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REVIEW

Cellular responses to unsaturated fatty acids mediated by their sensor Ubxd8

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Abstract **Fatty acids (FAs) are crucial nutrient for cell survival because they are required for synthesis of phospholipids in cellular membranes and for generation of energy. However, overaccumulation of FAs is toxic. In response to excessive FAs, cells are able to activate multiple reactions to prevent their overaccumulation. These reactions are mediated by Ubxd8, a cellular protein that specifically interacts with unsaturated but not saturated FAs. The selective interaction of Ubxd8 with unsaturated FAs may explain previous observations that only unsaturated but not saturated FAs are able to stimulate the regulatory reactions that prevent overaccumulation of FAs. Thus, understanding the mechanism through which Ubxd8 maintains cellular FA homeostasis may provide new insights into saturated FA-induced lipotoxicity.**

Keywords review, fatty acids, triglycerides, Ubxd8, Insig-1, SREBP

Introduction

Although fatty acids (FAs) are crucial for cell survival as they are the building blocks for phospholipid components of membranes and the major source for energy production, their overaccumulation is toxic to cells. When FAs accumulate in cells, they exert regulatory reactions to prevent their further accumulation. These actions include feedback inhibition of FA synthesis, which is achieved at least in part through inhibition of transcription of genes encoding proteins required for FA synthesis (Ntambi, 1992; Hannah et al., 2001; Ou et al., 2001). Excessive FAs also enhance their incorporation into triglycerides (TGs), a reaction that is critical to prevent overaccumulation of FAs (Farese and Walther, 2009). Remarkably, these regulatory functions are carried out by unsaturated but not saturated FAs (Hannah et al., 2001; Coll et al., 2008; Lee et al., 2008). The lack of ability for saturated FAs to control their homeostasis makes these FAs more toxic than unsaturated FAs. Indeed, overconsumption of saturated FAs in western diet is believed to be the major contributor for development of lipotoxicity (Unger et al., 2010).

Recently, Ubxd8 was identified as a cellular sensor for unsaturated FAs that controls unsaturated FA-induced inhibition in FA synthesis and activation in TG synthesis (Lee et al., 2010). The selective interaction of Ubxd8 with unsaturated but not saturated FAs may provide explanation for previous observations that only unsaturated but not saturated FAs are able to stimulate the reactions that prevent overaccumulation of FAs. The molecular mechanism through which Ubxd8 senses the presence of unsaturated FAs to maintain their homeostasis will be summarized in this article.

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Ubxd8-mediated feedback inhibition in fatty acid synthesis

Sterol regulatory element binding protein 1 (SREBP-1) is a transcription factor that activates all genes required for FA synthesis (Horton et al., 2003). Mammalian cells produce two isoforms of SREBP-1 (SREBP-1a and SREBP-1c) through the use of alternate promoters that produce transcripts with a different first exon (Hua et al., 1995). Together with SREBP-2 that is encoded by a separate gene, these proteins constitute a family of transcription factors designated as SREBPs (Brown and Goldstein, 1997).

SREBPs were originally identified as transcription factors that regulate cholesterol metabolism (Brown and Goldstein, 1997). Unlike typical transcription factors, SREBPs are synthesized as integral membrane proteins localized in the

endoplasmic reticulum (ER). The NH_2-terminal and the COOH-terminal domains of the proteins project into the cytosol (Fig. 1A). They are anchored to membranes by a central domain containing two membrane-spanning sequences separated by a short loop that projects into the lumen of the ER (Fig. 1A). The COOH-terminal domain of SREBPs is in complex with a polytopic membrane protein designated as Scap (Hua et al., 1996; Sakai et al., 1997) (Fig. 1). Scap is a cholesterol sensor in the ER (Radhakrishnan et al., 2004, 2008). In cells loaded with cholesterol, Scap binds to cholesterol and adopts a conformation enabling it to interact with Insig proteins, a reaction that retains the Scap/SREBPs complex in the ER (Yabe et al., 2002; Yang et al., 2002) (Fig. 1A). Mammalian cells contain two isoforms of Insig proteins, namely Insig-1 and Insig-2 (Yabe et al., 2002; Yang et al., 2002). Among the two isoforms, Insig-1 is the predominant isoform expressed in cultured cells (Sever et al., 2004). In cells depleted of cholesterol, the affinity between cholesterol-free Scap and Insig proteins is markedly reduced (Yang et al., 2002) (Fig. 1B). Insig-1 dissociated from Scap becomes ubiquitinated by a membrane-bound E3 ubiquitin ligase gp78 and is rapidly degraded by proteasomes (Gong et al., 2006; Lee et al., 2006). In the absence of interaction with the Insig proteins, Scap escort SREBPs from the ER to Golgi, where SREBPs are sequentially cleaved by Site-1 protease and Site-2 protease that reside in the Golgi complex (Rawson et al., 1997; Sakai et al., 1998; Rawson et al., 1999; Sakai et al., 2005) (Fig. 1B). These cleavages release the NH_2-terminal domain of SREBPs from membranes, allowing it to enter nucleus where it activates target genes leading to increased cholesterol synthesis and uptake (Horton et al., 2003) (Fig. 1B).

