Triacylglycerol Metabolism in *Drosophila melanogaster*

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ABSTRACT Triacylglycerol (TAG) is the most important caloric source with respect to energy homeostasis in animals. In addition to its evolutionarily conserved importance as an energy source, TAG turnover is crucial to the metabolism of structural and signaling lipids. These neutral lipids are also key players in development and disease. Here, we review the metabolism of TAG in the *Drosophila* model system. Recently, the fruit fly has attracted renewed attention in research due to the unique experimental approaches it affords in studying the tissue-autonomous and interorgan regulation of lipid metabolism *in vivo*. Following an overview of the systemic control of fly body fat stores, we will cover lipid anabolic, enzymatic, and regulatory processes, which begin with the dietary lipid breakdown and *de novo* lipogenesis that results in lipid droplet storage. Next, we focus on lipolytic processes, which mobilize storage TAG to make it metabolically accessible as either an energy source or as a building block for biosynthesis of other lipid classes. Since the buildup and breakdown of fat involves various organs, we highlight avenues of lipid transport, which are at the heart of functional integration of organismic lipid metabolism. Finally, we draw attention to some "missing links" in basic neutral lipid metabolism and conclude with a perspective on how fly research can be exploited to study functional metabolic roles of diverse lipids.

KEYWORDS *Drosophila* fat; triacylglycerol; lipogenesis; lipolysis; lipases; lipid transport; FlyBook

TABLE OF CONTENTS

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ALL animals are faced with large fluctuations in energy intake and demand on diurnal to seasonal scales. To maintain energy homeostasis during nonfeeding developmental stages or upon metabolic stress, animals have developed the ability to store a surplus of calories, which serve as flexible on-demand energy depots. The quantitatively most important energy reservoirs in many metazoans, including those in the model invertebrate *Drosophila melanogaster*, include the carbohydrate glycogen and neutral triacylglycerol (TAG) lipids. TAG represents the most concentrated form of chemically bound energy. On the one hand, the carbon atoms of TAG are in more reduced states than the carbon atoms of carbohydrates. This results in a greater caloric yield upon complete oxidation of TAG. Moreover, the weight to energy content ratio of TAG is favorable due to its apolar nature, which unlike glycogen allows its anhydrous storage in specialized intracellular organelles called lipid droplets (LDs) (Thiam *et al.* 2013; Zechner *et al.* 2017). Importantly, the metabolic function of TAG is not limited to energy storage. Fatty acid (FA) moieties stored in the form of TAG may also serve as building blocks for structural membrane lipids or signaling molecules. TAG also represents an essential metabolic sink that sequesters excess carbon units from dietary sugar or fat, which stress cells via their conversion to lipo- or glucotoxic metabolic intermediates (Garrido *et al.* 2015; Welte and Gould 2017; Zechner *et al.* 2017). These principal biological functions of TAG have been highly conserved during evolution, and are similar in *Drosophila* and higher metazoans. This makes the fruit fly an increasingly popular model to study the general (patho)physiology of TAG metabolism [reviewed in Baker and Thummel (2007), Owusu-Ansah and Perrimon (2014), and Musselman and Kühnlein (2018)].

Reflecting its central role in metabolic homeostasis, dynamic changes in TAG levels characterize all stages of *Drosophila* development (Carvalho *et al.* 2012; Guan *et al.* 2013). The accumulation and catabolism of TAG stores are essential for successful oogenesis and embryogenesis, respectively (Buszczak *et al.* 2002; Grönke *et al.* 2005). Embryos are provided with maternal TAG stores, which are largely depleted during embryogenesis (Carvalho *et al.* 2012; Guan *et al.* 2013; Tennessen *et al.* 2014). This catabolic phase is followed by a massive buildup of TAG stores during larval growth that ceases in the postfeeding stages of metamorphosis (Carvalho *et al.* 2012; Guan *et al.* 2013). In larval and adult stages, TAG stores represent an essential reservoir for surviving starvation (Grönke *et al.* 2003, 2007; Gutierrez *et al.* 2006; Bi *et al.* 2012). When food is abundant, complex diet–genotype interactions influence TAG storage in adult *Drosophila*, and the dietary history of parental or grandparental generations can influence the TAG phenotype of the offspring through epigenetic mechanisms (Öst *et al.* 2014; Palu *et al.* 2017). Fluctuations in energy intake are integrated by an endocrine system that coordinates catabolic and anabolic branches of TAG metabolism between several organ systems, which are central to the organismal lipid metabolism. Endocrine signals cause alterations in the activity of protein factors, such as transporters, enzymes, and scaffolding or regulatory proteins, which regulate the trafficking and metabolism of TAG at the molecular level. The enzymatic and transport machineries that govern TAG metabolism, and the principal regulatory mechanisms controlling these enzymatic activities in *Drosophila*, are the focus of this review. We begin with a brief overview of the major endocrine pathways and organ systems implicated in TAG metabolism, before we describe the proteins orchestrating the synthesis, degradation, and transport of TAG in detail.

Hormonal Control of *Drosophila* TAG Metabolism

The past decade has yielded a substantial increase in knowledge about endocrine systems that regulate TAG metabolism in *Drosophila*. A comprehensive description of these endocrine pathways is the subject of a number of recent excellent reviews (Rajan and Perrimon 2013; Lehmann 2018). Here, we briefly summarize the implications of some major hormonal pathways in the regulation of TAG storage that include insulin, adipokinetic hormone (Akh), juvenile hormone (JH), and ecdysone signaling.

Insulin signaling is a central nutrient-sensing pathway regulating growth, aging, stress responses, reproduction, and metabolism. In *Drosophila*, multiple insulin-like peptides (Dilps) serve as ligands for a single insulin receptor (InR), which relays the signal via a conserved cascade to downstream intracellular effectors such as kinases and transcription factors. Stimulation of these pathways translates into alterations in the expression and activities of key metabolic enzymes [for review, see Teleman (2010), Nässel and Broeck (2015), and Nässel *et al.* (2015)]. Binding of Dilps to InR is relayed to initial activation of the *Drosophila* ortholog of phosphatidylinositol-3-phosphate kinase (PI3K) Pi3K92E (Leevers *et al.* 1996; Oldham *et al.* 2002) and, subsequently, to Akt1 kinase (Verdu *et al.* 1999; Cho *et al.* 2001). Akt1 phosphorylates and regulates multiple protein targets including other kinases (Papadopoulou *et al.* 2004; DiAngelo and Birnbaum 2009), metabolic enzymes, and transcription factors (Jünger *et al.* 2003; Puig *et al.* 2003; Wang *et al.* 2008; Bolukbasi *et al.* 2017). Akt1 promotes phosphorylation and cytoplasmic retention of the transcription factor Forkhead box subgroup O (Foxo) and decreases its transcriptional output (Puig *et al.* 2003). Accordingly, Foxo plays a central role in connecting insulin signaling to TAG metabolism. Conversely, a reduction in insulin signaling, *e.g.*, upon starvation, promotes nuclear translocation and transcriptional activity of Foxo, which in turn alters expression of a wide range of metabolic genes (Puig *et al.* 2003; Alic *et al.* 2011). Here, Foxo promotes the expression of lipases and increases the enzymatic breakdown of TAG (Vihervaara and Puig 2008; Wang *et al.* 2011). Consequently, transcriptional repression of lipolysis by the insulin/Foxo axis is a critical tissue-autonomous determinant of TAG homeostasis in the *Drosophila* fat body (DiAngelo and

Birnbaum 2009; Wang *et al.* 2011). However, systemic defects in insulin signaling cause a complex array of growth-related, developmental, and metabolic phenotypes in *Drosophila* that typically includes increased organismal TAG stores (Böhni *et al.* 1999; Oldham *et al.* 2002; Werz *et al.* 2009; Grönke *et al.* 2010; Slack *et al.* 2010; Song *et al.* 2010).

The glucagon-like peptide Akh was named after its stimulatory effect on TAG mobilization and was first described in migratory locusts (Mayer and Candy 1969). The hormone is produced by the neuroendocrine cells of the corpora cardiaca and is released on demand to the hemolymph to bind to its cognate G protein-coupled Akh receptor (AkhR) on fat body cells. AkhR translates the Akh signal to downstream effectors via two distinct second-messenger systems (Park *et al.* 2002; Staubli *et al.* 2002). In the first system, it activates the adenylate cyclase-catalyzed production of cyclic adenosine-3′,5′-monophosphate (cAMP), an allosteric activator of protein kinase A (Pka), which promotes the phosphorylation of several downstream effectors including transcription factors, kinases, and LD proteins (Patel *et al.* 2005; Lee *et al.* 2018). In the second system, it activates a phospholipase C that converts phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol-1,4,5-trisphosphate (IP3), which, in turn, triggers activation of endoplasmic reticulum (ER)-resident IP3 receptors and increases cytosolic concentrations of the second messenger Ca^{2+} [for a review, see Gäde and Auerswald (2003)]. This Ca^{2+} response transmits the Akh signal via currently unknown mechanisms to a gene expression signature that favors TAG catabolism (described in more detail below; Baumbach *et al.* 2014a). Although many components of the Akh pathway were first characterized in other insects, research on *Drosophila* has been fundamental in determining the genetic basis of this process. Consistent with the "adipokinetic" nature of the pathway, ablation of either the *AkhR* or the *Akh* gene impairs TAG mobilization in *Drosophila* (Grönke *et al.* 2007; Bharucha *et al.* 2008; Gáliková *et al.* 2015; Sajwan *et al.* 2015). Further, *Drosophila* genetic studies have also confirmed the crucial role of Ca^{2+} in transmitting the Akh signal and identified the G proteins Gγ1, Gαq, and the phospholipase C Plc21C as signal transducers along this axis (Baumbach *et al.* 2014b). In addition, several proteins that either promote or antagonize intracellular Ca^{2+} levels, such as the ER sensor Stromal interaction molecule (Stim), the plasma membrane channel Olf186-F, the ER Ca^{2+} channels sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and Itp-R83a, and the Ca^{2+}-binding protein calmodulin (Cam), have been linked to TAG homeostasis in *Drosophila* (Baumbach *et al.* 2014a; Bi *et al.* 2014; Moraru *et al.* 2017). Notably, the Akh pathway intersects with several other endocrine axes including insulin signaling (Choi *et al.* 2015; Kim and Neufeld 2015; Rajan *et al.* 2017; Song *et al.* 2017).

JH is the umbrella term for a group of acyclic sesquiterpenoids that are produced by the cells of the corpora allata, and that regulate diverse insect traits such as development, reproduction, and aging [for a review, see Flatt *et al.* (2005)]. JHs likely act by directly binding to transcription factors such as Methoprene-tolerant (Met) and germ cell-expressed bHLH-PAS (gce; bHLH-PAS stands for basic helix-loop-helix-PER-ARNT-SIM) to regulate expression of JH-responsive genes in target tissues (Charles *et al.* 2011; Jindra *et al.* 2015). Recent studies implicate JH signaling in the regulation of TAG metabolism (Baumann *et al.* 2013; Kang *et al.* 2017). For example, flies lacking corpora allata cells or lacking *Met* show reduced TAG levels, whereas pharmacological treatment with the JH analog methoprene increases TAG levels in adult flies. Moreover, loss-of-function alleles of the JH effector *Kruppel homolog 1* (*Kr-h1*) have been shown to affect lipogenic and lipolytic gene expression, and TAG homeostasis in *Drosophila* larvae. The delineation of physical and genetic interactions between Kr-h1 and the insulin effector Foxo suggests an intersection of both pathways in the regulation of TAG homeostasis (Kang *et al.* 2017).

Ecdysteroids are a group of polyhydroxylated steroid hormones that are essential for molting and metamorphosis in *Drosophila* [reviewed in Tennessen and Thummel (2011)]. The "prototypical" ecdysteroid, ecdysone, is synthesized from cholesterol through several enzymatic steps in the prothoracic gland of *Drosophila* larvae and in currently unknown tissues in adults [reviewed in Schwedes and Carney (2012)]. It is converted to the active metabolite 20-hydroxyecdysone in several target tissues, and signals through a heterodimeric nuclear hormone receptor complex comprised of Ecdysone receptor (EcR) and Ultraspiracle (Koelle *et al.* 1991; Yao *et al.* 1992, 1993; Thomas *et al.* 1993). Activation of this heterodimer regulates the expression of ecdysone-responsive genes by binding to specific promoter sequences. More recently, ecdysone signaling has been implicated in the regulation of TAG metabolism in various developmental stages. EcR is required in the central nervous system for sexually dimorphic feeding behavior and TAG accumulation in mature female flies (Sieber and Spradling 2015). EcR also regulates lipid accumulation during oogenesis, a process that is essential for oocyte maturation (Carney and Bender 2000; Sieber and Spradling 2015). Likewise, ecdysone signaling has been shown to promote TAG accumulation during metamorphosis (Francis *et al.* 2010), whereas its role in third-instar larval TAG homeostasis is controversial (Wang *et al.* 2010; Kamoshida *et al.* 2012). Notably, ecdysone antagonizes insulin signaling and promotes nuclear translocation of the insulin-responsive transcription factor Foxo (Rusten *et al.* 2004; Colombani *et al.* 2005). Conversely, insulin signaling dampens ecdysone signaling by controlling the expression of the transcriptional coactivator Diabetes and obesity regulated (encoded by *DOR*), suggesting extensive cross talk between both pathways in the regulation of TAG metabolism (Francis *et al.* 2010).

