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CELL BIOLOGY SYMPOSIUM: Imaging the organization and trafficking of lipolytic effectors in adipocytes[1],[2]

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Abstract

The storage and mobilization of lipid energy are central functions of adipocytes. Lipid energy is stored as triglyceride in lipid droplet structures that are now recognized as bona fide organelles and whose functions are greatly influenced by members of the perilipin family of lipid droplet scaffolds. Recent work indicates that the signaling events underlying fatty acid mobilization involve protein trafficking to a specialized subset of lipid droplets. Furthermore, the core lipolytic machinery is composed of evolutionarily conserved proteins whose functions are conserved in avian and mammalian production species. Lipolysis affects many aspects of animal nutrition and physiology, which can have an important influence on growth efficiency, lactation, and meat quality. This review focuses on recent research that addresses the organization and trafficking of key players in hormone-stimulated lipolysis, and the central role of perilipin1A in adipocyte lipolysis. The review emphasizes recent work from the laboratories of the authors that utilizes imaging techniques to explore the organization and interactions among lipolytic effectors in live cells during lipolytic activation. A mechanistic understanding of lipolysis may lead to new strategies for promoting human and animal health.

Keywords

adipose triglyceride lipase; alpha/beta hydrolase domain-containing 5; fluorescence resonance energy transfer; hormone sensitive lipase; perilipin; protein complementation

INTRODUCTION

The storage and mobilization of lipid are fundamental cellular processes, and multicellular organisms, from insects to mammals, have evolved specialized cells that store excess lipid energy for mobilization in times of need. In mammals, adipose tissue functions as a highly specialized lipid energy buffer that stores excess energy as triglycerides (**TG**) for systemic mobilization in the form of FFA. Adipocytes are a key source of fatty acids under various physiological conditions, such as fasting, exercise, and lactation (Sumner and McNamara, 2007; Elkins and Spurlock, 2009; Frayn, 2010), and in disease states like diabetes mellitus

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(Unger, 1995). Thus, alterations in adipose tissue lipolysis can have an important influence on growth, energy partitioning, and insulin sensitivity. Energy flow into and out of adipocytes also has important implications in animal agriculture because body fat content and distribution are key factors in determining meat quality and production efficiency (Hausman et al., 2009). Several key players regulating adipocyte lipolysis are highly conserved evolutionarily, including in the chicken, quail (Lee et al., 2009; Serr et al., 2009), and pig (Deiuliis et al., 2008). Therefore, a better understanding of energy storage and mobilization in rodent adipocytes should shed light on new production strategies that would allow for selective lipid deposition into desirable fat depots, such as marbling adipose tissue, without sacrificing overall production efficiency.

Although it is well established that TG stores are dynamically regulated, the cellular and molecular bases of this dynamic regulation are just now being revealed. Recent work indicates that storage and mobilization of intracellular lipid involves the assembly of specialized subcellular structures (i.e., lipid droplets), the targeting of unique protein scaffolds and enzymes, and the dynamic trafficking of lipases and regulatory proteins (Granneman and Moore, 2008).

KEY PLAYERS IN ADIPOCYTE LIPOLYSIS

Adipocyte lipolysis is a multifaceted phenomenon that is subject to distinct temporal controls, many of which are poorly understood. This review addresses protein trafficking during rapid, hormone-stimulated lipolysis (for recent reviews on the general regulation of lipolysis see Langin and Arner, 2006; Duncan et al., 2007), and focuses on work from our laboratory that examines the organization and trafficking of lipolytic effector proteins. Interest in lipolytic protein trafficking originates from the seminal work of Londos et al. (1999, 2005). These investigators demonstrated that hormone-stimulated lipolysis is directly related to the amount of protein kinase A (**PKA**) activation (Honnor et al., 1985a,b). Thus, major signals that acutely inhibit (e.g., insulin and adenosine) or stimulate (e.g., catecholamines) lipolysis converge at the level of PKA (Figure 1). In addition, Londos and colleagues discovered perilipin (**PLIN1**) as a major lipid coat protein in adipocytes, and demonstrated that this protein is the dominant target of PKA activation (Greenberg et al., 1991). Lastly, Londos and colleagues showed that hormone-sensitive lipase (**HSL**), itself a target of PKA, translocates to lipid droplet surfaces during PKA activation (Egan et al., 1992). Together, these observations provided a conceptual framework whereby lipolysis is regulated by the PKA-dependent trafficking of proteins to lipid droplet surfaces (Figure 1).

Lipid Droplets and Droplet Scaffold Proteins

Virtually all cells can accumulate small amounts of neutral lipid in structures termed lipid droplets (**LD**) or lipid bodies. Until relatively recently, these structures were considered to be inert repositories of lipid; however, recent proteomic and lipidomic experiments have clearly established that LD are heterogeneous, dynamic organelles involved in the synthesis, movement, and degradation of lipid (Brasaemle et al., 2004; Liu et al., 2004; Bartz et al., 2007b; Zehmer et al., 2009).