In addition to activating genes participated in cholesterol synthesis, SREBP-1 is actually more active in driving genes involved in FA synthesis (Pai et al., 1998; Horton et al., 2003). The importance of SREBP-1 in activating FA synthesis in cultured cells is most clearly demonstrated by observations that mutant CHO cells deficient in proteases cleaving SREBPs or Scap are auxotrophic for both cholesterol and unsaturated FAs (Rawson et al., 1997; Rawson et al., 1998; Rawson et al., 1999). Thus, SREBP-1 is also subjected to feedback inhibition by FAs. Polyunsaturated fatty acids (PUFA) inhibit transcription of SREBP-1 but not SREBP-2 (Ou et al., 2001). This is because transcription of SREBP-1 is activated by liver X receptors (LXRs) (Repa et al., 2000; DeBose-Boyd et al., 2001) that are antagonized by PUFA (Ou et al., 2001). In mice, only *SREBP-1c* but not *SREBP-1a* contains the LXR response element in its promoter. Thus, only *SREBP-1c* transcription is inhibited by PUFA (Repa et al., 2000). This is not the case in human cells where the LXR response element is found in the promoter regions of both SREBP-1a and SREBP-1c. As a result, transcription of SREBP-1a is also inhibited by PUFA in human cells (Hannah et al., 2001).

Unsaturated FAs also inhibit proteolytic activation of SREBP-1 (Hannah et al., 2001). This activity is not limited to PUFA as mono-unsaturated FAs such as oleate inhibit SREBP-1 processing as well (Hannah et al., 2001). In contrast, saturated FAs are unable to suppress proteolytic activation of SREBP-1 (Hannah et al., 2001). Unsaturated