Physiology of *Drosophila* TAG Metabolism at a Glance

Figure 1 summarizes the TAG metabolism in major fly organs of glycerolipid absorption, storage, and utilization/processing.

TAG is a natural component of many standard laboratory diets used for the cultivation of *Drosophila*. After ingestion, dietary TAG is hydrolyzed by digestive lipase(s) into free FAs, glycerol, and/or other acylglycerol intermediates in the midgut lumen. These TAG-derived metabolites are then absorbed by enterocytes and reincorporated into complex lipids (Canavoso *et al.* 2001; Sieber and Thummel 2009; Carvalho *et al.* 2012). Enterocytes convert dietary FA and glycerol moieties to the acylglycerol intermediate diacylglycerol (DAG), the major transport form of neutral lipid in the hemolymph (Palm *et al.* 2012). Excess dietary FA is also converted to TAG and deposited in intracellular LDs for transient storage (Carvalho *et al.* 2012; Palm *et al.* 2012). In addition, midgut enterocytes convert acetyl-CoA units derived from the metabolism of ingested carbohydrates into FAs (a process termed *de novo* lipogenesis), which are also incorporated into DAG or TAG for transport into other tissues or for transient storage, respectively (Figure 1) (Song *et al.* 2014). Hemolymph circulates through an open circulatory system, and transports nutrients and humoral factors between tissues. DAG, exported into the hemolymph by the midgut, exists in the form of specific lipoprotein complexes that serve as reusable shuttles for lipid transport between tissues (Palm *et al.* 2012). The hemolymph exports lipids to the brain, oocytes, oenocytes, and imaginal discs, in addition to other tissues and organs (Parra-Peralbo and Culi 2011; Palm *et al.* 2012; Parvy *et al.* 2012). Most midgut-derived DAG is directed to the prime energy storing tissue, the fat body, which has a uniquely high capacity for storage of TAG. It utilizes ingested lipids and *de novo* synthesized FAs for TAG storage (Canavoso *et al.* 2001; Palm *et al.* 2012; Parvy *et al.* 2012). In times of starvation, fat body TAG reserves are mobilized by enzymatic hydrolysis in a process termed lipolysis and supply DAG to other tissues (Figure 1) (Chino and Gilbert 1964; Arrese and Wells 1997; Grönke *et al.* 2005, 2007). One prominent example is the oenocyte, which acquires neutral fat from fat body lipolysis specifically upon fasting and possesses unique biochemical pathways for processing FA units into cuticle structural lipids, some of which serve as pheromones (Gutierrez *et al.* 2006; Chatterjee *et al.* 2014; Makki *et al.* 2014) (Figure 1). Although the fat body is the primary TAG storage organ, most other tissues maintain autonomous TAG pools and typically express specific sets of enzymes for the synthesis of TAG (Chintapalli *et al.* 2007; Kühnlein 2011). Likewise, enzymatic machineries that catabolize TAG (and DAG) to FA moieties, which are required for further catabolism and resultant energy production, are believed to be a general feature of all fly tissues and organs.

De Novo Lipogenesis and Dietary Lipid Digestion

FA moieties that are stockpiled in TAG are either derived from the diet or synthesized *de novo* through a cascade of enzymatic reactions. The basic pathway of *de novo* FA formation requires two enzymes. First, Acetyl-CoA-carboxylase (ACC) catalyzes the formation of malonyl-CoA from acetyl-CoA. Second, the multienzyme complex FA synthase (FAS, encoded by *FASN*) sequentially condenses malonyl-CoA units with acetyl-CoA to produce long-chain FAs (Smith *et al.* 2003; Barber *et al.* 2005). The *Drosophila* genome encodes a single *ACC* gene and three distinct *FASN* genes (Parvy *et al.* 2012). *ACC* is ubiquitously expressed, with highest levels in oenocytes and fat body cells (Parvy *et al.* 2012). Three *FASN*-related genes—*FASN1* (*CG3523*), *FASN2* (*CG3524*), and *FASN3* (*CG17374*)—show distinct expression patterns and are differentially expressed in the fat body, oenocytes, midgut, and muscles (Garrido *et al.* 2015; Wicker-Thomas *et al.* 2015). Both, *ACC* and *FASN* functions are essential for development as genetic loss of the single *Drosophila ACC* gene, or a combined deletion of *FASN1* and *FASN2*, results in embryonic and larval lethality, respectively (Parvy *et al.* 2012; Garrido *et al.* 2015). These developmental deficits are in part due to oenocyte-specific functions of *de novo* FA synthesis (Parvy *et al.* 2012). The long-chain and very long-chain FA pools generated in oenocytes are further converted to cuticular hydrocarbons and other lipids, which are essential for the barrier functions of the cuticle and the trachea (Chung *et al.* 2014; Wicker-Thomas *et al.* 2015). Notably, the tissue-autonomous TAG pool of larval oenocytes accumulates even in the absence of *ACC* function, suggesting that tissue-autonomous storage lipid synthesis in oenocytes does not contribute to the developmental arrest evoked by defects in *de novo* FA synthesis (Parvy *et al.* 2012). Instead, ACC and FASN contribute to storage lipid synthesis mainly in two other tissues, the fat body and the midgut (Palm *et al.* 2012; Parvy *et al.* 2012). In the latter tissue, *de novo* lipogenesis contributes to the formation of DAG for export to the hemolymph and for transport of FA moieties to other tissues (Palm *et al.* 2012; described below). In the fat body, *de novo* lipogenesis generates a quantitatively important fraction of storage lipids (Parvy *et al.* 2012; Garrido *et al.* 2015; Wicker-Thomas *et al.* 2015). Both, fat body and midgut lipogenesis have important tissue-autonomous functions in maintaining whole-body energy homeostasis. Animals deficient in *de novo* lipogenesis due to combined mutations in *FASN1* and *FASN2* store less TAG in the larval as well as adult states, suggesting a continuous contribution of *de novo* FA synthesis to fat body TAG storage throughout development (Wicker-Thomas *et al.* 2015). This blockade of fat body lipogenesis renders larvae sensitive to excess sugar, and suggests a dual role of *de novo* lipogenesis in bulk energy storage and in sugar detoxification (Garrido *et al.* 2015). Sugar-stressed *FASN1/2* mutant cells display a cell-autonomous decrease in size, and accumulate advanced glycation end products that form by covalent bonding between amine and carbonyl groups of sugars, or their α-oxalaldehyde derivatives (Garrido *et al.* 2015). However, *FASN1* knockdown did not result in increased sugar sensitivity in another study (Havula *et al.* 2013), suggesting that specific features of the diet or genetic background might modulate this phenotype.

It is notable that genetic blockade of lipogenesis in skeletal muscle tissues impairs muscle function, and translates into

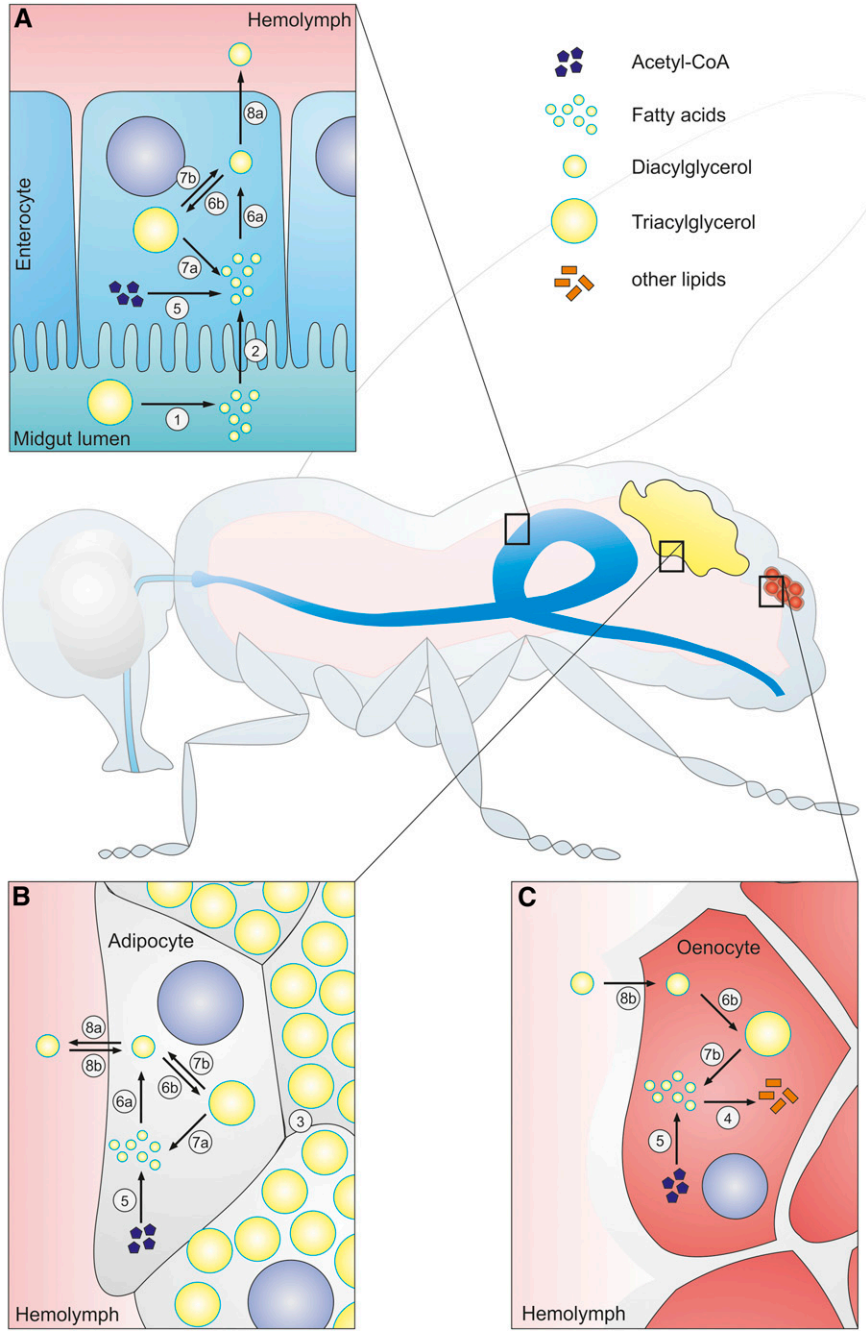

Figure 1 Triacylglycerol (TAG) metabolism in major fly organs of glycerolipid absorption, storage, and utilization/processing. (A) The midgut serves as major site of TAG digestion and lipid absorption. Dietary TAG is enzymatically processed to lipolytic products such as fatty acids (FAs) by digestive lipases prior to absorption by the enterocytes of the midgut (1–2). Enterocytes produce additional FAs through *de novo* synthesis from acetyl-CoA (5). FAs from both sources are converted to diacylglycerol (DAG) via enzymatic pathways (6a). DAG is exported to the hemolymph for lipid transport to other tissues (8a). Alternatively, DAG can be esterified with excessive FAs to TAG for storage (6b). TAG can be remobilized to FA and DAG by intracellular lipolysis (7a–b). (B) The fat body serves as a major energy reservoir that stores bulk TAG (3). Similar metabolic pathways as in the midgut direct the synthesis and turnover of FA, DAG, and TAG (5–7). Adipocytes control lipid transport via the hemolymph by the export and import of DAG (8a–b). (C) Oenocytes are an exemplary site of lipid utilization. FAs derived from *de novo* lipogenesis (5) or TAG lipolysis (7b) are converted to structural or signaling lipids (4). Oenocytes also convert surplus DAG acquired from the hemolymph (8b) to TAG for storage (6b). Note that TAG metabolism is not restricted to the organs displayed but is an inherent property of all fly tissues. In particular, the central nervous system, as well as cardiac and skeletal muscles, play important roles in organismal TAG homeostasis integration and/or show pathophysiological phenotypes in response to TAG dysregulation.

defects in larval motility and dietary-induced hyperactivity in adults (Katewa *et al.* 2012; Garrido *et al.* 2015). In adult flies, dietary restriction has been shown to increase steady-state whole-body TAG levels as well as overall TAG turnover rates. These metabolic adaptions, as well as life-extending effects of dietary restriction, were compromised by muscle-specific knockdown of *ACC* (Katewa *et al.* 2012). Unrestricted lipogenesis via *ACC* and *FASN* gene functions was identified as a pathogenic mechanism involved in cardiac dysfunction of flies with mutations in the *easily shocked* (*eas*) gene, which encodes an ethanolamine kinase (Lim *et al.* 2011). *Eas* mutant hearts exhibit elevated lipogenic gene expression and increased TAG content, likely due to a derepression of the transcription factor sterol regulatory element-binding protein (SREBP, discussed in further detail below). The increased cardiac TAG levels and cardiac dysfunction could be corrected by genetically interfering with the cardiac expression of lipogenic genes. However, it is currently not known if the phenotypes evoked by dysregulated lipogenesis in skeletal or cardiac muscle directly involve tissue-autonomous changes of TAG metabolism, or changes in the metabolism of other lipids. Notably, a recent study demonstrated that ceramide accumulation causes lipotoxic cardiomyopathy in flies and identified FASN1 as a ceramide-interacting

protein (Walls *et al.* 2018). Thus, ectopic TAG accumulation in cardiac muscle may be a metabolic signature, rather than a mechanistic cause, of FA-induced stress. In summary, *de novo* lipogenesis contributes a quantitatively important source of FAs for TAG storage in various developmental stages of *Drosophila*, but also provides tissue-specific pools of FAs for essential, as well as potentially lipotoxic, downstream metabolites that are unrelated to TAG.