Lipid droplets of adipocytes are thought to form in a specialized subdomain of the endoplasmic reticulum (Martin and Parton, 2006) and are sequentially bound by several proteins containing conserved **PAT** [perilipin, adipose differentiation-related protein, tail-interacting protein-47 (**TIP-47**)] domains as they migrate and enlarge (Wolins et al., 2003, 2006; Nagayama et al., 2007). The biology of PAT proteins is an active area of research, and accumulating evidence indicates that specific PAT proteins play specialized roles in LD biology (Londos et al., 2005; Wolins et al., 2006; Brasaemle et al., 2009). Members of the perilipin protein family have been functionally classified into those that translocate between

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the cytoplasm and LD surface and those that are constitutively bound to the LD surface (Wolins et al., 2006). Exchangeable PAT appear to be involved in the assembly and enlargement of LD and include PLIN4 (S3–12), PLIN3 (also known as TIP-47), and to some extent PLIN5 (also known as muscle lipid droplet protein and oxidative-PAT). Perilipin homologs that are constitutively bound to LD (i.e., cPAT) include PLIN1 (i.e., perilipin) and **PLIN2** (also known as adipose differentiation-related protein and adipophilin). Perilipin2 expression is upregulated during LD formation and is induced by conditions that expose adipocytes to increase quantity of fatty acid (Gao et al., 2000; Granneman et al., 2005); however, mature white adipocytes normally express little if any PLIN2 in vivo. Both PLIN1 and PLIN2 are degraded when cytosolic and are stabilized when targeted to LD (Brasaemle et al., 1997a,b).

Of the PAT proteins expressed in adipocytes, there is compelling evidence that perilipin1A (**PLIN1A**), a PLIN1 splice variant, plays a central role in orchestrating hormone-stimulated lipolysis in adipocytes (as discussed subsequently in detail). The major LD PAT protein of fully differentiated adipocytes is PLIN1 (Greenberg et al., 1991, 1993). Perilipin1A can be phosphorylated at up to 6 sites by PKA and is the major target of PKA phosphorylation in adipocytes (Greenberg et al., 1991). Recent evidence indicates that PLIN1 is a multifunctional protein, capable of reducing basal lipolysis, promoting lipolysis after PKA activation, and controlling LD fragmentation through mechanisms that are lipase dependent and lipase independent (Brasaemle et al., 2009).

Genetic deletion of *Plin* in mice largely abrogates hormone-stimulated lipolysis (Tansey et al., 2001; Miyoshi et al., 2006). Furthermore, expression of wild type PLIN1A, but not phosphorylation-defective mutants, restores PKA-dependent lipolysis in adipocytes differentiated from *Plin1* null mice (Miyoshi et al., 2006, 2007). Reconstitution experiments in heterologous (non-fat cell) systems demonstrate that PLIN1A confers the ability of PKA to increase HSL-dependent and HSL-independent lipolysis (Souza et al., 2002; Sztalryd et al., 2003; Tansey et al., 2003). It is important to note that PLIN1 regulation of lipolysis is complex and involves phosphorylation-dependent and phosphorylation-independent interactions among different functional domains of the protein (Zhang et al., 2003).

In summary, adipocytes express multiple PAT proteins depending on developmental and nutritional status, and these proteins play distinct roles in the generation and maintenance of LD. Of perilipin (**PLIN**) family members, PLIN1A is the most abundant in mature unilocular fat cells, where it is centrally involved in the organization and regulation of lipolytic effector interactions in both basal and stimulated states.

Lipases

HSL—Until recently, HSL (UniGene name: LIPE) was considered to be the major, if not exclusive, lipase mediating hormone-stimulated lipolysis (reviewed in Holm, 2003; Yeaman, 2004). Hormone-sensitive lipase exhibits strong diglyceride hydrolase activity that is 10- and 5-fold greater than its activity against TG and monoglyceride substrates, respectively (Belfrage et al., 1978). Interestingly, phosphorylation of HSL by PKA increases its activity toward TG, but not aqueous esterase substrates (Yeaman, 1990). Hormone-stimulated lipolysis (measured by cellular FFA release) is significantly impaired, but not eliminated, in HSL knockout mice. Adipocytes of HSL null mice are largely incapable of releasing glycerol and have massive accumulation of cellular diacylglycerol, clearly demonstrating the importance of HSL as a diglyceride lipase (Haemmerle et al., 2002).