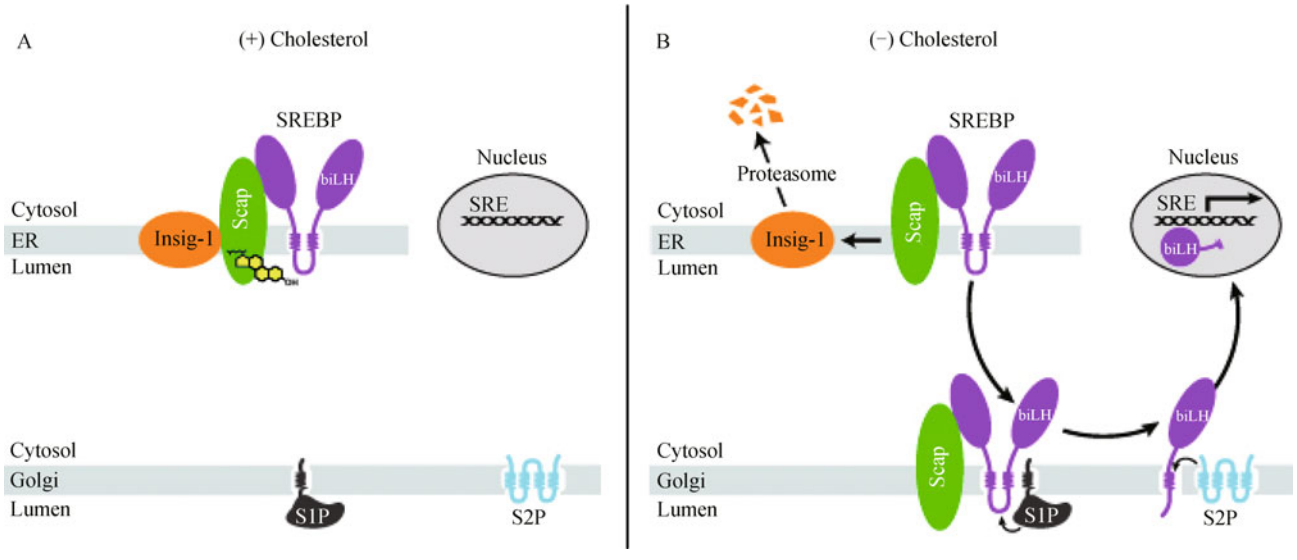

Figure 1 Regulation of proteolytic activation of SREBP by cholesterol. (A) Scap binds to cholesterol in cells loaded with the lipid. Under this circumstance Scap binds to Insig-1, a reaction that retains the Scap/SREBP complex in the ER thereby preventing cleavage of SREBP in the Golgi complex. (B) In cells depleted of cholesterol, the affinity between Scap and Insig-1 is reduced, and Insig-1 dissociated from Scap is rapidly degraded by proteasomes. Consequently, Scap/SREBP complex is transported from the ER to Golgi where SREBP is sequentially cleaved by S1P and S2P, two proteases that localize in the Golgi. The final cleavage catalyzed by S2P liberates the NH_2-terminal domain of SREBP from membranes, allowing it to enter the nucleus where it activates transcription of all genes required for cholesterol synthesis and uptake.

FAs inhibit SREBP-1 cleavage by blocking the proteasomal degradation of Insig-1 (Lee et al., 2008). Since more Insig-1 is required to inhibit proteolytic activation of SREBP-1 than that of SREBP-2 (Engelking et al., 2005), unsaturated FA-induced accumulation of Insig-1 has a more profound effect in blocking cleavage of SREBP-1 (Lee et al., 2008). Inasmuch as unsaturated FAs inhibit SREBP-1 cleavage by increasing the amount of Insig-1 protein, this inhibition is not independent of cholesterol, which is required for Insig-1 to bind Scap. Rather, by increasing the amount of Insig-1, unsaturated FAs make SREBP-1 more sensitive to the inhibitory effects of cholesterol (Lee et al., 2008).

Remarkably, unsaturated FAs and cholesterol inhibit Insig-1 degradation by different mechanisms. In cholesterol- and FA-depleted cells, Insig-1 binds to gp78, a membrane-bound E3 ubiquitin ligase that attaches polyubiquitin chains to Insig-1 (Lee et al., 2006) (Fig. 2). Insig-1 also binds to Ubxd8, a protein that recruits p97 complex to Insig-1 through its bridging interaction with both proteins (Lee et al., 2008) (Fig. 2). Both ubiquitination of Insig-1 and recruitment of p97 to Insig-1 are required for Insig-1 to be recognized and subsequently degraded by proteasomes (Ikeda et al., 2009) (Fig. 2). In response to cholesterol accumulation, Insig-1 binds to Scap, a reaction that precludes the binding of gp78 to Insig-1 (Lee et al., 2006) (Fig. 2). Consequently, ubiquitination of Insig-1 is inhibited (Gong et al., 2006; Lee et al., 2006). In contrast, cholesterol does not inhibit binding of the Ubxd8/p97 complex to Insig-1 (Lee et al., 2008), as ubiquitination of Insig-1 is not required for this interaction (Ikeda et al., 2009). Unsaturated FAs do not block Insig-1 ubiquitination, but they prevent the recruitment of p97 to Insig-1 by blocking the interaction between Ubxd8 and Insig-1 (Lee et al., 2008) (Fig. 2). Inasmuch as proteasome binding requires both ubiquitination and p97, either cholesterol or unsaturated FAs can block the degradation of Insig-1 (Fig. 2).

The model outlined in Fig. 2 demonstrates the importance of Ubxd8 in unsaturated FA-regulated degradation of Insig-1. Ubxd8 facilitates Insig-1 degradation in FA-depleted cells, allowing SREBP-1 to be efficiently cleaved to induce FA synthesis. As a result, knockdown of Ubxd8 inhibited FA synthesis in FA-depleted cells (Lee et al., 2010). Unlike unsaturated FAs, saturated FAs are unable to block the interaction between Ubxd8 and Insig-1 (Lee et al., 2008). This observation explains why saturated FAs are unable to stabilize Insig-1 (Lee et al., 2008), and to inhibit proteolytic activation of SREBP-1 (Hannah et al., 2001).