The diet constitutes an alternative source of FA for the synthesis of structural and storage lipids. The *Drosophila* midgut—the main site of digestion and nutrient absorption—is composed of defined subregions with distinct morphological, histological, physiological, and genetic properties (Buchon *et al.* 2013; Lemaitre and Miguel-Aliaga 2013). Digestive enzymes are frequently organized in genomic clusters that may have arisen by gene duplication followed by functional divergence. The corresponding gene products are often sequentially expressed in various gut regions, suggesting a tight regulation of nutrient digestion in each section of the digestive tract. The resorption of dietary lipids requires the digestion of complex lipids into simple building blocks by the action of dietary lipases to yield FAs, glycerol, and acylglycerol intermediates. The *Drosophila* genome encodes about a dozen genes with predicted lipase function whose expression in the midgut qualifies them as putative digestive enzymes (Horne *et al.* 2009; Sieber and Thummel 2009). However, relatively little is known about the biochemistry and enzymology of lipid digestion in *Drosophila*. Alterations in dietary status or food composition cause distinct expression responses for these putative midgut lipases (Zinke *et al.* 2002; Chintapalli *et al.* 2007; Sieber and Thummel 2009; Karpac *et al.* 2013). For example, expression of the pancreatic lipase-related genes *CG6277* and *CG6283* is repressed by dietary sugar, whereas expression of the gastric lipase-related *magro* gene is induced by feeding, and suppressed by starvation and cholesterol (Grönke *et al.* 2005; Horner *et al.* 2009; Chng *et al.* 2014; Mattila *et al.* 2015). Although the large number of putative midgut lipases may suggest substantial functional redundancy in dietary lipid breakdown, Magro has been ascribed a major function in lipid digestion (Sieber and Thummel 2009). Magro exhibits TAG and cholesterol ester hydrolase activity *in vitro*, and is expressed at high levels in the proventriculus from where it is secreted into the gut lumen. Knockdown of *magro* expression or pharmacological inhibition of midgut lipase activity decreases midgut TAG lipase activity and assimilation of dietary TAG, which manifests in decreased whole-body TAG storage and starvation sensitivity (Sieber and Thummel 2009, 2012).

Transcriptional Regulation of *De Novo* Lipogenesis and Digestion of Dietary TAG

The enzymatic control of *de novo* lipogenesis and lipid digestion is governed by changes in the dietary status of the animal, which are sensed and transmitted by transcription factors that respond to distinct dietary or hormonal cues. Among them, the SREBPs have an evolutionarily conserved function in controlling lipogenic gene expression (Dobrosotskaya *et al.* 2002; Seegmiller *et al.* 2002; McKay *et al.* 2003; Walker *et al.* 2011). SREBPs regulate transcription of a variety of genes involved in *de novo* lipogenesis and membrane biosynthesis, including *ACC* and *FASN*, and are targeted by numerous hormonal and dietary signals. SREBPs are membrane-bound bHLH leucine zipper (bHLH-Zip) transcription factors that are activated by proteolytic cleavage at specific sites that, in turn, facilitate the release and nuclear translocation of the active bHLH-Zip domains. A lipid-sensing chaperone termed SREBP cleavage-activating protein (SCAP), and specific proteases including site-specific proteases 1 and 2 (S1P and S2P) constitute the basic molecular machinery that controls SREBP activation. This machinery senses membrane lipids such as sterols and phospholipids, which act in a feedback mode to inhibit SREBP processing and activation [for reviews, see Horton (2002), Rawson (2003), and Osborne and Espenshade (2009)]. The *Drosophila* genome encodes single orthologs of SREBP, SCAP, S1P, and S2P (Seegmiller *et al.* 2002). Consistent with its lipogenic function, SREBP is mainly expressed in the midgut, fat body, and oenocytes (Kunte *et al.* 2006). *Drosophila SREBP* null mutants are FA auxotrophic and die as second-instar larvae unless supplemented with dietary FAs. Mutant larvae show decreased expression of lipogenic genes such as *ACC* and *FASN* (Kunte *et al.* 2006). Although TAG storage has not been investigated in *SREBP* mutant larvae, the lean phenotype of *Drosophila* mutants lacking components of the SREBP-processing machinery (SCAP or S2P) suggests that *SREBP* function is required for the buildup of organismal TAG stores (Matthews *et al.* 2010). Moreover, manipulations of *SREBP* expression in the larval midgut by ectopic expression or RNA interference (RNAi)-mediated knockdown have been shown to reciprocally affect midgut lipid stores and body TAG levels (Song *et al.* 2014). Thus, the impairments in TAG storage observed upon manipulations of *SREBP* expression largely recapitulate the phenotypes of *ACC* or *FASN* deficiency described above. At present, it is unclear as to what extent the developmental defects displayed by *SREBP* mutants are related to the reduced capacity to build up energy reserves or to defects in the synthesis of other lipids required for development (Kunte *et al.* 2006). The tissue-autonomous function of lipogenesis in larval oenocytes that generates essential TAG-unrelated lipids suggests that a similar mechanism may account for the *SREBP* mutant phenotype (Parvy *et al.* 2012). However, results of genetic rescue experiments argue against an oenocyte-autonomous role for SREBP, but rather point to essential functions of this protein in the midgut and fat body (Kunte *et al.* 2006). In accordance with the evolutionarily conserved function of SREBP proteins in sensing membrane lipids, the processing of *Drosophila* SREBP is inhibited by synthesis of the major *Drosophila* phospholipid phosphatidylethanolamine (PE) (Dobrosotskaya *et al.* 2002). RNAi-mediated interference with PE synthesis in the adult fat body causes enhanced *ACC* and *FASN* gene expression and

obesity (Baumbach *et al.* 2014a). Likewise, defective PE synthesis in *eas* mutants has been linked to elevated lipogenic gene expression and tissue-autonomous ectopic TAG deposition in cardiac muscle (Lim *et al.* 2011). In addition to membrane lipids, SREBP activity is also regulated by hormones. Enterocyte SREBP activity is induced by JH signaling in response to mating and is required for optimal fecundity (Reiff *et al.* 2015). Conversely, tachykinin, secreted from enteroendocrine cells upon fasting, represses midgut SREBP activity via activation of Pka (Song *et al.* 2014). Notably, SREBP control by the PI3K/Akt/TOR (target of rapamycin) pathway [reviewed by Krycer *et al.* (2010)] appears to be evolutionarily highly conserved among flies and mammals, exhibiting similar effects on cell growth in these organisms (Porstmann *et al.* 2008). Lipogenic gene expression is also controlled by the heterodimeric transcription factor Mondo/Bigmax (also abbreviated Mio/Mlx), which coordinates transcriptional responses to dietary sugar [reviewed in Mattila and Hietakangas (2017)]. *bigmax* mutant larvae have decreased *ACC* and *FASN* gene expression, and store less TAG. Upon increased dietary sugar, Mondo/Bigmax induces expression of the transcription factor Sugarbabe, which acts in a feedforward loop to transcriptionally activate lipogenic gene expression (Mattila *et al.* 2015). Notably, Mondo/Bigmax is also required for the sugar-dependent repression of certain genes. These include the putative gut lipases CG6283 and CG6277, suggesting that Mondo/Bigmax coordinates the expression of digestive enzymes with dietary needs and coordinates sugar metabolism, *de novo* lipogenesis, and lipid digestion (Mattila *et al.* 2015).

In addition to Mondo/Bigmax, several other transcription factors have been implicated in the regulation of dietary lipid digestion. The Hormone receptor-like in 96 (Hr96, also known as DHR96) acts in the midgut as a transcriptional regulator of genes involved in dietary lipid breakdown and uptake (Horner *et al.* 2009; Sieber and Thummel 2009, 2012). Hr96 is required for expression of the major digestive TAG lipase Magro. *Hr96* mutants are lean and genetic rescue experiments support the conclusion that Magro acts as main effector downstream of *Hr96* to control organismal TAG storage (Sieber and Thummel 2009). *Magro* expression is highly sensitive to alterations in food intake. Genetic models of hyperphagia in flies show increased *magro* expression and intestinal lipase activity (Subramanian *et al.* 2013). In contrast, the suppression of *magro* expression upon starvation is controlled by the insulin-responsive transcription factor Foxo (Karpac *et al.* 2013). Interference with intestinal *foxo* expression in adult flies increases *magro* expression and organismal TAG stores. Conversely, increased Foxo activity in the aging gut has been causally linked to decreased *magro* expression and compromised organismal TAG stores (Karpac *et al.* 2013).

Enzymatic Synthesis of TAG

Figure 2 summarizes the enzymatic steps and subcellular topology of TAG synthesis and its regulation. Excess FAs acquired via the diet or synthesized *de novo* need to be converted to complex lipids for transport or storage. This conversion involves the sequential esterification of FA moieties to a glycerol backbone. The canonical glycerol-3-phosphate (G3P) pathway (also called the Kennedy pathway) of TAG synthesis is initiated by G3P-*O*-acyltransferase (GPAT), which catalyzes the transfer of an FA unit from acyl-CoA to G3P [for review, see Bell and Coleman (1980), Liu *et al.* (2012), and Wang *et al.* (2017)]. The resultant lysophosphatidic acid (LPA, also termed 1-acylglycero-3-phosphate) is converted to phosphatidic acid (PA) in a second acyltransferase reaction by LPA acyltransferase (LPAAT; also termed 1-acylglycero-3-phosphate-*O*-acyltransferase, AGPAT). PA is converted by PA phosphatase (PAP) to DAG, which is the major transport unit of FA in the hemolymph of *Drosophila* (Palm *et al.* 2012). An alternative pathway of DAG synthesis involves the acylation of monoacylglycerol (MAG) by MAG-*O*-acyltransferase (MGAT). However, unlike the precursors of the G3P pathway, MAG is not synthesized *de novo*, but derived from the breakdown of complex lipids. Both the G3P and MGAT pathways converge at DAG (Liu *et al.* 2012). Finally, DAG-*O*-acyltransferase (DGAT) transfers FA from acyl-CoA to DAG, resulting in the formation of TAG. Each step is catalyzed by isoenzymes that differ in tissue expression and subcellular localization (Figure 2) (Chintapalli *et al.* 2007; Wilfling *et al.* 2013; Wang *et al.* 2017). TAG synthesis competes with several other biochemical pathways that consume FA, *e.g.*, FA oxidation or membrane lipid synthesis. The synthesis routes of glycerophospholipids share the initial steps with TAG, and branch off at the level of PA or DAG. Notably, the analysis of the *Drosophila* third-instar larval lipidome indicates a selective enrichment of medium-chain FAs in DAG and TAG as compared to membrane glycerophospholipids (Palm *et al.* 2012). It is presently unclear if this diversification involves the "channeling" of different FAs along *de novo* synthesis pathways toward either TAG or glycerophospholipids, *e.g.*, via different isoenzymes, or selective remodeling reactions after synthesis.

