Peroxisomal-PEX5 Controls Fasting-Induced Lipolysis

Ji Seul Han1, Kyung Hee Han1, and Jae Bum Kim1

Abstract
Lipid droplets (LDs) are dynamic subcellular organelles which play critical roles for lipid homeostasis upon change of nutritional state. Although several organelles such as mitochondria and peroxisomes are involved in lipid metabolism, physiological roles and mediators involved in the spatiotemporal regulation of these subcellular organelles for energy metabolism has largely remained elusive. Our recent study implicates the importance of peroxisomes in the translocation of lipases onto LDs upon fasting cues. Also, we found that peroxisomal protein PEX5 modulates PKA-induced lipolysis by escorting ATGL toward LDs. This is accompanied by KIFC3-mediated migration of peroxisomes, leading to the physical contact between peroxisomes and LDs. In adipocyte-specific PEX5-knockout mice, fasting induced lipolysis is attenuated due to defective ATGL recruitment onto LDs. These results show that PEX5 plays a pivotal role in PKA induced lipolysis that occurs upon nutritional deprivation. We further speculate that the contact between LDs and peroxisomes could facilitate lipid metabolism via exchange of lipid metabolites between the organelles in response to nutritional changes.

Keywords
lipolysis, peroxisome, lipid droplet, PEX5, ATGL, PKA

Lipid droplets (LDs) are unique subcellular organelles which are found in most organisms from bacteria to humans. LDs consist of neutral lipid metabolites such as triglycerides (TAG) and sterol esters surrounded by a phospholipid monolayer and LD-binding proteins. Depending on energy state and hormonal stimuli, LDs are dynamically modulated in their size and composition. When energy balance is positioned toward the anabolic condition, surplus energy is stored in LDs to prepare for an energy deficient state. On the other hand, upon energy demand, LDs are hydrolyzed by lipases to produce an energy source, in a process called lipolysis. In Caenorhabditis elegans (C. elegans), the contents of LDs in the intestine are sensitively and reversibly altered upon feeding and fasting stimuli (Jo et al., 2009). Catabolic cues trigger cAMP-dependent protein kinase A (PKA) signaling pathway to stimulate lipolysis. It has been reported that activated PKA promotes phosphorylation of its target proteins such as perilipin 1 (PLIN1), hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) to initiate lipolysis (Fruhbeck et al., 2014; Lee et al., 2016). As ATGL is the rate-limiting enzyme in lipolysis, the activity of ATGL is regulated by several cofactors such as comparative gene identification-58 (CGI-58), G(0)/G(1) switch gene 2 (G0S2) in mammals and lipid droplet protein 1 (LID-1) in C. elegans (Lass et al., 2006; Yang et al., 2010; Lee et al., 2014). On the contrary, lipolysis is suppressed by anabolic hormones and signals. For instance, insulin potently reduces the level of cAMP and ATGL, resulting in suppression of PKA-induced lipolysis (Fruhbeck et al., 2014; Lee et al., 2016). In addition, environmental stimuli such as hypoxia suppresses lipolysis through inhibition of PKA activity and ATGL regulation (Han et al., 2019).

Peroxisomes are single membrane organelles with variable sizes ranging from 0.1 to 1 μm. Peroxisomes have a wide spectrum of cellular metabolic processes which contribute to production and decomposition of hydrogen...
Peroxidase (Lodhi and Semenkovich, 2014). Peroxisomes not only have ROS related roles, but also participate in lipid metabolism (Baes and Van Veldhoven, 2012). Peroxisomes actively contribute to degradation of very long chain fatty acids and branched chain fatty acids which cannot be oxidized in mitochondria. In addition, peroxisomes also have anabolic roles by synthesizing diverse lipid metabolites such as ether lipids, cholesterol and bile acids. Due to these active roles, it is likely that dynamic interplay and communication could occur between subcellular organelles, including LDs, through physical contact.