Identification of Ubxd8 as a cellular sensor for unsaturated FAs

Ubxd8 belongs to a family of proteins that contain the UBX domain (Imai et al., 2002), which interacts with p97 (Buchberger, 2002), a protein required for ER-associated degradation (Halawani and Latterich, 2006). In addition to the UBX domain located at the COOH-terminus of the protein, Ubxd8 also contains a UBA and UAS domain (Fig. 3). While the UBA domain is known to interact with polyubiquitin

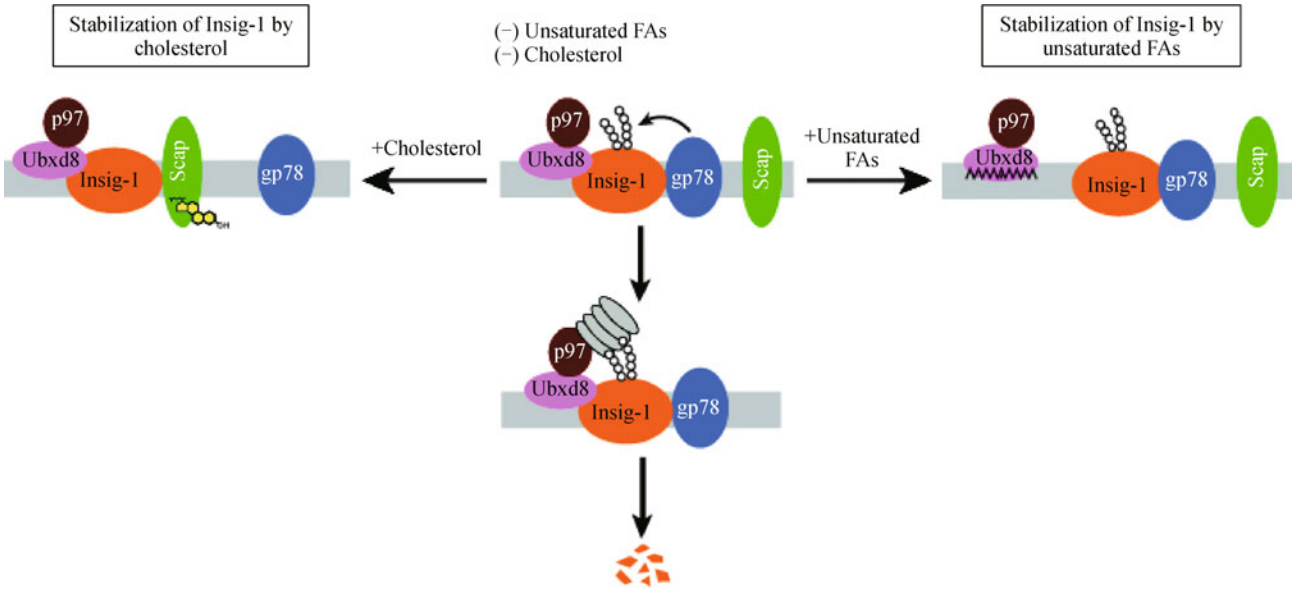

Figure 2 Unsaturated FAs and cholesterol stabilize Insig-1 through different mechanism. In cells depleted of cholesterol and unsaturated FAs, Insig-1 is ubiquitinated by gp78 and associated with p97 through the bridging interaction mediated by Ubxd8. These reactions allow Insig-1 to be recognized by proteasomes, resulting in rapid degradation of the protein. Cholesterol triggers the binding of Insig-1 to Scap, a reaction that displaces gp78 from Insig-1. As a result, ubiquitination of Insig-1 is inhibited and the protein is stabilized. Cholesterol does not affect the interaction between the Ubxd8/p97 complex and Insig-1. In contrast to cholesterol, unsaturated FAs do not inhibit Insig-1 ubiquitination but they block the interaction between Insig-1 and Ubxd8. Consequently, Insig-1 is stabilized owing to the lack of the recruitment of p97.