The *Drosophila* genome encodes three putative GPAT isoenzymes that belong to two different protein families and are structurally related to *bona fide* mammalian GPATs. The gene product of *minotaur* (*mino*), an ortholog of mammalian GPAT1 and GPAT2, has been named after its localization in the labyrinthine fusome network that connects nurse cells of *Drosophila* ovaries (Vagin *et al.* 2013). Interfering with *mino* function in ovaries causes defects in piwi-interacting RNA biosynthesis by a mechanism that is unrelated to its predicted enzymatic function. Consistent with an additional role in TAG formation, ectopic expression of *mino* increased fat storage in larval salivary glands (Tian *et al.* 2011; Pagac *et al.* 2016). However, the specific contribution of Mino to TAG synthesis has not been investigated in flies deficient for *mino*. The genes *CG15450* and *CG3209*/Gpat4 encode structurally related proteins with homology to mammalian GPAT3 and GPAT4, respectively. Whereas *CG15450* expression is restricted to the testis, Gpat4 is ubiquitously expressed

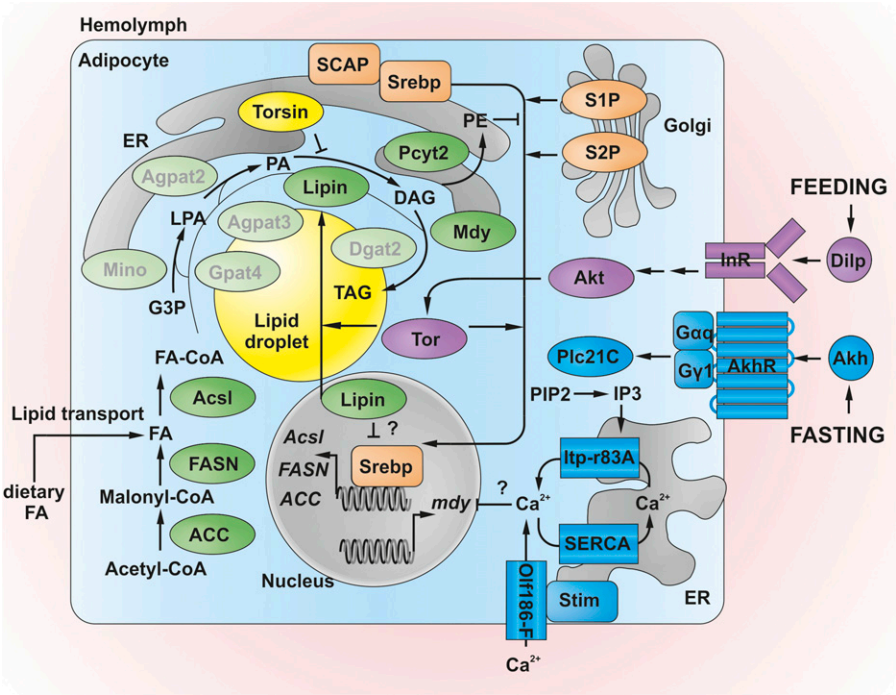

Figure 2 Triacylglycerol (TAG) synthesis in *Drosophila*: biochemistry and main regulatory pathways. Fatty acids (FAs) derived from the diet or synthesized *de novo* by Acetyl-CoA-carboxylase (ACC) and the FA synthase complex (FASN) are activated to FA-CoA by Acsl, and are esterified consecutively by glycerol-3-phosphate acyltransferases (GPATs) (possibly Gpat4 or Mino) to lysophosphatidic acid (LPA) and by LPA acyltransferases (possibly Agpat3, Agpat2) to phosphatidic acid (PA). Lipin dephosphorylates PA to diacylglycerol (DAG), which is further esterified by DAG acyltransferases (Mdy and possibly Dgat2) to TAG and stored in lipid droplets (LDs). Acyltransferases show differential localization to the endoplasmic reticulum (ER) or LDs, respectively. Lipin shuttling between the cytoplasm and the nucleus is controlled by the Tor kinase, which in turn is subject to insulin pathway regulation via Akt under feeding conditions. Lipin is also under the inhibitory control of Torsin. The transcription factor sterol regulatory element-binding protein (SREBP) promotes FA-CoA synthesis via positive control of *ACC*, *FASN*, and *Acsl* expression. The canonical pathway of SREBP activation requires its interaction with the escort factor SREBP cleavage-activating protein (SCAP), and its proteolytic processing via Golgi-resident S1P and S2P. SREBP activation is promoted by target of rapamycin (Tor) kinase and inhibited by the membrane lipid PE, which is synthesized from DAG by Pcyt2. Nuclear Lipin may also inhibit SREBP activity. Under fasting conditions, *mdy* expression is suppressed in a Ca^{2+}-dependent manner under the control of adipokinetic hormone (Akh) signaling. Akh receptor (AkhR) activation by Akh triggers store-operated entry of extracellular Ca^{2+} via small G proteins, IP3 signaling under the control of phospholipase C, and ER Ca^{2+} store depletion, which promotes interaction of Stim with the plasma membrane Ca^{2+} channel Olf186-F to result in extracellular Ca^{2+} influx until the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) restores ER Ca^{2+} levels. The proximal mechanisms of Ca^{2+}-dependent *mdy* repression are currently unknown. See main text for details and definitions of abbreviations. Proteins shown in faded green with gray lettering are based on homology-based activity predictions and await experimental validation. Note the antagonistic action of the lipoanabolic insulin/Tor pathway (in magenta) under feeding *vs.* the lipocatabolic Akh pathway (in blue) under fasting conditions.

(Chintapalli *et al.* 2007) and the protein localizes to LDs in *Drosophila* S2 cells (Wilfling *et al.* 2013). *Drosophila* Gpat4 mutants exhibit a semilethal phenotype. More than 80% of Gpat4 mutant animals do not develop into larval stages. Animals that escape early death exhibit severe metabolic defects and developmental delay, but give rise to adults of normal size (Yan *et al.* 2015). Of note, TAG levels were unaltered in surviving mutants, suggesting that the developmental phenotypes of flies lacking Gpat4 are unrelated to storage lipid metabolism. Depletion of Gpat4 expression by RNAi reduced LDs in glial cells of *Drosophila* larvae (Bailey *et al.* 2015) and reduced TAG formation of embryonic S2 cells (Wilfling *et al.* 2013), suggesting a cell type-specific contribution of GPAT4 to TAG synthesis, which awaits further characterization.

As for the initial step of the G3P pathway, the second step has also been attributed to a set of various isoenzymes with putative LPAAT/AGPAT function. The *Drosophila* genome encodes four genes of the LPAAT/AGPAT family including *CG4729/Agpat3*, *CG4753/Agpat4*, *fu12/Agpat2*, and *CG3812/Agpat1*. No mutant alleles of these genes have been characterized so far. The function of these genes is largely inferred by homology to *bona fide* mammalian AGPATs and lacks biochemical evidence. A specific role of *Agpat3* in TAG storage metabolism has been suggested by a cell-based study using *in vitro*-cultured S2 cells in which the protein was localized to LDs and contributed to FA-induced TAG formation (Wilfling *et al.* 2013). In addition, *in vivo* knockdown of *Agpat3* gene expression reduced LD formation in glial cells (Bailey *et al.* 2015). Whether this protein controls TAG formation in other cells or acts redundantly to other AGPATs remains to be investigated.

The third step in TAG formation is the dephosphorylation of PA to DAG. PA is a substrate for type I and type II PAP enzymes that are discriminated by their requirement for Mg^{2+} (Zhang and Reue 2017). The Mg^{2+}-dependent type I PAP activity is encoded by *Lipin* genes that have been assigned major roles in glycerolipid and TAG synthesis in diverse phyla (Péterfy *et al.* 2001; Han *et al.* 2006; Donkor *et al.* 2007). Apart from their enzymatic function in glycerolipid synthesis, Lipins have been shown to act as transcriptional cofactors in the nucleus (Finck *et al.* 2006; Peterson *et al.* 2011). *Drosophila* has a single *Lipin* gene (*Lpin*) that encodes at least three different protein isoforms with tissue- and developmental stage-specific expression patterns (Valente *et al.* 2010). *Lpin* mutants exhibit a plethora of phenotypes including developmental delay, pupal semilethality, and reduced fertility (Ugrankar *et al.* 2011). Consistent with a key function of Lipin in TAG formation, mutant larvae present with severe lipodystrophy,

reduced organismal TAG levels, and decreased LD size (Ugrankar *et al.* 2011). Conversely, TAG levels and LD size are increased in the fat body of young larvae mutant for the Lipin repressor Torsin, which affects Lipin localization (Grillet *et al.* 2016). This fat body phenotype is not limited to cellular TAG content, but also manifests as altered cell morphology, including fragmented nuclei, aberrant organelle structure, and altered cell shape (Ugrankar *et al.* 2011). Specifically, loss of *Lpin* is associated with a cell-autonomous reduction in insulin signaling and fat cell size (Schmitt *et al.* 2015). This phenotype is further enhanced upon genetic impairment of insulin and Tor signaling, suggesting that *Lpin* function is required for the normal insulin responsiveness of fat cells (Schmitt *et al.* 2015).

The last and only committed step in TAG synthesis is the conversion of DAG to TAG by DGAT. Eukaryotic DGAT isoenzymes belong to two unrelated protein families, membrane-bound *O*-acyltransferase (MBOAT) and DGAT2, respectively. Although the *Drosophila* genome encodes for proteins of both families, a single enzyme of the MBOAT family, Midway (Mdy), has been assigned a major role in *Drosophila* TAG metabolism. Midway is structurally related to mammalian DGAT1 and has been shown to possess DGAT activity *in vitro* (Buszczak *et al.* 2002). Mutations in the *mdy* gene were initially identified in a genetic screen for female sterile mutants (Schüpbach and Wieschaus 1991). *Mdy* mutants display reduced oocyte lipid stores and degeneration of egg chambers during midoogenesis (Buszczak *et al.* 2002). Since TAG serves as an energy resource of the developing embryo, it has been suggested that defects in TAG synthesis are causative for egg chamber degeneration in *mdy* mutants (Buszczak *et al.* 2002). Likewise, *mdy* lack-of-function mutations in adult fat-storing tissues cause a strong reduction in organismal TAG stores, arguing for a principle role of this enzyme in TAG synthesis in *Drosophila* (Beller *et al.* 2010; Baumbach *et al.* 2014a). The *Drosophila* genome contains three DGAT2 family members encoded by the uncharacterized genes *CG1941*, *CG1942/Dgat2*, and *CG1946*. The associated proteins are structurally related to mammalian DGAT2 and MGAT enzymes. Although recombinant CG1942/Dgat2 protein has been shown to localize to LDs in *Drosophila* S2 cells, a reduction in *CG1942/Dgat2* expression did not compromise TAG formation (Wilfling *et al.* 2013). Moreover, and in contrast to Midway, the enzymatic activities of *Drosophila* DGAT2 family members have not been characterized. Thus, it remains to be shown if *Drosophila* DGAT2 enzymes represent *bona fide* DGATs or, rather, function in the MGAT pathway of TAG synthesis. Notably, MGAT activity has been measured in fat body samples of the tobacco hornworm *Manduca sexta*, further arguing for the presence of this pathway in insects (Arrese *et al.* 1996).

Regulation of TAG Synthesis

Although data from genome-wide transcriptomics suggests nutritional regulation of several acyltransferases (Zinke *et al.* 2002; Harbison *et al.* 2005; Mattila *et al.* 2015) implicated in TAG synthesis, little is known about the underlying molecular mechanisms of these transcriptional responses or the physiological significance for storage lipid metabolism. *Mdy* gene expression levels decrease in response to genetic manipulations that increase cytosolic Ca^{2+} levels in fat body cells, such as knockdown of the ER Ca^{2+} pump SERCA (Bi *et al.* 2014). Conversely, depletion of cytosolic Ca^{2+} levels in the fat body tissue of adult flies by knockdown of the ER calcium sensor *Stim*, or by knockdown of the IP3 receptor gene *Itp-r83A*, increase *mdy* gene expression (Figure 2) (Baumbach *et al.* 2014a). This response is in part due to a tissue-nonautonomous effect on activation of the orexigenic brain short neuropeptide F (sNPF) and ensuing hyperphagia. Notably, a similar change in *mdy* transcript expression was also elicited by genetic manipulations that compromise Akh signaling in the fat body, which is in line with the observation that Ca^{2+} is an important second messenger mediating Akh responses in insects (Arrese *et al.* 1999; Baumbach *et al.* 2014a). Conversely, genetic activation of Akh signaling suppressed *mdy* expression. However, the molecular mechanisms that relay Akh/Ca^{2+} signaling to altered *mdy* transcript levels remain elusive (Figure 2). Unlike many other anabolic genes, transcriptional expression of *Lpin* is induced upon starvation, compared to the *ad libitum* fed state (Harbison *et al.* 2005; Ugrankar *et al.* 2011). In addition, fasting alters the subcellular localization of Lipin and increases its abundance in the nucleus (Figure 2). Although the nuclear functions of *Drosophila* Lipin are not fully understood, studies with mammalian orthologs suggest that nuclear Lipin acts as a transcriptional activator of genes involved in FA utilization and as a repressor of lipogenic genes (Finck *et al.* 2006; Peterson *et al.* 2011). Insulin- and TOR complex 1 (TORC1)-dependent phosphorylation of mammalian Lipins prevents nuclear translocation (Harris *et al.* 2007; Peterson *et al.* 2011). Likewise, nuclear translocation of *Drosophila* Lipin is suppressed by TORC1 signaling, whereas altered insulin pathway activity did not change Lipin localization *in vivo* (Schmitt *et al.* 2015). Although these observations suggest a dual role for Lipin in TAG synthesis and the regulation of gene expression, knowledge about putative transcriptional targets of *Drosophila* Lipin is currently lacking.