Adipose Triglyceride Lipase—The fact that loss of HSL does not abolish hormone-stimulated lipolysis indicated the existence of another TG lipase in adipose tissue. The identification of a second major lipase, adipose triglyceride lipase [**ATGL**; also known as

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desnutrin (TTS-2.1); UniGene name: PNPLA2; Jenkins et al. (2004); Villena et al. (2004); Zimmermann et al. (2004)] and the discovery that CGI-58 (UniGene name: **ABHD5**), a PLIN1-interacting protein (Subramanian et al., 2004; Yamaguchi et al., 2004), is a key co-activator of ATGL (Lass et al., 2006) greatly advanced our understanding of the biochemical basis of adipose tissue lipolysis. Adipose triglyceride lipase is a member of the patatin-domain-containing family of proteins (Kienesberger et al., 2009). Although most abundant in fat, ATGL is found in numerous tissues (Villena et al., 2004; Zimmermann et al., 2004). Adipose triglyceride lipase has strong TG hydrolase activity, but no activity against diglyceride or monoglyceride substrates (Zimmermann et al., 2004). Interestingly, ATGL has reduced, but detectable, transacylase and phospholipase activities (Jenkins et al., 2004; Notari et al., 2006); however, it is presently unclear how these activities of ATGL are regulated in adipocytes or if they are involved in hormone-stimulated lipolysis. Adipose triglyceride lipase is not a direct PKA target (Zimmermann et al., 2004); thus, its activation by hormones must involve indirect mechanisms.

Genetic disruption of *Atgl* in mice results in massive lipid accumulation in muscle (cardiac and skeletal), liver, kidney, and testes (Haemmerle et al., 2006). Adipose triglyceride lipase deletion in mice reduces adrenergic activation of lipolysis by more than 70% in white fat explants, corresponding to the loss in total TG hydrolase activity. Similar results have been observed using shRNA knock-down in cultured cells (Kershaw et al., 2006; Miyoshi et al., 2007). Adipose triglyceride lipase null mice are largely incapable of lipid mobilization during fasting and thus rely heavily on glucose as an energy source. Human mutations of ATGL have been discovered that result in ectopic lipid accumulation and myopathy, demonstrating the importance of this lipase in humans (Akiyama et al., 2007; Fischer et al., 2007; Kobayashi et al., 2008). Interestingly, a C-terminal truncation mutation of ATGL that abrogates activity in vivo does not inhibit activity in vitro; rather, the mutation results in mistargeting of the protein away from LD in vivo (Schweiger et al., 2008). These observations further reinforce the importance of lipase targeting and trafficking in the regulation of lipolysis.

ABHD5—Alpha/beta hydrolase domain-containing 5, also known as *CGI-58* and 1-acylglycerol-3-phosphate O-acyltransferase, belongs to the esterase/lipase subfamily of proteins containing α/β hydrolase folds. However, unlike bona fide lipases, the predicted catalytic serine within the consensus GXSXG motif contains asparagine (Lefèvre et al., 2001), and thus ABHD5 exhibits no lipase activity (Lass et al., 2006; Yamaguchi et al., 2007). Rare homozygous mutations of ABHD5/CGI-58 result in Chanarin-Dorfman syndrome (MIM 27630) that is characterized by ectopic lipid accumulation in numerous tissues (Lefèvre et al., 2001; Akiyama et al., 2003). A major advance in understanding the function of ABHD5 came with the discovery that ABHD5 dramatically increases the TG hydrolase activity of ATGL owing to a direct interaction between these proteins (Lass et al., 2006). A second key feature of ABHD5, discussed below, is its ability to associate with PAT proteins, in particular PLIN1 and PLIN5 (Subramanian et al., 2004; Yamaguchi et al., 2004; Granneman et al., 2009b). Recently, ABHD5 was found to mediate the acylation of lysophosphatidic acid (Ghosh et al., 2008; Montero-Moran et al., 2010). The significance of this activity is presently unclear; however, mutations of ABHD5 that result in Chanarin-Dorfman syndrome do not affect this acyltransferase activity. It is important to note that although null mutations of ABHD5 and ATGL both result in ectopic lipid accumulation, the phenotypes differ considerably and point to unique functions of these interacting proteins (Radner et al., 2010).

Other Lipases and the Biological Significance of HSL vs. ATGL—A proteomic survey using activity-based probes concluded that ATGL, HSL, and monoglyceride lipase are the major lipases in mouse adipose tissue (Birner-Gruenberger et al., 2005), whereas

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combined pharmacological, immunological and genetic approaches strongly indicate that ATGL and HSL constitute greater than 90% of TG hydrolase activity in fat cell extracts (Claus et al., 2005; Schweiger et al., 2006). It is important to remember, however, that TG hydrolase activities against artificial TG emulsions may not reflect activity against biological substrates within the cell (Schweiger et al., 2008). Adipose triglyceride lipase and HSL appear to work in concert during PKA activation because of their complementary enzymatic activities. Moreover, the relative contribution of each of these lipases is unlikely to be fixed, but rather will depend on nutritional and hormonal factors (Villena et al., 2004) and the relative abundance of ATGL activators, such as ABHD5, and inhibitors, such as G0S2 (Yang et al., 2010).

Lastly, it is important to note that the neutral lipid core of adipocyte LD is surrounded by a monolayer of phospholipid (Blanchette-Mackie et al., 1995), and it has been proposed that the monolayer phospholipid composition plays a role in the activity of lipolytic effectors (Okuda et al., 1994). In this regard, a functional genetic screen of genes involved in LD formation in Drosophila S2 cells demonstrated the significance of phospholipid metabolism on LD number and size (Guo et al., 2008). It is worth noting that both ABHD5 and ATGL can directly affect phospholipid metabolism, and it is conceivable that their roles as lipolytic effectors may involve alterations in the LD phospholipid monolayer.

