Expression of perilipin 5 promotes lipid droplet formation in yeast

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Neutral lipids are packed into dedicated intracellular compartments termed lipid droplets (LDs). LDs are spherical structures delineated by an unusual lipid monolayer and they harbor a specific set of proteins, many of which function in lipid synthesis and lipid turnover. In mammals, LDs are covered by abundant scaffolding proteins, the perilipins (PLIN1–5). LDs in yeast are functionally similar to that of mammalian cells, but they lack the perilipins. We have previously shown that perilipins (PLIN1–3) are properly targeted to LDs when expressed in yeast and that they promote LD formation from the ER membrane enriched in neutral lipids. Here we address the question whether PLIN5 (OXPAT) has a similar function. Both human and murine PLIN5 were properly targeted to yeast LDs, but the protein localized to the cytosol and its steady-state level was reduced when expressed in yeast mutants lacking the capacity to synthesize storage lipids. When expressed in cells containing high levels of neutral lipids within the membrane of the endoplasmatic reticulum, PLIN5 promoted the formation of LDs. Interestingly, PLIN5 was properly targeted to LDs, irrespective of whether these LDs were filled with triacylglycerol or steryl esters, indicating that PLIN5 did not exhibit targeting specificity for a particular subtype of LDs as was reported for mammalian cells.

We have previously shown that PLIN1–3 are properly targeted to LDs when expressed in yeast cells and that these proteins become cytosolic and lost their membrane association when expressed in cells lacking LDs.14 In addition, expression of these LD scaffolding proteins is sufficient to induce LD formation in cells having high levels of neutral lipids in the ER membrane, such as mutants lacking the phosphatidate phosphatase Pah1/Lipin.14 Here we extend these studies to one additional member of the perilipin family, PLIN5 (OXPAT).

cDNAs encoding both murine and human PLIN5 (OXPAT) were placed under transcriptional control of a constitutively active alcohol dehydrogenase 1 (ADH1) promoter and N-terminally fused in frame with GFP and expressed from a centromeric plasmid. When expressed in wild-type cells, PLIN5 stained circular intracellular structures that were co-stained with the lipophilic fluorescent dye Nile Red, indicating LD localization of PLIN5 (Fig. 1A). LDs formed in the presence of PLIN5, however, frequently appear to be slightly bigger (average diameter 0.85 μm) and clustered compared to those formed in wild-type cells expressing the LD marker Erg6-GFP (average diameter 0.6 μm) (Fig. 1B) When expressed in cells lacking the capacity to synthesize neutral lipids due to the deletion of 3 of the genes required for neutral lipid synthesis (are1Δ are2Δ dga1Δ) and
placement of the fourth gene under transcriptional control of the glucose repressible GAL1 promoter (GAL-LRO1), PLIN5 showed cytosolic staining and Nile Red stained endomembranes. Western blot analysis of PLIN5 indicated higher steady-state levels of the protein when expressed in cells containing LDs, suggesting that the proteins is unstable when expressed in cells lacking LDs (Fig. 1C). Analysis of protein turnover after treatment of cells with cycloheximide to block translation, however, indicates that PLIN5 turnover is not dramatically accelerated in cell lacking LDs, but that its steady-state levels are decreased (Fig. 1D). PLIN5 thus behaves similar to ADRP/PLIN2 and TIP47/PLIN3 in that the protein is stable even in the absence of LDs. This is in contrast to Oleosin and PLIN1, which are both rapidly degraded in cells lacking LDs.14

To examine whether PLIN5 could also induce formation of LDs, as we previously observed for both oleosin and PLINs,14 we expressed PLIN5 in mutants lacking the phosphatidate phosphatase Pah1, the yeast homolog of human lipin.15,16 Pah1p catalyzes the dephosphorylation of phosphatidic acid to diacyl-glycerol (DAG), which is then further converted to triacylglycerol. Cells lacking Pah1 display a defect in LD formation and show elevated levels of neutral lipids in the ER membrane.17 When stained with Nile Red, pah1Δ mutant cells did not show any detectable LDs and the dye instead stained the endomembranes, particularly the perinuclear ER (Fig. 2A). However, when these cells expressed
PLIN5, punctuate structures that stained with both Nile Red and GFP-tagged PLIN5 became apparent, indicating that PLIN5 was able to induce formation of LDs in a pab1Δ mutant background. The LDs formed under these conditions were not only enriched in neutral lipids as indicated by the Nile Red staining, but they also contained a bona fide LD marker protein, Erg6, as indicated by the colocalization of Erg6-RFP with GFP-tagged human PLIN5 (Fig. 2B).

In mammalian adrenocortical cells some perilipins are preferentially stabilized in the presence of exogenous lipids, either oleic acid or cholesterol, indicating that they specifically target to LDs enriched with either STE or TAG. PLIN5, for example, preferentially targets

![Figure 3](https://www.tandfonline.com/e1071728-3)

**Figure 3.** PLIN5 localizes to LDs irrespective of whether they are filled with either TAG or STE. GFP-PLIN5 was expressed in are1Δ are2Δ or in dga1Δ iro1Δ double mutant strains, cells were stained with Nile Red and analyzed by confocal microscopy as described for Figure 1. Arrows in the merge indicate colocalization. Bar, 5 μm.
to TAG containing LDs at the cell periphery. To examine whether PLIN5 would exhibit target-specificity also when expressed in yeast, we analyzed its localization in cells lacking the 2 ACAT (acyl-CoA cholesterol acyltransferase) enzymes Are1 and Are2, as well as in cells lacking the TAG biosynthetic enzymes Dga1 and Lro1. Both human and mouse PLIN5 co-localized with Nile Red positive punctuate structures in both double mutants strains, indicating that LD-targeting of PLIN5 did not exhibit a strong preference for LDs subtypes enriched with either STE or TAG (Fig. 3). In addition, the Nile Red stained punctuate structures to which PLIN5 localized in

**Figure 4.** PLIN5 co-localizes with the LD marker protein Erg6 on LDs filled with either TAG or STE. GFP-PLIN5 and Erg6-RFP were co-expressed in are1Δ are2Δ or in dga1Δ lro1Δ double mutant strains and cells were analyzed by confocal microscopy. Arrows in the merge indicate colocalization. Bar, 5 μm.
cells synthesizing either STE or TAG also contained the LD marker Erg6, indicating the formation of functional LDs (Fig. 4). Taken together, the results presented here indicate that PLIN5 is targeted to LDs when expressed in yeast, that the protein is expressed at increased steady-state levels in the presence of LDs and that it promotes LD formation from the ER membrane of a sensitized strain which accumulates high levels of neutral lipids within its ER bilayer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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