TAG Storage: Formation of LDs

Although the *Drosophila* fat body has a unique capacity to synthesize and store TAG, most other tissues harbor the protein machinery of TAG synthesis and storage. TAG storage has been observed in the midgut, brain, oenocytes (Gutierrez *et al.* 2006; Palm *et al.* 2012; Bailey *et al.* 2015), imaginal discs, ovaries (Parra-Peralbo and Culi 2011), and salivary glands (Thiel *et al.* 2013). At the cellular level, fat storage is accomplished by deposition of lipid esters into discrete globular organelles termed LDs (Welte 2015). The evolutionarily conserved organization of LDs is characterized by a hydrophobic core of storage lipid esters (largely TAG and sterol ester), surrounded by a closed phospholipid monolayer to which proteins are attached

(Welte 2015). Proteomic studies of LDs from *Drosophila* embryos, larvae, or *in vitro*-cultured cells have identified specific sets of LD proteins with functions in metabolism, membrane trafficking, signaling, and protein turnover (Beller *et al.* 2006; Cermelli *et al.* 2006; Krahmer *et al.* 2013). Although the general organization of LDs is highly conserved between distant phyla such as fungi, plants, or animals, there is morphological and functional heterogeneity between LDs, even within single cells or tissues (Wilfling *et al.* 2013; Thul *et al.* 2017). Although the exact mechanisms of LD biogenesis are incompletely understood, a prevalent model suggests that LDs arise through the deposition of neutral lipids between the leaflets of the ER lipid bilayer, developing nascent droplets that finally bud off from the ER membrane after reaching a critical size [reviewed in Walther *et al.* (2017)]. This process is regulated and assisted by distinct proteins whose dysfunction compromises normal LD morphology and function. Genome-wide RNAi screens in *Drosophila* cells were indispensable to the comprehensive identification of proteins required for normal LD structure (Beller *et al.* 2008; Guo *et al.* 2008). A prominent example is Seipin, which was initially identified as being deficient in human patients with congenital lipodystrophy (Magré *et al.* 2001). Orthologous proteins in diverse eukaryotic model organisms such as yeast, plants, and insects have shaped the view that Seipin has a conserved function in establishing and maintaining LD morphology (Grippa *et al.* 2015; Wang *et al.* 2016; Taurino *et al.* 2018). The single *Drosophila* Seipin ortholog is expressed mainly in the fat body, but also in the salivary gland, midgut, and muscle (Tian *et al.* 2011). *Drosophila* Seipin localizes at contact sites between the ER and LDs, and promotes distinct steps in LD maturation in *Drosophila* S2 cells (Wang *et al.* 2016). *Seipin* mutant flies display fat body-autonomous reductions in LD size and organismal TAG levels (Tian *et al.* 2011). Notably, this deficit in TAG storage has been linked to impaired activity of the ER Ca^{2+} pump SERCA (Bi *et al.* 2014) and subsequent mitochondrial dysfunction, which leads to reduced lipogenesis (Ding *et al.* 2018). The *Seipin* mutant also represents a striking example of ectopic fat accumulation in salivary glands, which is unrelated to the function of Seipin in the fat body. Genetic and biochemical studies have linked ectopic LD formation in *Seipin* mutant salivary glands to the accumulation of PA, and aberrant TAG synthesis via Lipin and Mdy, rather than to defective fat breakdown (Tian *et al.* 2011). Notably, lipodystrophy, as well as ectopic fat accumulation, are also major pathological manifestations of loss-of-function mutations in mammalian *Seipin* (Magré *et al.* 2001; Chen *et al.* 2012), highlighting the value of *Drosophila* as a model providing novel insights into human LD-associated pathologies.

TAG Mobilization by Lipolysis

During nonfeeding developmental stages, periods of increased energy demand, or nutrient deprivation, insects mobilize their energy stores. The mobilization of fat body TAG requires hydrolysis of the ester bonds in TAG by the action of lipases. The prevailing model of insect fat body lipolysis is that TAG hydrolysis directly generates DAG, which is exported into the hemolymph for transport into other tissues (Arrese and Wells 1997). A major executor of fat body lipolysis in *Drosophila* is the TAG hydrolase Brummer (Bmm), a member of the patatin-like domain-containing family of proteins (Grönke *et al.* 2005). Orthologous proteins in diverse phyla such as plants (Eastmond 2006), yeast (Athenstaedt and Daum 2003; Kurat *et al.* 2005), and mammals (Zimmermann *et al.* 2004) have been assigned analogous functions in lipid mobilization, highlighting the ancestral role of Bmm-related proteins in TAG breakdown. *Drosophila bmm* mutant flies accumulate excessive TAG during adulthood and display a reduced rate of TAG breakdown upon starvation. Due to the excessive fat depots and delayed TAG consumption, *bmm* mutants substantially outlive wild-type flies during nutrient deprivation, suggesting impaired, though still functional, lipid mobilization (Grönke *et al.* 2005). An alternative lipolytic system in *Drosophila* depends on Akh signaling that controls the expression of one or more currently uncharacterized TAG lipase(s), which complement Bmm lipase function (Grönke *et al.* 2007). The *bmm* gene is broadly expressed and abundant in several other tissues than the fat body, including the gut, salivary glands, heart, and oenocytes (Chintapalli *et al.* 2007). In line with a general function of Bmm in TAG breakdown, *bmm* mutants accumulate excessive TAG in peripheral tissues such as the midgut (Palm *et al.* 2012) and in the Malpighian tubules (P. Hehlert and R. P. Kühnlein, in preparation). Notably, *bmm* transcripts are maternally provided and expressed throughout embryogenesis, suggesting an essential function of Bmm during embryonic TAG mobilization. In line with this hypothesis, a lack of zygotic and maternal *bmm* function results in embryonic lethality (Grönke *et al.* 2005).

DAG that has been liberated from fat body TAG stores needs to be further hydrolyzed before FA can enter β-oxidative or anabolic pathways. Brummer lacks DAG lipase activity *in vitro* (Grönke *et al.* 2005) and is, therefore, unlikely to provide the activity necessary to catabolize DAG. A candidate enzyme for this function is the *Drosophila* hormone-sensitive lipase (Hsl), which has been named because of its homology to mammalian Hsl. Mammalian Hsl is a hydrolase with broad substrate specificity and acts as a key DAG lipase in adipose tissue, testis, and muscle (Haemmerle *et al.* 2002). *Drosophila* Hsl has been implicated in TAG mobilization in parallel with Bmm lipase in the larval fat body (Bi *et al.* 2012). As the enzymatic properties of *Drosophila* Hsl have not yet been characterized, it remains to be shown if *Drosophila* Hsl acts as a DAG lipase in peripheral tissues and to what extent it contributes to complete acylglycerol breakdown for energy production.

Regulation of Lipolysis

Consistent with its major physiological function with respect to energy metabolism, TAG mobilization in *Drosophila* is

nutritionally regulated both at the transcriptional and post-translational levels. For example, *bmm* transcription is suppressed by feeding and induced upon nutrient withdrawal. An important transcriptional inducer of *bmm* expression is the insulin-responsive transcription factor Foxo (Figure 3) (Wang *et al.* 2011). Nuclear Foxo promotes transcription of a plethora of fasting-induced genes while it suppresses others [Karpac *et al.* (2013) and see above]. The nuclear abundance of Foxo is regulated by post-transcriptional modifications, including phosphorylation and acetylation, that control its retention in the cytosol. In addition to the canonical inhibitory Foxo phosphorylation by Akt1, Salt-inducible kinase 3 (Sik3) acts as an important negative regulator of Foxo and indirectly controls its sequestration in the cytosol (Puig *et al.* 2003; Wang *et al.* 2011; Choi *et al.* 2015). Sik3 phosphorylates histone deacetylase 4 (HDAC4), which sequesters HDAC4 in the cytosol, rendering it unable to interact with and activate nuclear Foxo. This results in decreased Foxo activity in the nucleus and, consequently, decreased *bmm* expression (Figure 3) (Wang *et al.* 2011; Choi *et al.* 2015). The failure to suppress *bmm* expression causes reduced TAG levels and increased starvation sensitivity in *Sik3* mutant animals (Wang *et al.* 2011). Although the insulin pathway likely directly engages Sik3 by Akt1-dependent phosphorylation (Wang *et al.* 2011), Sik3 is also a direct target of the *Drosophila* ortholog of mammalian liver kinase B1 (Lkb1), which acts through an insulin-independent pathway, suggesting that various pathways converge at Sik3 to control *bmm* expression via Foxo (Choi *et al.* 2015). Additionally, Foxo-dependent *bmm* expression is repressed by the zinc finger transcription factor Kr-h1, a central effector of JH signaling (Kang *et al.* 2017). Notably, transcriptional control of *bmm* by Foxo is suggested to be evolutionarily highly conserved, as the mammalian Brummer ortholog adipose triglyceride lipase is subject to FoxO1 regulation (Chakrabarti and Kandror 2009). *Bmm* transcript levels show an antagonistic response to disturbed Akh and fat body Ca^{2+} homeostasis, as opposed to the lipogenesis gene *mdy* (Baumbach *et al.* 2014a). Genetic manipulations that deplete cytosolic Ca^{2+} or that result in a loss-of-function of AkhR reduce *bmm* transcript concentrations (Baumbach *et al.* 2014a), suggesting that Akh signaling via Ca^{2+} promotes *bmm* expression (Figure 3) (Baumbach *et al.* 2014a; Choi *et al.* 2015). As described above, hyperphagia driven by orexigenic sNPF contributes, in part, to this response, indicating a yet unknown nonautonomous tissue regulatory circuit controlled by fat body Ca^{2+}. Recently, Akh signaling via Ca^{2+} and Calcium/Cam-dependent protein kinase II (CaMKII) has been demonstrated to inhibit secretion of the adipokine Unpaired 2 (Upd2) from the fat body (Rajan *et al.* 2017). Upd2 triggers systemic insulin signaling from the central brain (Rajan and Perrimon 2012) and impairs TAG mobilization in the fat body, possibly via repressing Foxo-dependent *bmm* transcription (Rajan and Perrimon 2012; Rajan *et al.* 2017). Although not formally proven, this model implies that alterations in Upd2 and systemic insulin signaling underlie Akh/Ca^{2+}-dependent regulation of *bmm* expression.

Similar to *bmm*, the expression of *Hsl* transcripts is induced upon starvation, but the underlying molecular mechanisms are currently unknown (Bi *et al.* 2012). Post-transcriptional lipase regulation has been less studied in *Drosophila*, but two important mechanisms include Bmm transport to the LD surface, and the functional interaction of both Bmm and Hsl with LD coat proteins. Genome-wide RNAi screens in cultured *Drosophila* cell lines identified the Coat protein complex I (COPI) retrograde vesicle trafficking machinery as a key regulator of LD structure and TAG storage (Beller *et al.* 2008; Guo *et al.* 2008). Further studies identified defective trafficking of Bmm or orthologous proteins to the LD surface as a likely cause of the TAG overstorage phenotype provoked by defective COPI function (Beller *et al.* 2008; Wilfling *et al.* 2014). Notably, fat body-specific knockdown of the COPI-associated small GTPase adenosine diphosphate ribosylation factor at 79F (Arf79F) recapitulates the overstorage phenotype observed in cultured cells in adult flies (Baumbach *et al.* 2014a).

Perilipins are evolutionarily conserved LD-associated proteins with key functions in regulating LD structure and catabolism (Bickel *et al.* 2009). Studies of perilipin function in diverse phyla uncovered two canonical functions of this protein family by (1) limiting basal access of lipases to the LD surface, and (2) promoting lipase activity and LD access upon hormonal signaling [for review see Bickel *et al.* (2009), Kimmel and Sztalryd (2016), and Sztalryd and Brasaemle (2017)]. The *Drosophila* genome encodes two perilipin-related proteins termed Plin1 (also called Lipid storage droplet-1, Lsd-1) and Plin2 (also called Lipid storage droplet-2, Lsd-2), both of which contribute to the regulation of lipocatabolism (Figure 3). Plin2 acts in an antilipolytic way and opposes *bmm* function in a gene dosage-dependent manner (Grönke *et al.* 2003, 2005). Consequently, *plin2* mutants are lean and starvation-sensitive, whereas transgenic flies with increased *plin2* expression are obese and starvation-resistant (Grönke *et al.* 2003). Although the underlying molecular mechanism has not been formally addressed in *Drosophila*, these observations are in line with the canonical function of perilipins in limiting basal access of lipases to the LD surface, and preventing interactions between lipase and lipase substrates (Sztalryd and Brasaemle 2017). In contrast to Plin2, the second *Drosophila* perilipin Plin1 has been attributed with both pro- and antilipolytic functions. Genetic deficiency of *plin1* results in obesity of larvae and adult flies, and impaired yet functional lipid mobilization (Beller *et al.* 2010; Bi *et al.* 2012). A double deficiency in *plin1* and *bmm* further accentuated this phenotype resulting in flies completely lacking lipid mobilization competence (Beller *et al.* 2010). Loss of Plin1 impaired the starvation-induced enrichment of Hsl at larval LDs, suggesting that it acts as a docking interface for lipolytic factors (Bi *et al.* 2012). However, further genetic interaction studies demonstrated that Plin1 also contributes to limit basal Bmm activity (Beller *et al.* 2010). Both Plins are phosphorylated (Welte *et al.* 2005; Arrese *et al.* 2008; Beller *et al.* 2010), although the significance and dynamics of these modifications for the regulation of lipolysis in *Drosophila* are