ORGANIZATION OF LIPOLYTIC PROTEINS: IMMUNOHISTOCHEMCIAL AND BIOCHEMICAL ANALYSES

PLIN1

As mentioned previously, there is strong evidence that lipolysis occurs at LD that have a unique protein composition, and that PLIN1 is indispensable in regulating the access and activity of lipases at these LD surfaces. Thin-section immunoelectron microscopic analysis of adipocytes demonstrated that PLIN1 is highly targeted to the lipid droplet surface (Blanchette-Mackie et al., 1995). Given its location and effect on basal lipolysis, PLIN1 was initially hypothesized to form a protective barrier that shielded TG from cellular lipases, and it was also hypothesized that PKA phosphorylation led to the dissociation of PLIN1, loss of the protective barrier, and subsequent attack by intracellular lipases. However, recent results indicate that PLIN1 does not form a continuous barrier at the LD surface, but rather may provide a scaffold for the targeting and trafficking of lipolytic effectors. First, the abundance of PLIN1 protein is largely unrelated to the adipocyte LD surface area (D'Eon et al., 2005; Nikonova et al., 2008), and confocal immunofluorescence analysis of PLIN1 indicates a discontinuous pattern on the LD surface (Blanchette-Mackie et al., 1995; Moore et al., 2005). In cultured fat cells, PLIN1 is heavily targeted to small LD, whereas large LD, which contain the bulk of cellular TG, have relatively low concentrations (Moore et al., 2005). We have recently used Fluoronanogold immunocytochemistry to directly correlate fluorescence confocal images with high resolution transmission electron micrographs of the LD surface of primary mouse fat cells. The results indicate that although PLIN1 associates with a variety of intracellular structures and is heavily targeted to microdroplets, its density is low on vast regions of the LD surface (V. A. Kimler and J. G. Granneman, unpublished results). To the extent that PLIN1 defines where lipolysis occurs, these observations indicate there is heterogeneity with respect to amount and location of droplet structures that are competent for hormone-stimulated lipolysis (Moore et al., 2005).

ABHD5

Alpha/beta hydrolase domain-containing 5 was independently discovered by 2 groups as a PLIN1-interacting protein (Subramanian et al., 2004; Yamaguchi et al., 2004). Alpha/beta hydrolase domain-containing 5 is cytosolic in preadipocytes, which do not express PLIN.

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Endogenous expression in adipocytes or ectopic expression of PLIN in preadipocytes results in targeting of ABHD5 to LD (Subramanian et al., 2004). Double-label immunofluorescence analysis indicates that ABHD5 and PLIN are highly co-localized in un-stimulated adipocytes (Subramanian et al., 2004; Granneman et al., 2007). Phosphorylation of PLIN by PKA rapidly decreases its proximity to ABHD5, although almost all ABHD5 remains localized to PLIN1-containing LD during the onset of lipolysis. However, ABHD5 becomes progressively cytosolic, during sustained stimulation, whereas PLIN1 remains on LD surfaces (Granneman et al., 2007; Yamaguchi et al., 2007). Experiments performed in vitro demonstrated that ABHD5 binds directly to PLIN1, but not phosphorylated PLIN1 (Yamaguchi et al., 2004).

ATGL

In the basal state, ATGL is found in the cytoplasm (Villena et al., 2004; Notari et al., 2006; Granneman et al., 2007) and to a lesser degree on LD (Zimmermann et al., 2004; Bartz et al., 2007b), including those containing PLIN (Granneman et al., 2007). Stimulation elicits minor translocation of ATGL to LD (Granneman et al., 2007), but does not improve the co-localization of ATGL with PLIN. Unlike HSL, ATGL does not interact with PLIN1, as judged by fluorescence resonance energy transfer (**FRET**) and bimolecular fluorescence complementation assays (Granneman et al., 2007). Interestingly, immunofluorescence studies of cultured adipocytes indicate that forskolin increases the co-localization of ABHD5 and ATGL. Because stimulation does not alter the overall co-localization of ATGL and PLIN, the stimulation-induced increase in co-localization of ABHD5 and ATGL may involve subcellular structures that lack PLIN.

HSL

Biochemical experiments have shown that HSL is almost exclusively found in the cytoplasm in the basal state and translocates to neutral lipid fractions after PKA activation (Egan et al., 1992; Clifford et al., 2000; Martin et al., 2009). Detailed immunofluorescence analysis has shown that phosphorylation of HSL on serine 659 and 660 occurs within 5 s of cell stimulation and is required for the rapid accumulation of the lipase on LD surfaces (Su et al., 2003; Martin et al., 2009). Protein kinase A-induced HSL translocation to LD is impaired in cells lacking PLIN and is enhanced by ectopic PLIN expression (Sztalryd et al., 2003; Miyoshi et al., 2006). Interestingly, although PKA-induced HSL translocation in adipocytes requires PLIN, it does not require PLIN phosphorylation (Miyoshi et al., 2006).