chains (Buchberger, 2002), the function of the UAS domain remains to be identified. Ubxd8 is inserted into membranes via a stretch of hydrophobic amino acid residues located between the UBA and UAS domain (amino acid residues 90-118) that forms a hairpin loop in membranes (Lee et al., 2010) (Fig. 3). This type of membrane insertion allows Ubxd8 to migrate from the ER in cells depleted of FAs to surface of lipid droplets in cells loaded with FAs (Zehmer et al., 2009). Because Insig-1 is localized in the ER regardless of treatment with FAs, it is tempting to speculate that the translocation of Ubxd8 from the ER to lipid droplets in FA-treated cells may be the mechanism through which Insig-1 is dissociated from Ubxd8 in these cells. However, two observations argued against this hypothesis: 1) Unsaturated FAs inhibited the interaction between Insig-1 and Ubxd8 even in cells treated with triacsin C, an acyl-CoA synthetase inhibitor that blocks TG synthesis and subsequent formation of lipid droplets (Lee et al., 2008); and 2) Unsaturated FAs inhibited the interaction between Insig-1 and a mutant version of Ubxd8 in which the membrane localization domain was deleted (Ubxd8(Δ90-118)) (Lee et al., 2010). Unlike wild type Ubxd8, Ubxd8 (Δ90-118) is mainly a soluble cytosolic protein that is not associated with lipid droplets (Lee et al., 2010). Thus, disruption of the interaction between Insig-1 and Ubxd8 in cells treated with FAs is not a direct consequence of translocation of Ubxd8 from the ER to lipid droplets.

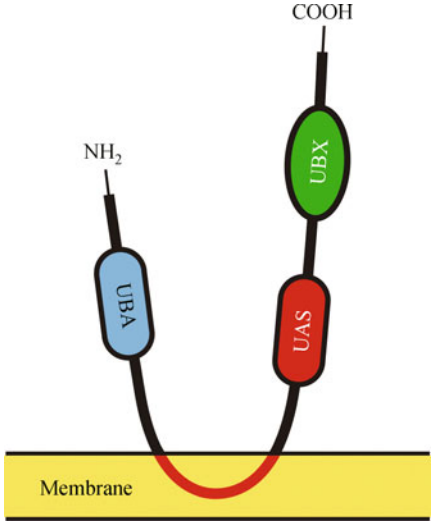

Figure 3 Diagram of Ubxd8. The three domains in Ubxd8 are indicated and the membrane localization sequence is highlighted in red.

Inasmuch as the interaction between Ubxd8(Δ90-118) and Insig-1 was still blocked by unsaturated FAs, the mutant Ubxd8 became a facile tool to study the interaction of Ubxd8 with unsaturated FAs in vitro as the recombinant Ubxd8(Δ90-118) was able to be purified to homogeneity in the absence of detergents, which frequently interfere with the in vitro binding assays with FAs. This analysis revealed that long chain unsaturated FAs altered the conformation of purified Ubxd8(Δ90-118), resulting in polymerization of the protein (Lee et al., 2010). This effect was specific to long chain unsaturated FAs as long-chain saturated FAs, median-chain unsaturated FAs, or long chain unsaturated alcohol failed to produce the same effect (Lee et al., 2010). The *in vitro* characterization performed with Ubxd8(Δ90-118) is likely to be a good indication of the full length Ubxd8 protein because the specificity of FAs and their analogs to stimulate polymerization of Ubxd8(Δ90-118) *in vitro* matches their specificity to stabilize Insig-1 inside cells (Lee et al., 2010). These results suggest that Ubxd8 is a cellular sensor for long-chain unsaturated FAs.

Ubxd8-mediated inhibition of TG synthesis in FA-depleted cells

As a sensor for unsaturated FAs, Ubxd8 also regulates activation of TG synthesis, another regulatory reaction that maintains FA homeostasis. The synthesis of TGs begins with attachment of FAs to glycerol-3-phosphate, leading to the generation of diacylglycerols (DAGs), a class of precursors that can be converted to either phospholipids or TGs (Yen et al., 2008). Diacylglycerol acyltransferases (DGATs) catalyze the final step in TG synthesis by adding the third FA molecule to DAGs (Yen et al., 2008). This step is inhibited in FA-depleted cells, and this inhibition depends on Ubxd8 as knockdown of Ubxd8 markedly increased conversion of DAGs to TGs in these cells (Lee et al., 2010). This regulatory action of Ubxd8 makes limited amount of FAs in these cells available for incorporation into phospholipids by inhibiting their diversion into TGs (Fig. 4A). When long-chain unsaturated FAs are supplied externally, the FAs change the conformation of Ubxd8, promoting its polymerization, and inhibiting its activity (Lee et al., 2010). Consequently, TG synthesis increases so that the excess exogenous FAs are stored as TGs in lipid droplets (Fig. 4B).