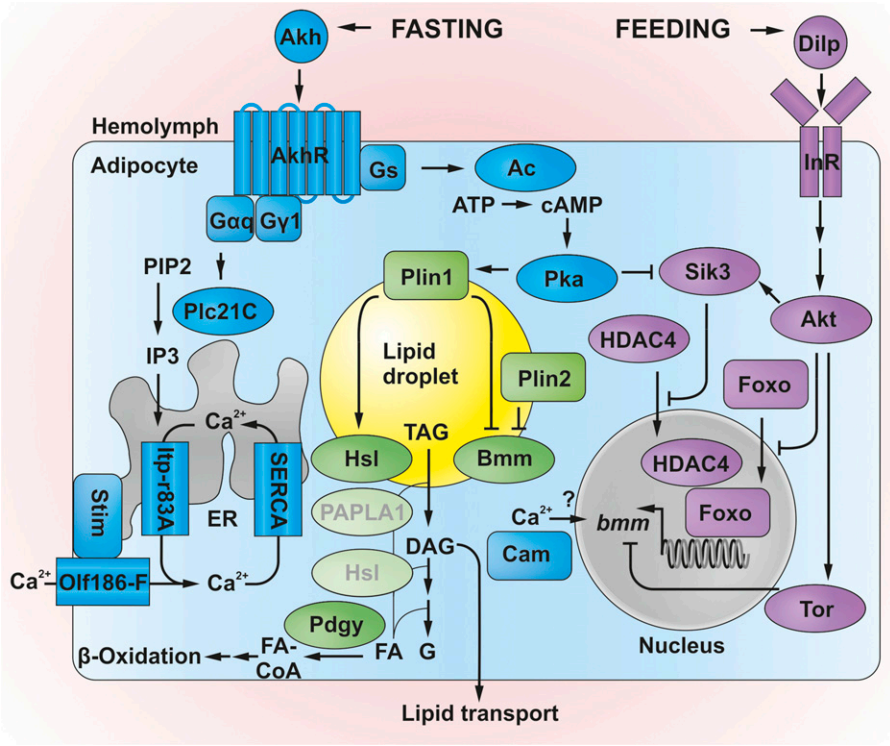

Figure 3 Triacylglycerol (TAG) lipolysis in *Drosophila*: biochemistry and main regulatory pathways. Mobilization of lipid droplet (LD) fat stores in adipocytes requires TAG hydrolysis by Bmm and Hsl [possibly also by PAPLA1 (Phosphatidic Acid Phospholipase A1)] to fatty acids (FAs) and diacylglycerol (DAG), which become exported to peripheral tissues, or are subjected to further lipolytic cleavage (possibly by Hsl) to release glycerol (G) and FAs. The latter can be catabolized via β-oxidation after activation to FA-CoA by Pdgy. LD coat proteins Plin1 and Plin2 restrict Bmm activity. Plin1 phosphorylation by protein kinase A (Pka) may also promote adipokinetic hormone (Akh)-stimulated TAG mobilization by facilitating the activity of Hsl and/or other currently unknown lipases. Transcription of *bmm* is under antagonistic control of the Akh pathway during fasting and under the insulin signaling pathway during feeding conditions. Insulin signaling operates via activation of Akt, which represses *bmm* expression by promoting phosphorylation and cytoplasmic retention of the transcription factor Foxo. In parallel, Akt activates Sik3, which promotes the phosphorylation and cytoplasmic retention of the Foxo-activator HDAC4. Target of rapamycin (Tor) kinase, which is positively regulated by insulin signaling, represses *bmm* transcript levels by a currently unknown mechanism. The insulin and Akh pathways converge at Sik3, which is suppressed by Pka. Pka, in turn, depends on the cyclic AMP (cAMP) branch of Akh/AkhR (Akh receptor) signaling, which operates via G_s and adenylate cyclase (Ac). Lipolysis also involves the Ca^{2+} branch of AkhR activation (for details see Figure 2 legend and main text). Genetic data support the idea that proteins such as Cam relay the rise in intracellular Ca^{2+}, resulting in *bmm* expression by unknown mechanisms. Proteins shown in faded green with gray lettering are based on homology-based activity predictions and await experimental validation. See main text for definitions of protein name abbreviations. Note the antagonistic action of the lipoanabolic insulin/Tor pathway (in magenta) under feeding *vs.* the lipocatabolic Akh pathway (in blue) under fasting conditions.

not completely understood (see below). A recent report linked ectopic TAG accumulation in skeletal muscle to defective proteolytic degradation of Plin2, suggesting that post-translational regulation of Plins contributes to TAG homeostasis (Yan *et al.* 2017). As noted above, Akh signaling influences the expression of key lipolytic/lipogenic effectors such as *bmm* and *mdy*, but also acts acutely via a second, currently unknown lipolytic effector (Grönke *et al.* 2007). The *bmm* and *Akh/AkhR* lipocatabolic systems show a strong genetic interaction and double mutants are extremely obese, unable to mobilize lipid stores, and highly starvation-sensitive (Grönke *et al.* 2007). Although the molecular events that relay Akh signaling to acute alterations in lipolytic output are understudied in *Drosophila*, other insect models have contributed important mechanistic insights into this process. In the *M. sexta* fat body, Akh signaling promotes elevated cAMP levels and subsequent activation of Pka, which phosphorylates the *Manduca* homolog of Plin1 (Arrese *et al.* 1999; Patel *et al.* 2005). This phosphorylation event renders Plin1-coated LDs susceptible to lipolytic activity by a cytosolic TAG lipase termed triglyceride lipase (TGL) (Patel *et al.* 2005; Arrese *et al.* 2006). Hsl and PA Phospholipase A1 (PAPLA1) are among the candidate Akh-dependent lipases in *Drosophila* (Figure 3). Whereas Hsl, together with Bmm, has been shown to coordinate TAG mobilization in larval stages, its function in adults remains unclear (Bi *et al.* 2012). *Drosophila* PAPLA1 is an ortholog of *Manduca* TGL and has been shown to possess TAG hydrolase activity *in vitro* (Arrese *et al.* 2006). *PAPLA1* mutant animals show a complex spectrum of phenotypes including impaired fecundity and fertility, decreased photoreceptor sensitivity, and metabolic abnormalities (Kunduri *et al.* 2014; Gáliková *et al.* 2017). However, TAG storage and mobilization are comparable in *PAPLA1* mutants and wild-type animals (Gáliková *et al.* 2017), which makes the hypothesis of *in vivo* relevance of PAPLA1 in *Drosophila* TAG mobilization controversial.

With notable exceptions, the steps of DAG breakdown to FA, and its subsequent degradation by mitochondrial or peroxisomal β-oxidation, have not been comprehensively characterized in the fly with respect to the involved genes and their corresponding regulatory mechanisms. However, the starvation-responsive nuclear receptor HNF4 is a critical regulator of lipid catabolism because it coordinates numerous genes involved in larval lipolysis, FA activation, and β-oxidation (Palanker *et al.* 2009). HNF4-induced genes include acyl-CoA synthetase (ACS) genes such as *bubblegum* (*bgm*). In the absence of *bgm* gene function, larvae accumulate lipids in the gastric caecae (Sivachenko *et al.* 2016). The importance of FA activation by acyl-CoA formation in global TAG control is further emphasized by the involvement of the

Foxo-dependent ACS gene *pudgy* (*pdgy*) in TAG homeostasis. *Pudgy* mutants accumulate excessive storage fat and display compromised TAG mobilization (Xu *et al.* 2012). In contrast, mutations of the carnitine pamitoyltransferases 1 and 2, which transport activated FA into mitochondria, have comparably mild phenotypes. These mutants are starvation-sensitive, and store ectopic lipids in brain and flight muscles without an effect on global body TAG content (Schulz *et al.* 2015). In contrast, flies deficient for the mitochondrial trifunctional protein α or β subunits, which assemble the enzyme complex responsible for the last three steps in β-oxidation, accumulate increased body fat stores (Kishita *et al.* 2012). Collectively, single-gene analyses to date reveal complex roles for β-oxidation genes with respect to fly TAG homoeostasis, which might, in part, be due to functional redundancies among multiple gene family members [for an overview see Palanker *et al.* (2009)]. Notably, adult flies incapable of TAG mobilization are viable on a regular, carbohydrate-rich diet, but are starvation-hypersensitive (Grönke *et al.* 2007). This finding predicts that dietary challenge might reveal more severe consequences of genetic β-oxidation impairment.

Lipid Transport

The interorgan transport of *Drosophila* lipids via hemolymph involves specific lipoprotein carriers. Upon feeding, this system carries dietary or *de novo*-synthesized lipids from the midgut to tissues as energy supply, or to the fat body for storage. Upon starvation, the same protein system delivers lipids from the fat body to peripheral tissues (Palm *et al.* 2012). Like *de novo* lipogenesis, hemolymph lipid transport is essential, and deficiency of lipid transport components leads to developmental arrest in preadult stages (Palm *et al.* 2012). Hemolymph contains distinct lipoproteins of various densities that have various qualitative and quantitative roles in lipid transport. Lipophorin (Lpp) is the major lipoprotein in fly hemolymph and carries $>$ 95% of total hemolymph lipids in feeding third-instar larvae (Palm *et al.* 2012). The protein fraction of Lpp consists of the two apoproteins ApoLI and ApoLII, which derive from proteolytic cleavage of a single Apolipophorin (Apolpp) precursor (encoded by the *apolpp* gene) and assemble into lipoprotein complexes in a 1:1 M ratio (Sundermeyer *et al.* 1996; Canavoso *et al.* 2001; Panáková *et al.* 2005; Palm *et al.* 2012). This protein component binds a mixture of polar and neutral lipids, depending on the developmental and dietary status of the animal (Palm *et al.* 2012). DAG is the primary neutral lipid of *Drosophila* Lpp and serves as the major transport form of FA in hemolymph. In contrast, only trace amounts of TAG and free FA have been identified in hemolymph. The remaining Lpp lipids include PE, free sterol, phosphatidylcholine (PC), sphingolipids, and hydrocarbons (Fernando-Warnakulasuriya and Wells 1988; Carvalho *et al.* 2012). Lpp-associated DAG in feeding third-instar larvae is markedly enriched in medium-chain FA chains, which are typically not found in PE or other phospholipids, suggesting channeling of specific FAs in glycerolipid synthesis pathways (Palm *et al.* 2012). Although the structure of *Drosophila* Lpp is currently unknown, studies in other insect species suggest that Lpp comprises a spherical particle with a hydrophobic core of neutral lipids and a surface covered by phospholipids, apolipoproteins, and a small fraction of DAG (Kawooya *et al.* 1991). Genetic studies in *Drosophila* third-instar larvae indicate that the fat body is the major site of Lpp synthesis and secretion (Palm *et al.* 2012). This observation confirms earlier biochemical studies in other insect species that demonstrate Lpp synthesis and release from isolated fat body explants (Prasad *et al.* 1986; Weers *et al.* 1992). Although the midgut is the dedicated site of lipid absorption, *de novo* synthesis, and export to the circulatory system, it lacks the capacity to synthesize lipoproteins, but relies on lipoprotein secretion from other tissues, principally the fat body (Palm *et al.* 2012). However, recent data suggest that, in response to nutritional challenges such as high fat levels, cardiac muscle cells deliver an additional pool of Lpp to the hemolymph (Lee *et al.* 2017).

In the fat body of feeding *Drosophila* third-instar larvae, Lpp is assembled as a PE-rich, but DAG-poor, precursor particle (Palm *et al.* 2012). The maturation of Lpp requires the presence of microsomal TAG transfer protein (Mtp), an ER-resident lipid transport protein with an evolutionarily conserved chaperoning function in the secretion of apoB-containing lipoproteins (Sellers *et al.* 2003). Accordingly, RNAi-mediated depletion of Mtp results in the accumulation of immature Apolpp precursors in the fat body and a deficiency of mature Lpp in hemolymph (Palm *et al.* 2012). Unlike its mammalian ortholog, *Drosophila* Mtp lacks TAG transfer activity, but displays phospholipid transfer activity *in vitro* consistent with its function in the biogenesis of the PE-enriched precursor Lpp (Rava and Hussain 2007). After its secretion by the fat body or cardiac muscle, Lpp is recruited to the midgut, where it is loaded with diet-derived DAG and sterols that are then redistributed to other tissues. As a consequence, RNAi-mediated interference of the hemolymph Lpp system manifests in a massive accumulation of LDs in the midgut of feeding third-instar larvae (Panáková *et al.* 2005; Palm *et al.* 2012), and in a concomitant depletion of lipid in other tissues such as the brain or imaginal discs (Palm *et al.* 2012; Rodríguez-Vázquez *et al.* 2015). Biochemical studies in non-*Drosophila* insects indicate that lipid delivery to tissues via Lpp occurs without concomitant degradation of ApoLI/II proteins, suggesting that Lpp acts as a reusable lipid shuttle (Downer and Chino 1985; van Heusden *et al.* 1991; Arrese *et al.* 2001).