DYNAMIC IMAGING OF LIPOLYTIC EFFECTORS IN LIVE CELLS

Immunochemical and biochemical experiments indicate a model in which PLIN1 acts as a dynamic scaffold that regulates the trafficking and activity of lipolytic effectors at the LD surface. We recently proposed a model of hormone-stimulated lipolysis in which PLIN1 regulates lipolysis by direct and indirect mechanisms (Figure 2), and we are testing the model using dynamic imaging techniques in live cells. First, we propose that PLIN1 indirectly regulates ATGL activity by controlling the availability of its protein activator, ABHD5, in a PKA-dependent fashion. Second, we hypothesize that PLIN1 facilitates HSL-mediated lipolysis by providing a docking site on the LD where HSL gains access to TG and diglyceride substrates.

According to the model, an important function of PLIN1 is to sequester ABHD5 from ATGL in the un-stimulated state and thereby suppress basal lipolysis. Protein complementation assays were used to assess the protein-protein interactions in live cells and demonstrated that the interaction of ABHD5 with PLIN1 in the basal state is far stronger that its interaction with ATGL (Granneman et al., 2009a). Importantly, ABHD5 can interact

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with PLIN1 or ATGL, but not with both simultaneously. Thus, expression of PLIN1 dramatically reduces the interaction of ABHD5 with its target lipase and provides a mechanism whereby PLIN1 can suppress lipolysis without acting as a barrier. This mechanism was confirmed by siRNA experiments showing that disinhibition of lipolysis after knockdown of PLIN1 requires both ATGL and ABHD5 (Granneman et al., 2009a).

We have used FRET to image the dynamic interaction of fluorescently tagged PLIN1 and ABHD5 live cells (Granneman et al., 2007). The FRET signals are generated when proteins are in extremely close proximity (<8 nm), as occurs during direct interactions. Figure 3 illustrates the results of a typical experiment. In the basal state, PLIN1 and ABHD5 are nearly perfectly co-localized, and the interaction of PLIN1 and ABHD5 supports strong energy transfer. Brief stimulation of PKA with forskolin rapidly (i.e., within 1 min) decreases the proximity between these proteins as evidenced by the pronounced decrease in the FRET signal. Interestingly, over the time period in which lipolysis is maximally activated (i.e., <10 min) no loss of ABHD5 or PLIN1 from the LD surface is observed and ABHD5 may remain as a complex with PLIN1A during the initiation of lipolysis. As mentioned previously, PLIN1A has 6 potential sites for PKA phosphorylation and mutational analysis indicates that phosphorylation of either PKA site 5 or 6 is sufficient to reduce the proximity between ABDH5 and PLIN1 (Granneman et al., 2009a).

Biochemical analyses have demonstrated that ATGL is critical for triggering hormone stimulated lipolysis and that PLIN1 is involved in this process (Miyoshi et al., 2007). Unlike HSL, ATGL is not a direct target of PKA, nor does ATGL bind to PLIN1. However, release of ABHD5 provides a mechanism whereby PLIN1 can indirectly regulate ATGL in a PKA-dependent fashion. Tests of this model using protein complementation demonstrated that PKA regulates the interaction of ABHD5 with its target lipase in a manner that requires phosphorylation of PLIN1 on the same sites that promote release of ABHD5. Previous immunocytochemical analyses, which assess static subcellular co-localization, suggested that ABHD5 and ATGL interact on structures lacking PLIN1. However, bimolecular fluorescence complementation (**BiFC**) experiments, which monitor dynamic protein-protein interactions directly, demonstrated that the interaction of ABHD5 and ATGL occurs mainly on LD that contain PLIN1 (Granneman et al., 2009a). This is illustrated in Figure 3 (middle row), in which the BiFC signal is reduced in the basal state and is rapidly increased upon stimulation. Furthermore, the increased BiFC signals co-localize precisely with that of fluorescently tagged PLIN1A.

There are substantial biochemical and immunochemical data demonstrating that phosphorylation of HSL provokes its translocation to lipid droplets and that PLIN1 is involved in this process. An example of dynamic imaging of fluorescently tagged HSL is shown in the Figure 3 (bottom row). Tagged HSL is largely cytosolic in the basal state and stimulation leads to its rapid accumulation on PLIN1A-containing LD (Granneman et al., 2007). Translocation of HSL begins within 1 min after forskolin stimulation and is essentially complete by 5 min. Interestingly, accumulation of HSL on individual LD occurs in direct proportion to PLIN1 concentration. Experiments using FRET, protein complementation, and chemical cross-linking indicate that PKA activation generates a complex containing PLIN1 and HSL (Miyoshi et al., 2006; Granneman et al., 2007; Shen et al., 2009). However, analysis of phosphorylation-defective mutants of PLIN1 in adipocytes strongly indicates that HSL translocation is not sufficient to initiate lipolysis in the absence of PLIN phosphorylation (Miyoshi et al., 2006). Thus, PLIN phosphorylation affects HSL activity beyond its ability to promote translocation, but it is not known whether this effect involves direct interactions with phosphorylated PLIN or is brought about indirectly by such factors as ATGL activation (and generation of diglyceride substrate) or droplet remodeling.