Unlike unsaturated FAs, saturated FAs are unable to interact with Ubxd8. As a result, Ubxd8-mediated inhibition in conversion of DAGs to TGs is not relieved in cells treated with only saturated FAs (Fig. 4C). Thus, saturated FAs are preferentially incorporated into DAGs instead of TGs (Coll et al., 2008; Lee et al., 2010) (Fig. 4C). Consistent with this reasoning, knockdown of Ubxd8 markedly increased the conversion of DAGs to TGs in cells treated with palmitate, a saturated FA (Lee et al., 2010). Co-incubation of palmitate-treated cells with oleate, an unsaturated FA that inhibits Ubxd8, also produced the similar result (Lee et al., 2010).

Recent evidences have suggested that accumulation of DAGs is responsible for the development of insulin resistance (Erion and Shulman, 2010). The effect of DAGs on insulin resistance was most extensively studied in skeletal muscle cells in which accumulation of DAGs was triggered by excessive saturated FAs (Coll et al., 2008). Thus, a

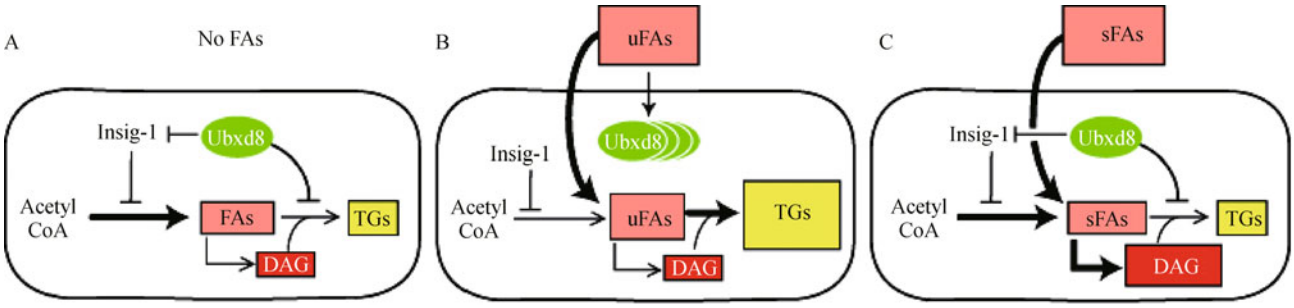

Figure 4 Ubxd8 mediates cellular responses to exogenous Fas. (A) In cells depleted of FAs, Ubxd8 facilitates Insig-1 degradation thereby activating FA synthesis. Ubxd8 also inhibits conversion of DAGs to TGs to prevent diversion of FAs into TGs. (B) Excess unsaturated FAs trigger Ubxd8 polymerization, which presumably inactivates the protein. As a result, Insig-1 is stabilized to inhibit FA synthesis. Inhibition in TG synthesis is also relieved so that the excess FAs can be incorporated into TGs for storage in lipid droplets. (C) Since saturated FAs do not interact with Ubxd8, the protein is not inhibited in the presence of these FAs. Consequently, Insig-1 is still rapidly degraded, and FA synthesis is not inhibited. The blockage in the conversion between DAGs to TGs is still intact so that majority of saturated FAs are incorporated into DAGs instead of TGs.

pharmacologic agent that mimics unsaturated FAs in inactivating Ubxd8 might enhance the conversion of DAGs to TGs and thus relieve insulin resistance caused by excessive uptake of saturated FAs.

While the molecular mechanism through which Ubxd8 regulates FA synthesis has been identified, exactly how the protein controls TG synthesis remains unclear. Two DGAT enzymes, namely DGAT1 and DGAT2, catalyze the final step of TG synthesis by converting DAGs into TGs (Harris et al., 2011). It remains to be determined whether either one of the DGAT enzymes or proteins that activate the enzymes is degraded through Ubxd8 in cells depleted of FAs, and whether this degradation is inhibited in cells exposed to excess unsaturated FAs.