Several molecular components are required for the interaction of Lpp with target tissues and/or lipid transfer. Lipid transfer particle (LTP, encoded by the *Apoltp* gene) is synthesized in the fat body as an ApoLTP precursor and, analogous to Lpp, becomes processed to ApoLTP-I and ApoLTP-II via proteolytic cleavage before secretion into hemolymph. As for Lpp, the maturation and secretion of LTP depend on the activity of

Mtp (Ryan *et al.* 1988; Palm *et al.* 2012). Although circulating LTP is associated with only 1% of hemolymph lipids, it is required for bulk lipid transport by facilitating lipid transfer between Lpp and tissues. Genetic depletion of LTP in *Drosophila* third-instar larvae compromises Lpp recruitment to the midgut and its loading with DAG, resulting in ectopic LD accumulation in the midgut epithelium (Palm *et al.* 2012). LTP activity has also been implicated in lipid transfer from lipid-loaded Lpp to the ovaries and wing discs (Rodríguez-Vázquez *et al.* 2015).

In addition to LTP, lipid transport between hemolymph and tissues depends on members of the evolutionarily conserved low-density lipoprotein receptor family of proteins. The *Drosophila* genome encodes seven members of this family, of which Lpp receptors 1 and 2 (LpR1 and LpR2) share the highest sequence homology to LpR from *Locusta migratoria*, and to the mammalian very low-density lipoprotein-like receptor (Parra-Peralbo and Culi 2011). LpR2 directs the recruitment and extracellular stabilization of both LTP and Lpp to target tissues, suggesting that LpRs transiently form ternary complexes to facilitate lipid transport (Parra-Peralbo and Culi 2011; Rodríguez-Vázquez *et al.* 2015). Through the use of alternative promoters and alternative splicing mechanisms, the *Drosophila LpR1* and *LpR2* genes are expressed as multiple isoforms, with tissue-specific expression patterns and various capacities for lipid transfer (Parra-Peralbo and Culi 2011; Parvy *et al.* 2012). LpR2 is the major receptor for lipid uptake into nurse cells and oocytes during vitellogenesis, with minor contributions of LpR1 to this process (Parra-Peralbo and Culi 2011; Sieber and Spradling 2015). Loss of both receptors results in oocyte degeneration during midoogenesis. This phenotype is only partially tissue-autonomous as germline-specific deficiency causes reduced oocyte storage lipid content, but not midoogenesis degeneration (Parra-Peralbo and Culi 2011). In addition, LpR2 has also been assigned a predominant role in lipid uptake into larval oenocytes upon fasting, whereas both LpR1 and LpR2 act redundantly in mediating lipid acquisition of the wing disc (Parvy *et al.* 2012; Rodríguez-Vázquez *et al.* 2015). Notably, whole-body TAG content and mobilization were unaffected by genetic deficiency of *LpR1* and *LpR2*, suggesting that the major routes of lipid transport are not grossly impaired. In *LpR1 LpR2* double mutants, LTP failed to accumulate at nurse cell plasma membranes or wing imaginal discs, but was still recruited to many other tissues, suggesting that LpR1/LpR2-independent lipid transport pathways contribute to lipid transport (Parra-Peralbo and Culi 2011; Rodríguez-Vázquez *et al.* 2015).

Despite the identification of key molecular components of intertissue lipid transport in *Drosophila*, little is known about the molecular mechanisms of this process. Biochemical studies in other insects suggest that LTP acquires limited amounts of lipid from a donor lipoprotein or a tissue, and transfers it to a receptor (Blacklock *et al.* 1992). Consistent with this hypothesis, depletion of the Lpp acceptor by RNAi increases the density of LTP particles (Palm *et al.* 2012). Notably, the ability of LTP to transfer lipids to Lpp appears to be tissue-selective. For example, LTP transfers lipids from the explanted midgut, but not from the explanted fat body of feeding *Drosophila* third-instar larvae to Lpp (Palm *et al.* 2012). Although Lpp receptors have been shown to induce Lpp endocytosis in cells and insect tissues (Dantuma *et al.* 1999; Van Hoof *et al.* 2002, 2003; Callejo *et al.* 2008), the functional relevance of this observation for lipid transport mechanisms is unclear and may again be tissue-specific. Genetic experiments that interfered with the endocytosis machinery suggest that lipid transport to the oocyte is endocytosis-independent (Rodríguez-Vázquez *et al.* 2015), whereas Lpp loading at the midgut of third-instar larvae partly depends on functional endocytosis (Palm *et al.* 2012). How lipid gets transported across the endosomal or plasma membranes, and if this process involves lipid processing (*e.g.*, by lipoprotein or lysosomal lipases), is currently unknown.

Future Perspectives

The preponderance of human lipid storage-associated disorders such as diabetes, obesity, and cardiovascular disease has spurred efforts to investigate TAG metabolism in genetically tractable model organisms. Metabolic phenotyping of *Drosophila* mutants, in combination with the use of genetic screens, have been fundamental tools in the identification of evolutionarily conserved regulators of TAG storage (Pospisilik *et al.* 2010; Reis *et al.* 2010; Baumbach *et al.* 2014a). The past decades of research on *Drosophila* TAG metabolism have shaped the view that the molecular processes of TAG formation, degradation, and storage are remarkably similar between *Drosophila* and higher organisms. Nevertheless, a comprehensive understanding of the molecular, cellular, and organismal principles of *Drosophila* TAG metabolism requires continued efforts among the steadily growing research community active in this field. Several important directions in this area are likely to fill those knowledge gaps and contribute novel insights into TAG metabolism, with relevance to basic and translational research.

Characterizing the basic enzymology and biochemistry of *Drosophila* TAG metabolism is a promising field. Genetic evidence indicates the presence of yet unknown enzymatic effectors in TAG formation and degradation in flies. However, functional annotations of enzymes are often based on homology to mammalian proteins and require confirmatory biochemical evidence. Moreover, enzyme isoforms may differentially or redundantly contribute to tissue-autonomous TAG metabolism and/or act during specific developmental stages. With clustered regularly interspaced short palindromic repeats/Cas9-based genome editing tools, the study of redundant genetic control by multiple genes is now accessible to functional analyses. Complementation of such *in vivo* functional analysis with the comprehensive enzymatic characterization of *Drosophila* TAG metabolism promises to be a solid framework for addressing open fundamental questions in lipid biology. Some of the important issues to be

addressed include: which isoenzymes catalyze TAG formation or breakdown in the fat body and peripheral tissues, how do they incorporate specific FAs into DAG and TAG as opposed to glycerophospholipids, and what is the contribution of lysosomes to the breakdown of lipoproteins or cytosolic LDs?

FAs are precursors for both structural and storage lipids. Accordingly, metabolic pathways of TAG are not independent from the effects of the presence of structural lipids such as glycerophospholipids or sphingolipids, which may interact with TAG metabolism at multiple levels. A prominent example of this interconnection is the repression of SREBP activity by the major *Drosophila* membrane lipid PE, which leads to tissue-autonomous or global alterations in *de novo* lipogenesis and TAG storage (Lim *et al.* 2011; Meltzer *et al.* 2017; Ziegler *et al.* 2017). Likewise, PC, the second most abundant glycerophospholipid in *Drosophila* membranes, has been identified as a critical modulator of TAG storage. PC acts as a structural component of the phospholipid monolayer surrounding LDs, and depletion of PC synthesis enzymes favors LD coalescence, and increases LD size and TAG levels (Krahmer *et al.* 2011; Moessinger *et al.* 2014). In analogy to glycerophospholipid metabolic pathways, sphingolipid formation and breakdown have also been implicated in TAG storage (Bauer *et al.* 2009; Kohyama-Koganeya *et al.* 2011; Nirala *et al.* 2013; Walls *et al.* 2013; Sociale *et al.* 2018). Reciprocal to the concept that membrane lipids affect the storage lipid pools, FAs liberated from TAG stores may also affect synthesis of sphingolipids or glycerophospholipids, with profound effects on membrane structure and function. This dual role of TAG lipolysis in providing FAs for β-oxidation, as well as providing structural lipids, might be particularly relevant during nonfeeding stages of development or during tissue remodeling as, for example, during embryogenesis or metamorphosis. The advent of lipidomic and metabolic labeling techniques in *Drosophila* developmental research provides unique tools for future studies of these processes (Carvalho *et al.* 2012; Guan *et al.* 2013; Musselman *et al.* 2013).

The regulation of TAG homeostasis in a constantly changing environment requires elaborate endocrine mechanisms that orchestrate intertissue communication. Research in *Drosophila* has contributed significantly to answer the question of how organs sense and communicate fluctuations in energy status (Colombani *et al.* 2003; Géminard *et al.* 2009). These studies have recently highlighted the role of cytokines and other humoral factors, which are released in tissue-specific patterns to communicate changes in the nutrient status and adjust TAG homeostasis, often via cross talk with insulin or Akh signaling pathways (Rajan and Perrimon 2012; Agrawal *et al.* 2016; Delanoue *et al.* 2016; Song *et al.* 2017; Zhao and Karpac 2017). However, how these pathways relay the information to direct changes in the expression or activity of enzymatic effectors that control TAG storage and mobilization is still poorly understood. One attractive hypothesis of coordinated, environmental-responsive gene regulation involves microRNAs (miRs). Notably, starvation reduces the levels of miR machinery components, implying the possible coupling of miR effectors to energy status (Barrio *et al.* 2014). Several miRs have been implicated in TAG storage control, *e.g.*, mir-278 (Teleman *et al.* 2006) and mir-14 (Xu *et al.* 2003; Varghese *et al.* 2010), which both modulate energy homeostasis via insulin signaling. Whether or not miRs control TAG metabolism more directly at the level of enzymes is an important issue to be resolved in future research.

The function of TAG synthesis and TAG deposition in LDs is not limited to energy storage, and may be highly tissue-specific. One of the most active fields in fly lipid research currently addresses the roles of TAG metabolism in neurodegeneration. Early molecular characterization of *Drosophila* neurodegeneration mutants, such as *swiss cheese* (*sws*) (Kretzschmar *et al.* 1997) and *bubblegum* (*bgm*) (Min and Benzer 1999), has already indicated the importance of lipid metabolism in neuronal integrity with relevance to human neurodegenerative disorders [reviewed in Lessing and Bonini (2009)]. More recent research has demonstrated that LDs fulfill a developmental function in brain glial cells by serving as a sink to protect unsaturated FAs, which are instrumental for neuroblast function, from excessive lipid peroxidation (Bailey *et al.* 2015). Similarly, transient LD accumulation in adult brain glia has been suggested to act neuroprotectively by scavenging peroxidated lipids under conditions of mitochondrial dysfunction in neurons (Liu *et al.* 2015). In this context, glial LDs participate in an evolutionarily conserved glia–neuron lactate shuttle (Liu *et al.* 2017). Under stress conditions, neurons use glia-derived lactate for the production of lipids, which are subject to peroxidation and become exported to build up glial LDs. Conditional ectopic LD formation is not restricted to the nervous systems, as exemplified by the larval salivary gland. In this tissue, ectopic LDs appear upon genetically compromised function of *Seipin* or of the cytidine 5′-diphosphate-DAG synthase gene *Cds*. In these cases, ectopic LD formation has been proposed to result from rerouting of glycerophospholipid precursors such as PA into TAG (Tian *et al.* 2011; Liu *et al.* 2014). In line with the concept of "lipotoxicity," these studies suggest a role of TAG synthesis and LD formation as a tissue-specific metabolic buffer for excess lipid. Yet, ectopic LD formation is highly dependent on the (patho)physiological circumstances, and on the cellular context. Collectively, the role of ectopic LDs as a trigger, bystander, or buffer of cellular stress is still controversial, which makes them an active and promising area of TAG metabolism research in the fly.

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Literature Cited

Agrawal, N., R. Delanoue, A. Mauri, D. Basco, M. Pasco *et al.*, 2016 The Drosophila TNF eiger is an adipokine that acts on insulin-producing cells to mediate nutrient response. Cell Metab. 23: 675–684. https://doi.org/10.1016/j.cmet.2016.03.003

Alic, N., T. D. Andrews, M. E. Giannakou, I. Papatheodorou, C. Slack *et al.*, 2011 Genome-wide dFOXO targets and topology of the transcriptomic response to stress and insulin signalling. Mol. Syst. Biol. 7: 502. https://doi.org/10.1038/msb.2011.36

Arrese, E. L., and M. A. Wells, 1997 Adipokinetic hormone-induced lipolysis in the fat body of an insect, Manduca sexta: synthesis of sn-1,2-diacylglycerols. J. Lipid Res. 38: 68–76.

Arrese, E. L., B. I. Rojas-Rivas, and M. A. Wells, 1996 Synthesis of sn-1,2-diacylglycerols by monoacylglycerol acyltransferase from Manduca sexta fat body. Arch. Insect Biochem. Physiol. 31: 325–335. https://doi.org/10.1002/(SICI)1520-6327(1996)31:3<325::AID-ARCH7>3.0.CO;2-W

Arrese, E. L., M. T. Flowers, J. L. Gazard, and M. A. Wells, 1999 Calcium and cAMP are second messengers in the adipokinetic hormone-induced lipolysis of triacylglycerols in Manduca sexta fat body. J. Lipid Res. 40: 556–564.

