Complex Lipid Biosynthesis: Phospholipid Synthesis

into account the extra protein (about 2 kDa) from
the vector, this value of 44 kDa agreed well
with the predicted protein size.

The availability of high amounts of active pea
choline kinase will enable us to study its enzymatic

References
43–144
Physiol. 35, 853–863
4 Tanaka, K., Tolbert, N. E. and Gohike, A. F. (1966)
Plant Physiol. 41, 307–312
Biophys. 260, 102–108

We are grateful to the Saudi Arabian Government for a
studentship to A. Al-M.

7 Wharfe, J. and Harwood, J. L. (1979) in Advances in
Biochemistry and Physiology of Plant Lipids (Appelqvist,
Scientific Publications, Amsterdam
8 Jones, P. L., Willey, D. L., Gacesa, P. and Harwood, J. L.
Plant Physiol. 110, 1197–1205

Received 28 June 2000

Intracellular transport of phosphatidic acid and phosphatidylcholine into lipid bodies: use of fluorescent lipids to study lipid-body formation in an oleaginous fungus

Y. Kamisaka¹ and N. Noda
National Institute of Bioscience & Human Technology, Tsukuba, Ibaraki 305-8566, Japan

Abstract
Fluorescent phosphatidic acid and phosphatidylcholine were used to characterize lipid-transport pathways into lipid bodies in an oleaginous fungus, Mortierella ramanniana var. angulispora.

Introduction
Mortierella ramanniana var. angulispora, an oleaginous fungus, accumulates large amounts of triacylglycerol (TAG) and lipid bodies [1]. TAG biosynthetic enzymes had different subcellular distributions between the lipid-body fraction and the membrane fraction the enzymes catalysing the later steps were enriched in the lipid-body fraction [2]. Lipid bodies enlarged with lipid accumulation to a diameter of about 2–3 μm [3]. To further elucidate mechanisms of lipid-body formation, we used short-chain fluorescent lipids, which have proved to be valuable tools to study intracellular lipid transport in mammalian cells [4]. Our initial studies indicated that the use of fluorescent phosphatidic acid (PA) and phosphatidylcholine (PC) analogues containing boron dipyrromethene difluoride (BODIPY), 1-palmitoyl-2-[5-(5,7-dimethyl BODIPY)-1-pentanoyl]-phosphatidic acid (C₅-DMB-PA) and 1-palmitoyl-2-[5-(5,7-dimethyl BODIPY)-1-pentanoyl]-phosphatidylcholine (C₅-DMB-PC), could monitor lipid-body formation in this fungus [3]. Based on the established system using these fluorescent lipids, the present study dissected the intracellular lipid transport into lipid bodies and revealed regulatory factors for lipid-body formation in this fungus.

Materials and methods
M. ramanniana var. angulispora (IFO 8187) was obtained from the culture collection of the In-
stitute of Fermentation (Osaka, Japan). Aliquots of cultures (1.5 ml of 42 h culture, about 10 mg of dry cell weight) were incubated with C$_2$-DMB-PA, C$_2$-DMB-PC or radiolabelled lipids in a rotary shaker for microtitre plates (MBSS-500, Marubishi Bioengineering, Tokyo, Japan) as described elsewhere [3]. After incubation, fungal cells were used for observation with a laser-scanning confocal microscope (LSM410, Zeiss) or lipid analysis.

**Results**

We investigated the effect of incubation temperature on fluorescent-lipid incorporation into lipid bodies in this fungus. Lowering incubation temperature decreased C$_2$-DMB-PA incorporation into lipid bodies, although no organelles other than lipid bodies were prominently labelled by fluorescence. This indicates that C$_2$-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$_2$-DMB-PC incorporation into lipid bodies and accumulated fluorescence in intracellular membranes, presumably endoplasmic reticulum.

Taking advantage of the low-temperature block of C$_2$-DMB-PC from intracellular membranes into lipid bodies, we set up pulse-chase experiments in which fungal cells were pulse-incubated with C$_2$-DMB-PC at 15 °C, washed and chased at 15 or 30 °C. 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 labelled, whereas lipid bodies remained unlabelled after a chase at 15 °C. This clearly indicated temperature-dependent transport of C$_2$-DMB-PC and its derivatives from intracellular membranes into lipid bodies. C$_2$-DMB-PC was metabolized into diacylglycerol, PA and TAG during its transport from intracellular membranes into lipid bodies under the pulse-chase conditions. Further experiments using several drugs suggested that C$_2$-DMB-PC transport was ATP-dependent, but microtubules and actin filaments were not substantially involved in C$_2$-DMB-PC transport.