Other functions of Ubxd8

Ubxd8 was originally identified as a protein overexpressed in human peripheral blood CD3-positive cells from patients with atopic dermatitis, but the role of Ubxd8 in development of the disease was not identified (Imai et al., 2002). Ubxd8 is known to accelerate degradation of proteins other than Insig-1. These proteins include Class I major histocompatibility complex heavy chains (Mueller et al., 2008), neurofibromin (Phan et al., 2010), and apolipoprotein B (Suzuki et al., 2012). At least one of these proteins, namely apolipoprotein B, requires production of lipid droplets for its degradation (Suzuki et al., 2012). Recent studies have suggested that recruiting certain ER membrane or luminal proteins to lipid droplets is a prerequisite step before these proteins can be extracted to cytosol for degradation by proteasomes (Ploegh, 2007; Hartman et al., 2010; Suzuki et al., 2012). Since Ubxd8 is an adaptor protein for p97 that is known to play an important role to extract ER membrane proteins to cytosol for proteasomal degradation (Halawani and Latterich, 2006), localization of Ubxd8 to lipid droplets may play an important role to recruit p97 to lipid droplets to degrade these ER proteins.

Perspective

The identification of Ubxd8 as a sensor for unsaturated FAs explains previous observations that only unsaturated but not saturated FAs are able to stimulate the regulatory reactions that maintain FA homeostasis. This discovery also opens new territory to be explored. While the effect of Ubxd8 on FA metabolism in cultured mammalian cells is clear, the physiologic function of the protein in living mammals remains to be determined. Since Ubxd8 mainly functions in FA-depleted cells to promote FA synthesis and to inhibit incorporation of FAs into TGs, the protein is expected to be critical for animals to survive conditions in which serum FA concentration is low. This condition can be achieved in mice through chronic calorie restriction (Zhao et al., 2010). It will be interesting to determine whether mice deficient in Ubxd8 are less likely to survive chronic calorie restriction.

Another interesting question raised by the discovery is why mammalian cells express a sensor like Ubxd8 that only senses unsaturated but not saturated FAs. A plausible explanation is that mammalian cells express acyl-CoA desaturases so that endogenously synthesized FAs are predominantly unsaturated FAs. Thus, before industrialized food products that are enriched in saturated FAs become readily available, sensing saturated FAs may not be evolutionally important.

Ubxd8 is conserved in all animal species and an ortholog of the protein, namely Ubx2, is found in yeast *Saccharomyces cerevisiae* (Schuberth and Buchberger, 2005). Similar to Ubxd8, Ubx2 shuttles between ER and lipid droplets (Wang and Lee, 2012). However, inhibition of Ubx2 led to a decrease in TG synthesis (Wang and Lee, 2012) rather than an increase in TG synthesis observed in mammalian cells in which Ubxd8 was knocked down (Lee et al., 2010). Ubx2 is

required for proper localization of lro1, an enzyme catalyzes TG synthesis in yeast (Wang and Lee, 2012). Since mammalian cells do not express orthologs of lro1, this effect of Ubx2 on TG synthesis appears to be unique to the yeast. The effect of Ubx2 on FA synthesis has not been determined. Unlike mammalian cells, *Saccharomyces cerevisiae* do not contain orthologs of Insig-1, SREBPs, and proteases that cleave SREBPs. Instead, they produce a membrane-bound transcription factor Spt23. In the absence of FAs, Spt23 is subjected to limited proteasomal digestion so that the NH_2-terminal fragment of the protein is liberated from the ER membrane and enters the nucleus to drive transcription of genes required for synthesis of unsaturated FAs (Hoppe et al., 2000). This limited proteasomal cleavage of spt23 depends on p97 complex (Rape et al., 2001). In the presence of exogenously added unsaturated FAs, proteolytic activation of Spt23 by proteasomes does not occur (Hoppe et al., 2000). Inasmuch as both yeast and mammalian cells use the ubiquitin-proteasome system to regulate synthesis of unsaturated FAs, a common factor in the ubiquitin-proteasome pathway may be a sensor for unsaturated FAs. Further studies are required to determine whether Ubx2 is the sensor for unsaturated FAs in yeasts to regulate proteolytic activation of Spt23.

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