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SUMMARY AND CONCLUSIONS

A growing body of evidence indicates that PLIN1 plays a critical role in organizing LD proteins and regulating trafficking at the LD surface. Perilipin1 clearly regulates the activity and accessibility of cellular lipases during PKA activation; however, the biochemical and biophysical bases are uncertain. In this regard, it is unclear what roles the conserved acyltransferase activity of ABHD5 or phospholipase activity of ATGL might play in lipolysis and lipid droplet dynamics. Additionally, PLIN1 mediates lipase-independent LD fragmentation and dispersion, suggesting additional functions of the protein (Marcinkiewicz et al., 2006).

The PLIN1 is expressed almost exclusively in adipocytes, yet most mammalian cells store and mobilize small amounts of TG. How the interactions of lipolysis are regulated in tissues lacking PLIN1, such as muscle and liver, is an important area of future research. Available evidence indicates that specific PLIN homologs contribute to cell-specific regulation of stored TG. In this regard, we have recently shown that PLIN5, which is enriched in muscle and liver, binds ABHD5; however, PLIN5 facilitates the interaction of ABHD5 and ATGL in the basal state, whereas Plin1 suppresses it (Granneman et al., 2009a,b). This interaction between ABHD5 and PLIN5 appears to promote basal lipolysis, but may not be involved in stimulated lipolysis because forskolin does not affect FRET between ABHD5 and PLIN5 (J. G. Granneman, unpublished results).

Much of the basic research into the molecular components of lipolysis has been performed on rodents or in cell culture models. Nonetheless, the basic elements of the lipolytic machinery are evolutionarily conserved, and research in production animals is beginning to emerge. Adipose triglyceride lipase is present in poultry, pigs, and cattle (Deiuliis et al., 2008; Nie et al., 2009) and is upregulated by fasting in chickens and pigs, as it is in rodents (Villena et al., 2004; Serr et al., 2009; Zhao et al., 2009). Furthermore, SNP in the ATGL gene have been associated with growth and fat content in chickens (Nie et al., 2010), and variations in ATGL mRNA expression have been correlated with leanness in pigs (Zhao et al., 2009). These observations suggest that a greater understanding of LD biology and lipolytic protein interactions may lead to new strategies for improving growth efficiency and meat quality of production animals.

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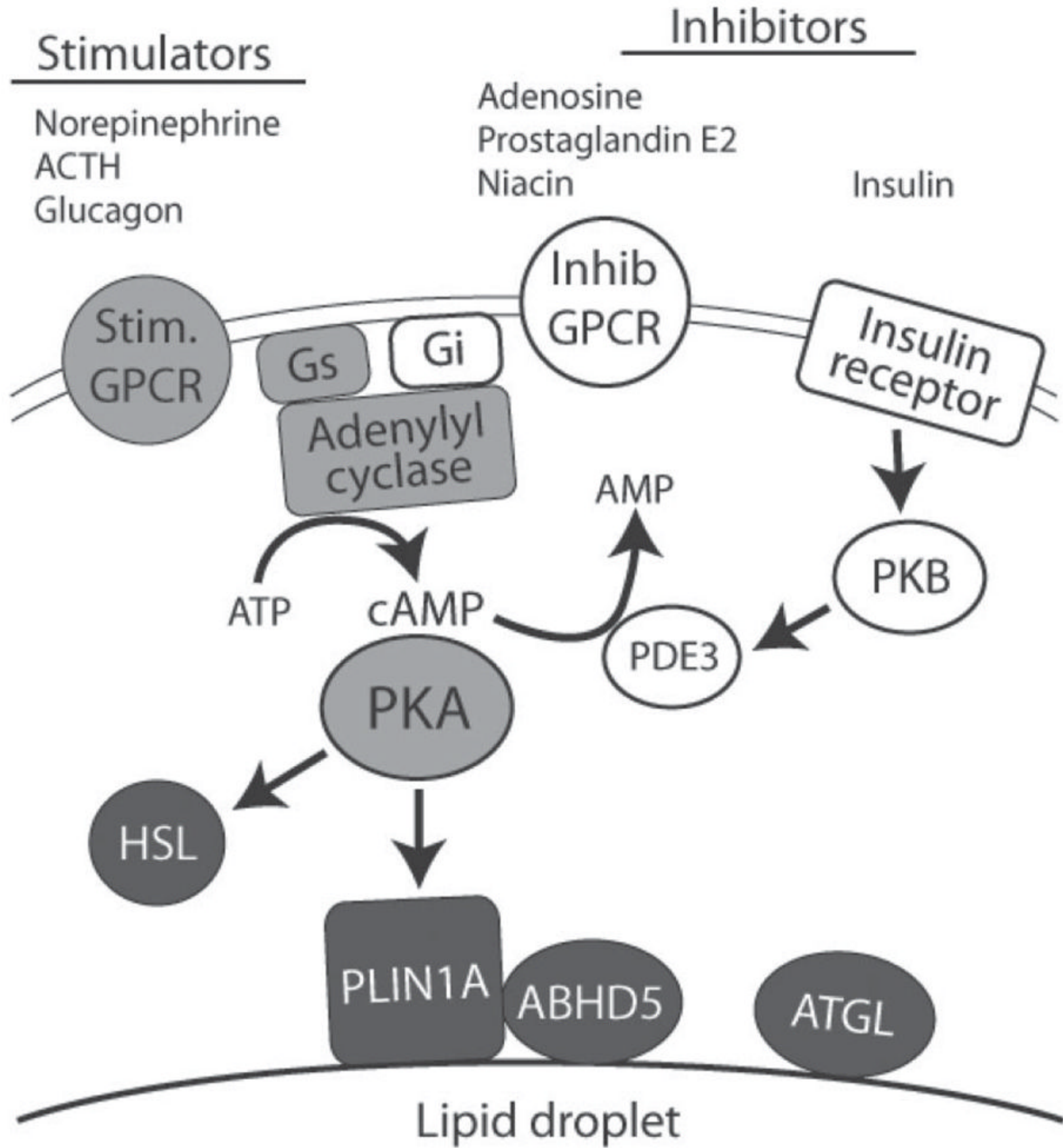

