*24*, *25*), the transport of C_5-DMB-PC and its derivatives to lipid bodies in this fungus may use intermediate filaments. Low temperature inhibited C_5-DMB-PC metabolism to DG, PA, and TG in addition to its transport to lipid bodies, suggesting that C_5-DMB-PC metabolism is blocked unless it is on the track of transport to lipid bodies.

We confirmed C_5-DMB-PC metabolism and transport using radiolabeled fatty acids and glycerol. Since these radiolabeled lipid precursors were incorporated into different intracellular sites from C_5-DMB-PC and labeled a variety of lipids, metabolism of radiolabeled lipids was more complex. Nevertheless, temperature-shift study revealed metabolism of PC, PE, and TG that was compatible with C_5-DMB-PC metabolism and transport to lipid bodies. One exception was ^{14}C-DG metabolism: ^{14}C-DG in the membrane fraction accumulated at low temperature and was decreased by 30°C chase incubation (Fig. 10), which differed from the change of fluorescent DG and ^{14}C-DG in whole cells following the temperature shift. The ^{14}C-DG that accumulated in the membrane fraction at low temperature may be derived from sources other than PC and other phospholipids.

The PC transport mechanism from intracellular membranes to lipid bodies preferentially used PC containing more unsaturated fatty acids such as linoleic acid. Thus, this transport mechanism may provide unsaturated fatty acids from PC to TG. Since polyunsaturated fatty acids such as linoleic acid and γ-linolenic acid were enriched and probably synthesized in phospholipids of this fungus (*26*), the mechanism preferentially transporting PC containing polyunsaturated fatty acids is plausible. Channeling of PC containing polyunsaturated fatty acids to TG was also described in plant seeds (*27*). Other phospholipids were transported by this mechanism, but similar preference for polyunsaturated fatty acids was not found in PE.

Blocking of PC transport into lipid bodies at low temperature may have another physiological significance: it prevents the flow of polyunsaturated fatty acids from PC and hence enriched these fatty acids in PC at low temperature. PC transport may therefore be involved in membrane fluidity maintenance at low temperature by increasing polyunsaturated fatty acids in PC.

In conclusion, the present study dissected intracellular transport of PA and PC into lipid bodies in an oleaginous *Mortierella* fungus. PA transport depicted by C_5-DMB-PA is assumed to be involved in *de novo* TG biosynthesis, whereas PC transport depicted by C_5-DMB-PC represents a remodeling pathway for TG biosynthesis. Using these fluorescent lipid systems, proteins involved in transport into lipid bodies will be able to be identified and characterized.

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