Recently, we have demonstrated that the crosstalk between peroxisomes and LDs is important in the regulation of lipolysis upon energy demand (Kong et al., 2020). To mediate spatiotemporal regulation of peroxisomes and LDs, kinesin family member C3 (KIFC3) plays a major role in the migration of peroxisomes toward LDs upon fasting. Notably, lipolytic activity was suppressed when peroxisomal movement was attenuated via inhibition of microtubule polymerization or downregulation of KIFC3. For pertinent lipolysis, spatiotemporal regulation of lipases and LD-binding proteins are crucial to modulate intricately regulated lipolysis depending on energy status. Although it has been shown that mobilization of ATGL and HSL onto LDs is stimulated during PKA-induced lipolysis, the molecular mechanisms underlying translocation of lipases have been largely unknown. We found that as peroxisomes translocate toward LDs, ATGL is escorted to peroxisomes and subsequently located to the contact sites between peroxisomes and LDs. These observations are evolutionarily well conserved from worms to mammalian adipocytes. Among the peroxisomal proteins, we identified PRX-5, an ortholog of mammalian peroxisomal biogenesis factor 5 (PEX5), as one of the possible peroxisomal proteins of C. elegans that escort ATGL to the contact points between peroxisomes and LDs in the presence of fasting cues (Figure 1).

Figure 1. Schematic Model of Pex5-Dependent Translocation of ATGL and Peroxisome Onto the Lipid Droplet Upon Fasting Cues. The fatty acids released by lipolysis are oxidized in both peroxisome and mitochondria. However, VLCFA and BCFA are oxidized in the peroxisome prior to being transferred to mitochondria to be completely oxidized. TAG, triacylglycerol; VLCFA, very long chain fatty acid; BCFA, branched chain fatty acid; LCFA, long chain fatty acid; SCFA, short chain fatty acid.
complex with ATGL to mediate lipolysis, ATGL does not have the PTS1 motif in its C-terminal end. Thus, it is plausible that PEX5 escorts ATGL onto the surface of peroxisomes, probably through non-canonical pathways. It has been reported that PEX5 not only functions via PTS1-dependent translocation, but also appears to recognize its cargo with less characterized motifs such as the ARL sequence motif (Benjamin and Hall, 2013). As ATGL and HSL have putative ARL sequence motifs, we cannot exclude the possibility that these lipases might be translocated by PEX5 in a PTS1-independent manner. Meanwhile, translocation of ATGL onto LDs was markedly promoted upon fasting cues. As the interaction between ATGL and PEX5 was enhanced by PKA activation, it is feasible to propose that PKA-dependent phosphorylation of PEX5 might promote translocation of ATGL onto LDs. In silico analysis revealed that PEX5 has at least 10 potential PKA-induced phosphorylation sites. Further study is required to identify PKA-dependent phosphorylation site(s) in PEX5, which might be crucial for the interaction with ATGL to mediate lipid metabolism upon energy states and hormonal regulation.

In order to maintain energy balance, LDs should be timely and dynamically regulated by crosstalk with other subcellular organelles for efficient energy storage and usage. As lipolysis takes place on the surface of LDs by lipases, it is reasonable to speculate that lipolytic products including free fatty acids and glycerol could affect functions of subcellular organelles interacting with LDs. During lipolysis, it is feasible that increased lipid metabolites could be consumed by peroxisomes and mitochondria through fatty acid oxidation. As the interaction between LDs and mitochondria is enhanced under nutrient deprived conditions, it seems that transfer of fatty acids might occur at the contact sites between LDs and mitochondria (Rambold et al., 2015). In addition to mitochondria, emerging evidence has proposed a physical interaction between LDs and peroxisomes which plays key roles in lipid metabolism within the peroxisome (Binns et al., 2006; Joshi et al., 2018; Chang et al., 2019). In yeast, an extension of the peroxisome into the core of LDs, termed pexopodia, seems to occur in parallel with the activation of lipid metabolism (Binns et al., 2006). Furthermore, it has been reported that M1 Spastin tethers peroxisomes to LDs for fatty acid trafficking (Chang et al., 2019). Intriguingly, peroxisomes moved more rapidly than mitochondria toward LDs upon PKA activation, in worms and mammals (Kong et al., 2020). Given that peroxisomes oxidize lipid metabolites prior to being transferred to mitochondria for complete oxidation, it is very likely that the contact between LDs and peroxisomes should precede the interaction between LDs and mitochondria. Further investigation is required to determine physiological roles of the crosstalk between peroxisomes and LDs beyond lipolysis regulation.