Figure 1.
Rapid regulation of adipocyte lipolysis of protein kinase A (PKA). Adipocyte lipolysis is regulated by diverse stimulatory (Stim.) and inhibitory (Inhib) ligands acting through G-protein coupled receptors (GPCR) that transverse the plasma membrane bilayer. G-protein coupled receptor-generated signals are integrated by adenylyl cyclase, which generates cAMP. In addition, insulin suppresses lipolysis through protein kinase B (PKB)-dependent activation of phosphodiesterase 3 (PDE3), which degrades cyclic adenosine monophosphate (cAMP) to adenosine monophosphate (AMP). Signals controlling lipolysis converge at PKA, which directly phosphorylates hormone-sensitive lipase (HSL) and perilipin1A (PLIN1A), the major PKA substrate in fat cells. Perilipin1A is targeted to the surface of lipid droplets that are covered by a phospholipid monolayer. Adipose triglyceride lipase (ATGL) and its co-activator, α/β hydrolase domain-containing 5 (ABHD5), are key

elements PKA-regulated lipolysis, yet neither appears to be a direct target of PKA. Gs and Gi are the stimulatory and inhibitory G-proteins, respectively, of adenylyl cyclase.

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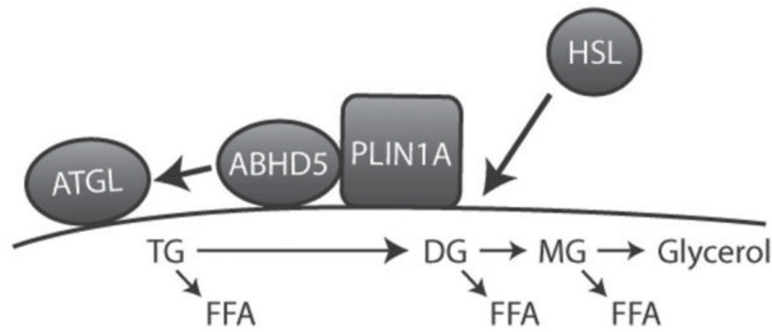

Figure 2.

Protein trafficking during initiation of lipolysis. Under basal conditions, perilipin1A (PLIN1A) and α/β hydrolase domain-containing 5 (ABHD5) form a complex on the surface of lipid droplets (LD). Hormone-sensitive lipase (HSL) is mainly cytosolic, whereas adipose triglyceride lipase (ATGL) is localized to LD, including those containing perilipin (PLIN). Stimulation by protein kinase A (PKA) activation leads to trafficking of HSL and ABHD5 (arrows). Phosphorylation of PLIN1A induces a conformational change that decreases its proximity to ABHD5, which allows ABHD5 to activate ATGL. Because ATGL acts exclusively on triglyceride (TG), it is likely that ATGL initiates generation of FFA. Phosphorylation of HSL promotes its translocation and tight association with PLIN1. A major component of HSL activity likely depends on generation of diglyceride (DG) substrate from the action of ATGL, whereas monoglyceride lipase (MG) acts to liberate glycerol and the final FFA. Not illustrated are potential effects of PLIN phosphorylation on the biophysical properties of the LD surface and the effects of sustained activity on LD fragmentation and movement.

