Lipid droplets (LDs) are key lipid storage structures in cells. One might think that they simply result from lipid molecules separating out within the aqueous intracellular environment. However, closer investigation has revealed that LDs are well-organized organelles surrounded by proteins that tightly control lipid entry into and exit from these organelles.

One LD-associated protein is perilipin 1 (PLIN1) \(^\dagger\). It is a highly abundant protein in fat cells (adipocytes) and the first protein identified on the LD surface (Fig. 1), as reported in a JBC paper recognized as a Classic here \(^\ddagger\), authored by Andrew Greenberg (Fig. 2), his late mentor Constantine Londos, and colleagues at the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

JBC reached out to Greenberg to learn about the discovery of PLIN1 and how this research milestone has spurred further investigations into fat storage and metabolism.

**How did you become interested in studying LDs?**

When we started this work, LDs had been identified under the microscope, but their roles in regulating lipid metabolism in cells and whole organisms were unclear. We knew little about the intracellular architecture of neutral lipid metabolism and the nature and localization of the proteins that regulate LD assembly and maintenance.

In earlier studies, LDs were found only in steatotic (fatty) liver and in the foam cells of atherogenic plaques. But as we studied the literature, we saw that they are present in almost all cell types. So we hypothesized that LDs serve as active centers for the storage and breakdown of neutral lipids.

These metabolic processes are critical for maintaining energy homeostasis in the body. For example, during feeding, the adipocyte rapidly stores food energy that exceeds the bodies’ current energy needs. Conversely, during fasting, adipocytes quickly release fatty acids into circulation.

So, by taking a closer look at LDs, we wanted to study these fundamental metabolic processes and identify the adipocyte proteins that regulate neutral lipid metabolism in response to fasting and feeding.

**What was known before your discovery of perilipin?**

We had some early clues that at least one protein strongly associates with lipids, but its identity remained unknown until our 1991 discovery. A 1978 JBC paper had reported the presence of a 62-kDa protein in the lipid fraction of cellular homogenates \(^\S\). Its size and phosphorylation behavior suggested in hindsight that it was PLIN.

**What prompted your study?**

In 1990, we became interested in investigating the role of a phosphatase that dephosphorylates hormone-sensitive lipase (HSL) and contributes to insulin actions that inhibit lipid breakdown (lipolysis) in adipocytes. To home in on the phosphorylation/dephosphorylation mechanisms, we loaded rat adipocytes with \(^{32}\)P-labeled P\(_\text{i}\) and incubated them with varying concentrations of the \(\beta\)-adrenoreceptor agonist isoproterenol in the absence and presence of insulin.

As reported in an earlier JBC paper \(^\dagger\), we observed that after cAMP-dependent protein kinase A (PKA) activation, the major hyperphosphorylated protein migrates as a 65–67-kDa protein doublet on SDS-PAGE. What surprised us was that when we fractionated the \(^{32}\)P-labeled adipocytes by homogenization and centrifugation, the phosphoprotein localized exclusively to the fat cake, which sparked our interest in learning more about its potential role in adipocyte metabolism.

**What did you find?**

We made several major observations \(^\dagger\). Over 95% of all radiolabeled phosphate that was incorporated into cellular proteins after the PKA stimulation went into PLIN1, making it the predominant PKA substrate in adipocytes. Immunocytochemical studies in differentiated 3T3-L1 adipocytes revealed that anti-perilipin immunoreactivity was present in well-defined ringlike patterns surrounding both large and small intracellular lipid droplets.
Our initial study uncovered PLIN1 as the first protein that’s localized specifically to the LD surface, suggesting that PLIN1 has an important role as a gatekeeper that regulates triglyceride metabolism in LDs. We also found that even in the absence of PKA activation, PLIN1 migrates as a 62-kDa phosphoprotein that also coats the LD surface. Finally, PLIN1 was most robustly expressed in adipocytes.

We also found that PLIN1 is specifically expressed in adipocytes. Later studies showed that PLIN1 is also expressed in sterologenic cells (reviewed in Ref. 5), in steatotic human livers (6), and in human foam cells of ruptured atherosclerotic plaques (7).

It seems that other kinases can also phosphorylate PLIN1; what are they, and what signaling pathways are involved?

Since our JBC paper on PLIN1, it has been demonstrated that, in addition to PKA, the activated form of cGMP-dependent protein kinase also hyperphosphorylates PLIN1 in adipocytes (8). Catecholamine-mediated activation of β-adrenergic receptors increases adenylyl cyclase activity, which raises the levels of cAMP and thereby activates PKA.