We verified the existence of PC transport from intracellular membranes into lipid bodies depicted by C$_2$-DMB-PC using radiolabelled lipids. Fungal cells were pulse-labelled with [14C]oleic acid, [14C]linoleic acid and [14C]glycerol, and then washed and chased under the same conditions as C$_2$-DMB-PC incorporation. These radiolabelled lipid precursors were incorporated into phospholipids and TAG, and the amounts of 14C in these lipids under the pulse-chase conditions were measured. Results indicate that 14C-PC accumulated and 14C-TAG decreased at 15 °C compared with the 30 °C pulse, and that 14C-PC decreased and concomitantly 14C-TAG increased after 30 °C chase incubation, which was consistent with the metabolism of C$_2$-DMB-PC under the same culture conditions. Although the basic metabolism was similar, several differences were found due to [14C]fatty acid; PC labelled by [14C]linoleic acid accumulated much more after 15 °C pulse-incubation than PC labelled by [14C]oleic acid. These observations indicate that the mechanism operating under pulse-chase conditions preferentially metabolized particular molecular species of PC containing linoleic acid.

**Discussion**

The present study dissected intracellular transport of PA and PC into lipid bodies in an oleaginous fungus, *M. ramanniana* var. angulispora. Intracellular lipid transport probed by C$_2$-DMB-PA was not completely blocked by low temperature. Since no apparent fluorescence accumulated within fungal cells at low temperatures, it is likely that decreased labelling of lipid bodies at low temperatures was not due to PA transport into lipid bodies, but to reduced uptake of C$_2$-DMB-PA through cell walls. The temperature dependence suggests that PA is transported to lipid bodies by a PA-specific protein-facilitated mechanism, but not by vesicular transport. Low-temperature-resistant C$_2$-DMB-PC transport into intracellular membranes, presumably endoplasmic reticulum, was also observed, which may be catalysed by another protein-facilitated mechanism. C$_2$-DMB-PC transported to intracellular membranes was then transported to lipid bodies, indicating that exogenous PC transport into lipid bodies was at least divided into two steps. The second step into lipid bodies was more clearly characterized using the pulse-chase experiments. It is temperature- and ATP-dependent, consistent with vesicular transport, although it was slower than protein secretory transport. PC transport by
vesicular mechanisms was also reported in mammalian cells [5] and yeasts [6].

The lipid transport into lipid bodies in this fungus may share some important characteristics with oil-body formation in plant seeds [7]. \(C_2\)-

DMB-PC transport into lipid bodies in this fungus represents a remodelling pathway for TAG biosynthesis, which may be similar to channelling of PC containing polyunsaturated fatty acids to TAG in plants [8]. \(C_2\)-DMB-PA transport into lipid bodies is assumed to be involved in de novo TAG biosynthesis. Thus, lipid-transport systems are important regulatory factors for TAG assembly in addition to enzymes involved in TAG biosynthesis. Fluorescent lipid analogues provide useful tools to evaluate lipid-transport systems for TAG assembly. Identification and characterization of proteins involved in the lipid transport depicted by these fluorescent lipid analogues will be very helpful in understanding lipid-body formation.

References

Received 23 June 2000

Metabolism of glycosylphosphatidylinositol-anchored proteins in Arabidopsis
L. Darjania*, N. Ichise*, S. Ichikawa*, T. Okamoto†, H. Okuyama† and G. A. Thompson, Jr*†
* Molecular Cell and Developmental Biology, University of Texas, Austin, TX 78712, U.S.A., and † Laboratory of Environmental Molecular Biology, Hokkaido University, Kita-ku, Sapporo, Japan

Abstract
Although glycosylphosphatidylinositol (GPI)-anchored proteins have now been found in several plants, very little is known regarding their metabolism there. This report describes studies of the biosynthesis and turnover of arabinogalactan proteins, a class of abundant GPI-anchored proteins secreted by cultured Arabidopsis cells.

Introduction
Lipids have many physiological functions that were unheard of a decade ago. Among these recently recognized roles is the targeting of proteins to the extracellular face of the plasma membrane by covalently-attached glycosylphosphatidylinositol (GPI) anchors. This role is well-established in animal and yeast cells [1], but only now are examples being discovered in plants. Following our report on GPI-anchored phosphatase in Spirodella oligovrheza [2] several other higher plants were shown to utilize this lipid anchor for protein trafficking. However, because putative GPI-anchored proteins of plants are generally present in very low abundance it has so far been impossible to achieve a complete characterization or to learn much about their metabolism.

Very recently proteins of the classical arabinogalactan protein (AGP) family were reported to be GPI-anchored in several plant species [3,4], making these the first abundant proteins to be so identified. AGPs are large (50 to approx. 200 kDa), unusual proteins, typically containing 90-95%, O-linked carbohydrate and only 4-15% protein by weight, and are localized in the extracellular space of plant tissues. Although mutant studies have showed them to be essential, their precise functions are uncertain. We have begun a study of AGP metabolism in cultured cells of Arabidopsis thaliana with the aim of characterizing the GPI-anchor involvement.

Key words: arabinogalactan protein, GPI-anchored protein. Abbreviations used: GPI, glycosylphosphatidylinositol; AGP, arabinogalactan protein; PE, phosphatidylethanolamine.
1To whom correspondence should be addressed (e-mail guythom@utvm.s.cc.utexas.edu).