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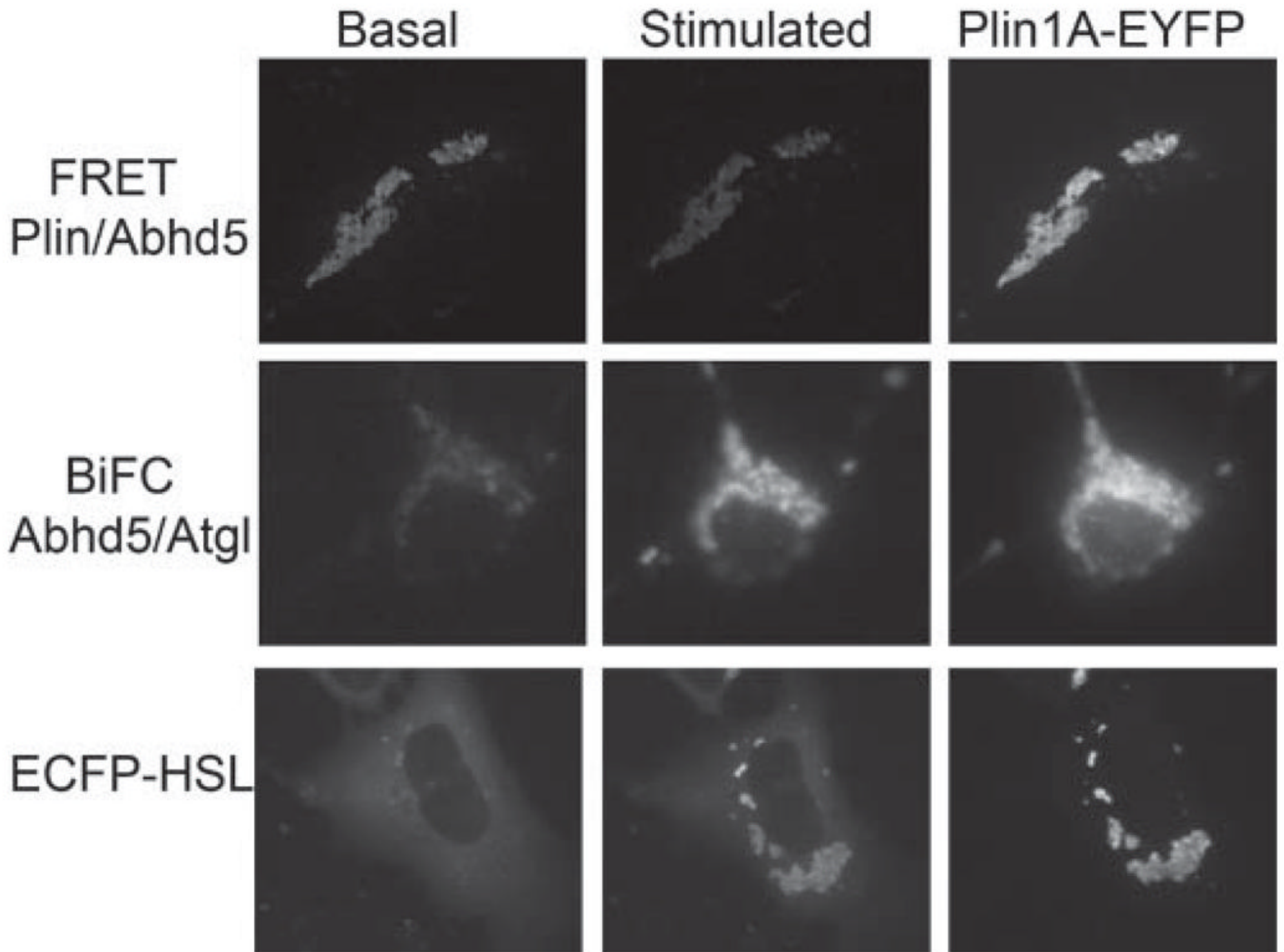

Figure 3.

Imaging protein trafficking in live cells by confocal fluorescence microscopy. Top row: Imaging the interaction of α/β hydrolase domain-containing 5 (ABHD5) and perilipin1A (PLIN1A) by fluorescence resonance energy transfer (FRET). In the basal state, ABHD5 and PLIN1A are in close molecular proximity and exhibit strong fluorescence energy transfer between enhanced cyan fluorescent protein (ECFP) and enhanced yellow fluorescent protein (EYFP) tags. Stimulation with forskolin rapidly decreases the association and is indicated by diminished FRET signal. Perilipin1A fused to EYFP shows the intracellular location of lipid droplets (LD). Middle row: Imaging the interaction of ABHD5 and adipose triglyceride lipase (ATGL) using bimolecular fluorescence complementation (BiFC). In the basal state, there is low interaction between ABHD5 and ATGL as indicated by weak fluorescence. Stimulation greatly increases the interaction, resulting in stronger BiFC signal, which co-localizes precisely to LD marked with PLIN1A-EYFP. Lower panel: Stimulationinduced accumulation of HSL on LD containing PLIN1A. In the basal state, ECFP-hormone sensitive lipase (HSL) is largely cytosolic, and stimulation leads to rapid and precise accumulation on LD-containing PLIN1A-EYFP.