Conversely, metabolites such as adenosine bind to their own receptors and reduce adenylyl cyclase activity (2, 4). Additionally, when insulin binds to its receptor on adipocytes, this can activate cyclic nucleotide phosphodiesterase, which degrades cAMP, attenuating PKA activity and PLIN1 hyperphosphorylation (4, 9). Finally, the laboratory of Stephen Yeaman has demonstrated that the protein phosphatases PP1 and PP2A dephosphorylate PLIN1 within intact adipocytes (10).

What’s the main function of PLIN1 in LD maintenance?

PLIN1 mainly acts as a dynamic scaffold for proteins involved in the storage and hydrolysis of neutral lipids in LDs. It’s a very dynamic interplay; PKA-mediated hyperphosphorylation of PLIN1 enhances lipolysis by activating adipose tissue triglyceride lipase (ATGL) and localizes PKA-phosphorylated HSL to the LD surface (11). Without PKA activation, PLIN1 binds and sequesters the LD-binding protein CGI-58 (12). With PKA activation and ensuing PLIN1 phosphorylation, CGI-58 is released from PLIN1 and then binds to and increases ATGL activity (1).

Is PLIN1 the only LD-associated protein?

Since our 1991 paper in JBC describing PLIN1, four more proteins have been identified that resemble PLIN1 in both sequence and conserved motifs. Among these, PLIN2 and PLIN3 are expressed ubiquitously. PLIN1 and PLIN2 are present exclusively on LDs, whereas PLIN3, PLIN4, and PLIN5 have been found on the LD surface, the cytoplasm, and, in some cases, the nucleus.

PLINs have quite distinct roles in different cell types. For instance, depending on cell type, PLIN3 or PLIN4 binds initially to the nascent LD. In nonadipocytes, PLIN2 then binds to and displaces PLIN3 on the mature LDs. In adipocytes, after PLIN2 associates with developing LDs, PLIN1 binds to and displaces PLIN2 from the mature LD.

There are many other proteins at the LD surface, including the cell death–inducing DFFA-like effector (CIDE) proteins, which also inhibit the hydrolysis of stored triglycerides. Our knowledge about the properties of LD-coating proteins is growing, and investigations are under way to further define their roles in stabilizing LD-associated hydrophobic neutral lipids within the hydrophilic cytoplasm.

How is PLIN expression regulated?

PLIN protein expression is regulated at multiple levels. The nuclear transcription factor peroxisomal proliferator–activated receptor γ (PPARγ) binds the PLIN1 gene promoter and increases its activity (13). We have shown that proinflammatory cytokines such as tumor necrosis factor α (TNFα) block PPARγ activities and decrease PLIN1 mRNA and protein expression in adipocytes (14). Additionally, neutral lipids have been demonstrated to stabilize PLIN1 levels through a posttranslational mechanism (15).

What’s the main focus of current research into PLINs?

Researchers want to understand how PLINs are involved in regulating the activities of ATGL and autophagy that hydrolyze stored triglycerides and to delineate the functions of the PLIN isoforms.

Research on LDs has expanded into LD biogenesis, the role of phospholipids associated with the LD surface, identification and function of other proteins that associate with and traffic to LDs, and interactions of LDs with other intracellular organelles such as mitochondria.
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A growing body of work is investigating how PLINs or other LD proteins may act as nutrient and lipid sensors to link changes in LD metabolism to downstream signaling pathways.

Does PLIN1 have a role in metabolic disorders such as obesity and diabetes?

In obesity, inflammation in adipose tissues is marked by elevated levels of cytokines such as TNFα, which reduce the expression of both the PLIN1 gene and protein, resulting in increased adipocyte lipolysis and release of fatty acids into circulation. These fatty acids are taken up by the liver and can cause nonalcoholic fatty liver disease and hypertriglyceridemia (1).

These fatty acids can also alter insulin-signaling pathways and result in insulin resistance in hepatocytes and skeletal muscle cells, which may lead to diabetes. In steatotic human livers, PLIN1 expression is increased, and PLIN1 is localized around hepatocyte lipid droplets, likely promoting triglyceride accumulation (6).

Have any therapeutic strategies or agents emerged that target PLIN1?

We have shown that a class of antidiabetic drugs, the thiazolidinediones, bind to and activate PPARγ and can increase the levels of PLIN1 mRNA and protein and block TNFα’s effects on PLIN1 expression and rates of lipolysis (16). Thus, both PLIN1 and cytokines represent potential targets for ameliorating inflammation caused by fatty acid release from adipocyte LDs.

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