1. INTRODUCTION: STORAGE AND MOBILIZATION OF FAT IN ADIPOCYTES

When food intake provides more energy than is needed immediately, animals temporarily store the excess energy as triacylglycerol (TG) in adipocytes. On energy demand, the stored TG is hydrolyzed and supplied to various tissues as fatty acid (FA), a process called lipolysis. Coordination of energy storage and utilization is critical for maintaining a proper metabolic balance, and its failure results in prevalent diseases such as obesity, type 2 diabetes, and metabolic syndrome.

It has been known for more than 40 years that catecholamines can elicit a lipolytic response in adipocytes. However, the intracellular mechanism of lipolysis was not clear until recently. Catecholamines bind to \( \beta \)-adrenergic receptors (especially \( \beta \)-3 in adipocytes) on the cell surface. Adenylate cyclase is then activated through the action of a stimulatory G-protein (\( G_{\alpha s} \)), the intracellular level of cAMP is elevated, and then cAMP-dependent protein kinase (PKA) is activated. PKA phosphorylates two key proteins involved in lipolysis: hormone-sensitive lipase (HSL) and perilipin (Fig. 1).\(^1,2\) HSL was the first identified adipocyte lipase and was long thought to be the rate-limiting enzyme for TG degradation in adipocytes. It was initially believed that lipolytic activation in adipocytes results from an increase in the catalytic activity of HSL upon phosphorylation by PKA. However, recent studies revealed that the phosphorylation-dependent translocation of HSL from the cytosol to the surface of LDs in the regulation of fat mobilization in cells.

2. FUNCTION OF CGI-58 IN ADIPOCYTES

Perilipin: A Major Target of Catecholamine Signaling

Perilipin, an LD-associated protein, plays a critical role in the translocation of HSL. This idea arose from studies on perilipin-null mice.\(^3–5\) These mice are lean and have a markedly smaller mass of adipose tissue (ca. 70% smaller) compared with wild-type mice. This results from the high level of constitutive lipolysis in adipocytes. On the other hand, in perilipin-deficient adipocytes, lipolytic activation is reduced by hormonal stimulation. Importantly, HSL was partially distributed on the surface of LDs even in perilipin-deficient adipocytes in a quiescent state, which was not enhanced by hormonal stimulation. These observations led to the conclusion that perilipin blocks the access of HSL to LDs in quiescent adipocytes and thus restricts hydrolytic attack. On the contrary, upon lipolytic stimulation, multiphosphorylated perilipin loses its barrier function but facilitates the access of HSL to LDs, thereby promoting lipolysis (Fig. 1). Thus, perilipin plays a central role in adipocyte metabolism by switching between lipid storage and utilization. Indeed, the human
ATGL: A TG Lipase Responsible for Adipocyte Lipolysis

For many years, it was believed that HSL is mostly responsible for TG degradation in adipocytes. HSL-null mice, however, are not obese. They retain about a half of their TG lipase activity in adipocytes compared with wild-type mice but accumulate an abnormally large amount of intracellular diacylglycerol (DG).\(^{13,14}\) Hence, another lipase was assumed to be responsible for TG lipolysis, and ATGL (also termed desnutrin or iPLA$_2$\(\zeta\)) was identified by several groups.\(^{15–17}\) ATGL is predominantly expressed in adipose tissue and catalyzes the hydrolysis of TG to DG and FA. ATGL seems to account for the remaining lipolytic activity in HSL-null mice. ATGL is localized in the cytosol and on the surface of LDs; the C-terminal region likely being responsible for the regulation of ATGL-association with LD.\(^{18}\) ATGL-null mice display a twofold increase in whole-body fat mass and exhibit enlarged adipose fat depots compared with normal mice.\(^{19}\) Furthermore, isoproterenol-stimulated lipolysis is severely reduced in their adipocytes, and TG hydrolysis activity is decreased by ca. 80%. These results indicate that ATGL is rate-limiting in the catabolism of cellular TG, whereas HSL is mainly responsible for the hydrolysis of DG rather than TG.\(^{20}\) This notion is supported by the results of in vitro lipase assays.\(^{15}\)

ATGL requires CGI-58 for efficient enzyme function.\(^{9,10}\) Although CGI-58 itself does not have lipase activity, it interacts with ATGL and activates the enzyme activity of the latter in vitro. Thus, CGI-58 functions as a protein cofactor of ATGL, and mutant forms of CGI-58 associated with CDS lose the capacity to activate ATGL.\(^{9}\)

Functional Interplay of Proteins on Adipocyte LDs

The current view of the mechanism of regulation of lipolysis can be summarized as follows. In quiescent adipocytes, perilipin binds to CGI-58 on the surface of LDs, hence preventing CGI-58 from interacting with ATGL. Upon lipolytic stimulation by catecholamines, PKA phosphorylates perilipin, and phosphorylated perilipin releases CGI-58. CGI-58 is now free to interact with ATGL, and the resulting ATGL/CGI-58 complex efficiently degrades TG to DG and FA. DG is then hydrolyzed to monoacylglycerol (MG) and FA by monoglyceride lipase, and the reaction products finally enter the circulation.