Our in vitro and in vivo studies have revealed that peroxisomal protein PEX5 has evolutionarily well-conserved roles in ATGL-translocation onto LDs upon fasting cues from nematode to mammals (Kong et al., 2020). Collectively, these findings explicitly propose that peroxisomes and PEX5 could form a novel axis for lipid homeostasis in response to fasting signals, which could shed new light on the molecular mechanism of lipid homeostasis.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (No. NRF-2020R1A3B2078617).

ORCID iD
Jae Bum Kim https://orcid.org/0000-0003-2337-6935

References
Baes M, Van Veldhoven PP (2012). Mouse models for peroxisome biogenesis defects and beta-oxidation enzyme deficiencies. Biochim Biophys Acta 1822, 1489–1500.
Benjamin D, Hall MN (2013). TSC on the peroxisome controls mTORC1. Nat Cell Biol 15, 1135–1136.
Binns D, Januszewski T, Chen Y, Hill J, Markin VS, Zhao YM, Gilpin C, Chapman KD, Anderson RGW, Goodman JM (2006). An intimate collaboration between peroxisomes and lipid bodies. J Cell Biol 173, 719–731.
Chang CL, Weigel AV, Ioannou MS, Pasolli HA, Xu CS, Peale DR, Shtengel G, Freeman M, Hess HF, Blackstone C, et al. (2019). Spastin tethers lipid droplets to peroxisomes and directs fatty acid trafficking through ESCRT-III. J Cell Biol 218, 2583–2599.
Fruhbeck G, Mendez-Gimenez L, Fernandez-Formoso JA, Fernandez S, Rodriguez A (2014). Regulation of adipocyte lipolysis. Nutr Res Rev 27, 63–93.
Han JS, Lee JH, Kim JB (2017). Hypoxia restrains lipid utilization via protein kinase A and adipose triglyceride lipase downregulation through hypoxia-inducible factor. Mol Cell Biol 39, e00390–18.
Jo H, Shin J, Lee JH, Lee J, Kim JB (2009). IRE-1 and HSP-4 contribute to energy homeostasis via fasting-induced lipases in C. elegans. Cell Metab 9, 440–448.
Joshi AS, Nebenfuehr B, Choudhary V, Satpute-Krishnan P, Levine TP, Golden A, Prinz WA (2018). Lipid droplet and peroxisome biogenesis occur at the same ER subdomains. Nat Commun 9, 1–12.
Kim PK, Hettema EH (2015). Multiple pathways for protein transport to peroxisomes. J Mol Biol 427, 1176–1190.
Kong J, Ji Y, Jeon YG, Han JS, Han KH, Lee JH, Lee G, Jang H, Choe SS, Baes M, et al. (2020). Spatiotemporal contact between peroxisomes and lipid droplets regulates fasting-induced lipolysis via PEX5. Nat Commun 11, 578.
Lass A, Zimmermann R, Haemmerle G, Riederer M, Schoiswohl G, Schweiger M, Kienesberger P, Strauss JG, Gorkiewicz G, Zechner R (2006). Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman syndrome. Cell Metab 3, 309–319.
Lee JH, Han JS, Kong J, Ji Y, Lv X, Lee J, Li, P, Kim JB (2016). Protein kinase a subunit balance regulates lipid metabolism in Caenorhabditis elegans and mammalian adipocytes. J Biol Chem 291, 20315–20328.
Lee JH, Kong J, Jang JY, Han JS, Ji Y, Lee J, Kim JB (2014). Lipid droplet protein LID-1 mediates ATGL-1-dependent lipolysis during fasting in Caenorhabditis elegans. Mol Cell Biol 34, 4165–4176.
Lodhi IJ, Semenkovich CF (2014). Peroxisomes: A nexus for lipid metabolism and cellular signaling. Cell Metab 19, 380–392.
Rambold AS, Cohen S, Lippincott-Schwartz J (2015). Fatty acid trafficking in starved cells: Regulation by lipid droplet lipolysis, autophagy, and mitochondrial fusion dynamics. Dev Cell 32, 678–692.
Yang X, Lu X, Lombes M, Rha GB, Chi YI, Guerin TM, Smart EJ, Liu J (2010). The G(0)/G(1) switch gene 2 regulates adipose lipolysis through association with adipose triglyceride lipase. Cell Metab 11, 194–205.