Future Questions

An intriguing observation in adipocyte lipolysis is the dynamic morphological change of LDs upon catecholamine stimulation. During the course of stimulated lipolysis, large central LDs begin to fragment and disperse, whereas numerous micro-LDs coated with perilipin emerge within several hours (Fig. 2A).\(^{21}\) Additionally, using coherent anti-Stokes Raman Scattering (CARS) microscopy, which visualizes LDs in living cells, we observed micro-LDs emerging within 10 min after catecholamine stimulation of 3T3-L1 adipocytes.\(^{20}\) Micro-LDs may be physiologically significant in light of the possibility that they may increase the efficiency of lipolysis by increasing the total surface area of LDs. The distribution of LD-associated proteins to micro-LDs, including ATGL, HSL, and CGI-58, has also been reported.\(^{22}\) Further studies are required to clarify the physiological significance of micro-LDs in terms of the exact intracellular origin of micro-LDs as well as the translocation of micro-LD-associated proteins. We demonstrated that micro-LDs appeared in all areas of the cytosol, though not necessarily in the vicinity of pre-existing large LDs, raising the possibility that they are formed from organelles other than large LDs, e.g., the endoplasmic reticulum.\(^{10}\) On the other hand, given that the fragmentation of large LDs depends on the phosphorylation of perilipin on Ser492 and that the micro-LDs produced contain perilipin,\(^{23}\) it is also possible that micro-LDs are derived from large central LDs coated with perilipin. The current hypothetical model is depicted in Fig. 2B. The location of active lipolysis in adipocytes, large LDs, micro-LDs, or even the cytosol may be an important issue. The roles of individual LD-binding proteins should
the acylation of lysophosphatidic acid. Indeed, there is a CDS fibroblasts, and CGI-58 was suggested to be involved in lesion of phospholipid synthesis from TG was observed in each other both in adipocytes and in other cell types. A study, including the possibility that they act independently of obese. The functions of ATGL and CGI-58 need further Interestingly, patients with these diseases are not typically symptoms differ between deficiencies of ATGL and CGI-58. and problems in other organs are also often reported. Thus, further studies are required to determine whether the function of CGI-58 in lipoprotein assembly in hepatocytes is related to its putative function as a cofactor of ATGL or to acyltransferase activity.

The most prominent symptom of CDS is ichthyosis, which results from the malformation of a permeability barrier in the skin. Truncation of CGI-58 protein results in abnormal lamellar granule (LG) formation in CDS. LGs are multifunctional structures involved in lipid transport and secretion in keratinocytes and have an important role in forming the epidermal lipid barrier. CGI-58 is localized to LGs in keratinocytes and its expression is upregulated during keratinocyte differentiation and development of the skin in humans. Furthermore, CGI-58 knockdown reduces the expression of keratinocyte differentiation markers in cultured human keratinocytes. Thus, CGI-58 plays crucial roles in epidermal keratinocyte differentiation and LG lipid metabolism, thereby contributing to the formation of the skin’s lipid barrier.

4. CONCLUDING REMARKS

LDs have long been regarded simply as storage depots for excess lipid in cells. However, recently they have been recognized as independent organelles occurring in almost all types of eukaryotic cells and play important roles in lipid homeostasis. In the clinical field, it has long been known that LD functions are associated with disorders such as obesity, inflammation, and atherosclerosis. Many studies have shown that proteins associated with LDs regulate their multiple functions. There is little evidence of any direct relationship between a particular disease and an LD-associated protein; however, CGI58, the gene responsible for CDS, would be the first example of an association between a disorder and defects in the function of an LD-associated protein. CGI-58 is a key molecule for TG degradation in adipocytes and in almost all human cell types, although the molecular mechanisms underpinning the actions of this protein may not be uniform. CGI-58 facilitates TG hydrolysis as a coactivator of ATGL, likely via direct interaction between these proteins. On the other hand, CGI-58 acts as a Co-A-dependent lysophosphatic acid acyltransferase and produces phosphatic acid in the cell. It is unclear whether the role of CGI-58 as a coactivator of ATGL requires acyltransferase activity or whether CGI-58 has a separate function mediated by a protein-protein interaction between CGI-58 and lipase. Further understanding of the function of CGI-58 at the molecular level, such as the functional and structural interplay involving CGI-
58, ATGL, and PAT proteins in adipocytes as well as nonadipose cells, should contribute to both basic biology and the search for a suitable approach to the clinical treatment of CDS.

REFERENCES