Arrese, E. L., L. E. Canavoso, Z. E. Jouni, J. E. Pennington, K. Tsuchida *et al.*, 2001 Lipid storage and mobilization in insects: current status and future directions. Insect Biochem. Mol. Biol. 31: 7–17. https://doi.org/10.1016/S0965-1748(00)00102-8

Arrese, E. L., R. T. Patel, and J. L. Soulages, 2006 The main triglyceride-lipase from the insect fat body is an active phospholipase A1: identification and characterization. J. Lipid Res. 47: 2656–2667. https://doi.org/10.1194/jlr.M600161-JLR200

Arrese, E. L., L. Rivera, M. Hamada, S. Mirza, S. D. Hartson *et al.*, 2008 Function and structure of lipid storage droplet protein 1 studied in lipoprotein complexes. Arch. Biochem. Biophys. 473: 42–47. https://doi.org/10.1016/j.abb.2008.02.036

Athenstaedt, K., and G. Daum, 2003 YMR313c/TGL3 encodes a novel triacylglycerol lipase located in lipid particles of Saccharomyces cerevisiae. J. Biol. Chem. 278: 23317–23323. https://doi.org/10.1074/jbc.M302577200

Bailey, A. P., G. Koster, C. Guillermier, E. M. Hirst, J. I. MacRae *et al.*, 2015 Antioxidant role for lipid droplets in a stem cell niche of Drosophila. Cell 163: 340–353. https://doi.org/10.1016/j.cell.2015.09.020

Baker, K. D., and C. S. Thummel, 2007 Diabetic larvae and obese flies—emerging studies of metabolism in Drosophila. Cell Metab. 6: 257–266. https://doi.org/10.1016/j.cmet.2007.09.002

Barber, M. C., N. T. Price, and M. T. Travers, 2005 Structure and regulation of acetyl-CoA carboxylase genes of metazoa. Biochim. Biophys. Acta 1733: 1–28. https://doi.org/10.1016/j.bbalip.2004.12.001

Barrio, L., A. Dekanty, and M. Milán, 2014 MicroRNA-mediated regulation of Dp53 in the Drosophila fat body contributes to metabolic adaptation to nutrient deprivation. Cell Rep. 8: 528–541. https://doi.org/10.1016/j.celrep.2014.06.020

Bauer, R., A. Voelzmann, B. Breiden, U. Schepers, H. Farwanah *et al.*, 2009 Schlank, a member of the ceramide synthase family controls growth and body fat in Drosophila. EMBO J. 28: 3706–3716. https://doi.org/10.1038/emboj.2009.305

Baumann, A. A., J. B. Benoit, V. Michalkova, P. O. Mireji, G. M. Attardo *et al.*, 2013 Juvenile hormone and insulin suppress lipolysis between periods of lactation during tsetse fly pregnancy. Mol. Cell. Endocrinol. 372: 30–41. https://doi.org/10.1016/j.mce.2013.02.019

Baumbach, J., P. Hummel, I. Bickmeyer, K. M. Kowalczyk, M. Frank *et al.*, 2014a A Drosophila in vivo screen identifies store-operated calcium entry as a key regulator of adiposity. Cell Metab. 19: 331–343. https://doi.org/10.1016/j.cmet.2013.12.004

Baumbach, J., Y. Xu, P. Hehlert, and R. P. Kühnlein, 2014b Gαq, Gγ1 and Plc21C control Drosophila body fat storage. J. Genet. Genomics 41: 283–292. https://doi.org/10.1016/j.jgg.2014.03.005

Bell, R. M., and R. A. Coleman, 1980 Enzymes of glycerolipid synthesis in eukaryotes. Annu. Rev. Biochem. 49: 459–487. https://doi.org/10.1146/annurev.bi.49.070180.002331

Beller, M., D. Riedel, L. Jänsch, G. Dieterich, J. Wehland *et al.*, 2006 Characterization of the Drosophila lipid droplet subproteome. Mol. Cell. Proteomics 5: 1082–1094. https://doi.org/10.1074/mcp.M600011-MCP200

Beller, M., C. Sztalryd, N. Southall, M. Bell, H. Jäckle *et al.*, 2008 COPI complex is a regulator of lipid homeostasis. PLoS Biol. 6: e292. https://doi.org/10.1371/journal.pbio.0060292

Beller, M., A. V. Bulankina, H.-H. Hsiao, H. Urlaub, H. Jäckle *et al.*, 2010 PERILIPIN-dependent control of lipid droplet structure and fat storage in Drosophila. Cell Metab. 12: 521–532. https://doi.org/10.1016/j.cmet.2010.10.001

Bharucha, K. N., P. Tarr, and S. L. Zipursky, 2008 A glucagon-like endocrine pathway in Drosophila modulates both lipid and carbohydrate homeostasis. J. Exp. Biol. 211: 3103–3110. https://doi.org/10.1242/jeb.016451

Bi, J., Y. Xiang, H. Chen, Z. Liu, S. Grönke *et al.*, 2012 Opposite and redundant roles of the two Drosophila perilipins in lipid mobilization. J. Cell Sci. 125: 3568–3577. https://doi.org/10.1242/jcs.101329

Bi, J., W. Wang, Z. Liu, X. Huang, Q. Jiang *et al.*, 2014 Seipin promotes adipose tissue fat storage through the ER Ca2+-ATPase SERCA. Cell Metab. 19: 861–871. https://doi.org/10.1016/j.cmet.2014.03.028

Bickel, P. E., Tansey J. T., and Welte M. A., 2009 PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. Biochim. Biophys. Acta 1791: 419–440. https://doi.org/10.1016/j.bbalip.2009.04.002

Blacklock, B. J., M. Smillie, and R. O. Ryan, 1992 Insect lipid transfer particle can facilitate net vectorial lipid transfer via a carrier-mediated mechanism. J. Biol. Chem. 267: 14033–14037.

Böhni, R., J. Riesgo-Escovar, S. Oldham, W. Brogiolo, H. Stocker *et al.*, 1999 Autonomous control of cell and organ size by CHICO, a Drosophila homolog of vertebrate IRS1–4. Cell 97: 865–875. https://doi.org/10.1016/S0092-8674(00)80799-0

Bolukbasi, E., M. Khericha, J. C. Regan, D. K. Ivanov, J. Adcott *et al.*, 2017 Intestinal fork head regulates nutrient absorption and promotes longevity. Cell Rep. 21: 641–653. https://doi.org/10.1016/j.celrep.2017.09.042

Buchon, N., D. Osman, F. P. David, H. Y. Fang, J. P. Boquete *et al.*, 2013 Morphological and molecular characterization of adult midgut compartmentalization in Drosophila. Cell Rep. 3: 1725–1738 (erratum: Cell Rep. 3: 1755). https://doi.org/10.1016/j.celrep.2013.04.001

Buszczak, M., X. Lu, W. A. Segraves, T. Y. Chang, and L. Cooley, 2002 Mutations in the midway gene disrupt a Drosophila acyl coenzyme A: diacylglycerol acyltransferase. Genetics 160: 1511–1518.

Callejo, A., J. Culi, and I. Guerrero, 2008 Patched, the receptor of Hedgehog, is a lipoprotein receptor. Proc. Natl. Acad. Sci. USA 105: 912–917. https://doi.org/10.1073/pnas.0705603105

Canavoso, L. E., Z. E. Jouni, K. J. Karnas, J. E. Pennington, and M. A. Wells, 2001 Fat metabolism in insects. Annu. Rev. Nutr. 21: 23–46. https://doi.org/10.1146/annurev.nutr.21.1.23

Carney, G. E., and M. Bender, 2000 The Drosophila ecdysone receptor (EcR) gene is required maternally for normal oogenesis. Genetics 154: 1203–1211.

Carvalho, M., J. L. Sampaio, W. Palm, M. Brankatschk, S. Eaton *et al.*, 2012 Effects of diet and development on the Drosophila lipidome. Mol. Syst. Biol. 8: 600–617. https://doi.org/10.1038/msb.2012.29

Cermelli, S., Y. Guo, S. P. Gross, and M. A. Welte, 2006 The lipid-droplet proteome reveals that droplets are a protein-storage

depot. Curr. Biol. 16: 1783–1795. https://doi.org/10.1016/j.cub.2006.07.062
Chakrabarti, P., and K. V. Kandror, 2009 FoxO1 controls insulin-dependent adipose triglyceride lipase (ATGL) expression and lipolysis in adipocytes. J. Biol. Chem. 284: 13296–13300. https://doi.org/10.1074/jbc.C800241200
Charles, J.-P., T. Iwema, V. C. Epa, K. Takaki, J. Rynes *et al.*, 2011 Ligand-binding properties of a juvenile hormone receptor, Methoprene-tolerant. Proc. Natl. Acad. Sci. USA 108: 21128–21133. https://doi.org/10.1073/pnas.1116123109
Chatterjee, D., S. D. Katewa, Y. Qi, S. A. Jackson, P. Kapahi *et al.*, 2014 Control of metabolic adaptation to fasting by dILP6-induced insulin signaling in Drosophila oenocytes. Proc. Natl. Acad. Sci. USA 111: 17959–17964. https://doi.org/10.1073/pnas.1409241111
Chen, W., B. Chang, P. Saha, S. M. Hartig, L. Li *et al.*, 2012 Berardinelli-seip congenital lipodystrophy 2/seipin is a cell-autonomous regulator of lipolysis essential for adipocyte differentiation. Mol. Cell. Biol. 32: 1099–1111. https://doi.org/10.1128/MCB.06465-11
Chino, H., and L. I. Gilbert, 1964 Diglyceride release from insect fat body: a possible means of lipid transport. Science 143: 359–361. https://doi.org/10.1126/science.143.3604.359
Chintapalli, V. R., J. Wang, and J. A. T. Dow, 2007 Using FlyAtlas to identify better Drosophila melanogaster models of human disease. Nat. Genet. 39: 715–720. https://doi.org/10.1038/ng2049
Chng, W.-B. A., M. S. B. Sleiman, F. Schüpfer, and B. Lemaitre, 2014 Transforming growth factor β/Activin signaling functions as a sugar-sensing feedback loop to regulate digestive enzyme expression. Cell Rep. 9: 336–348. https://doi.org/10.1016/j.celrep.2014.08.064
Cho, K. S., J. H. Lee, S. Kim, D. Kim, H. Koh *et al.*, 2001 Drosophila phosphoinositide-dependent kinase-1 regulates apoptosis and growth via the phosphoinositide 3-kinase-dependent signaling pathway. Proc. Natl. Acad. Sci. USA 98: 6144–6149. https://doi.org/10.1073/pnas.101596998
Choi, S., D.-S. Lim, and J. Chung, 2015 Feeding and fasting signals converge on the LKB1–SIK3 pathway to regulate lipid metabolism in Drosophila. PLoS Genet. 11: e1005263. https://doi.org/10.1371/journal.pgen.1005263
Chung, H., D. W. Loehlin, H. D. Dufour, K. Vaccarro, J. G. Millar *et al.*, 2014 A single gene affects both ecological divergence and mate choice in Drosophila. Science 343: 1148–1151. https://doi.org/10.1126/science.1249998
Colombani, J., S. Raisin, S. Pantalacci, T. Radimerski, J. Montagne *et al.*, 2003 A nutrient sensor mechanism controls Drosophila growth. Cell 114: 739–749. https://doi.org/10.1016/S0092-8674(03)00713-X
Colombani, J., L. Bianchini, S. Layalle, E. Pondeville, C. Dauphin-Villemant *et al.*, 2005 Antagonistic actions of ecdysone and insulins determine final size in Drosophila. Science 310: 667–670. https://doi.org/10.1126/science.1119432
Dantuma, N. P., M. Potters, M. P. De Winther, C. P. Tensen, F. P. Kooiman *et al.*, 1999 An insect homolog of the vertebrate very low density lipoprotein receptor mediates endocytosis of lipophorins. J. Lipid Res. 40: 973–978.
Delanoue, R., E. Meschi, N. Agrawal, A. Mauri, Y. Tsatskis *et al.*, 2016 Drosophila insulin release is triggered by adipose Stunted ligand to brain Methuselah receptor. Science 353: 1553–1556. https://doi.org/10.1126/science.aaf8430
DiAngelo, J. R., and M. J. Birnbaum, 2009 Regulation of fat cell mass by insulin in Drosophila melanogaster. Mol. Cell. Biol. 29: 6341–6352. https://doi.org/10.1128/MCB.00675-09
Ding, L., X. Yang, H. Tian, J. Liang, F. Zhang *et al.*, 2018 Seipin regulates lipid homeostasis by ensuring calcium-dependent mitochondrial metabolism. EMBO J. 37: e97572. https://doi.org/10.15252/embj.201797572
Dobrosotskaya, I. Y., A. C. Seegmiller, M. S. Brown, J. L. Goldstein, and R. B. Rawson, 2002 Regulation of SREBP processing and membrane lipid production by phospholipids in Drosophila. Science 296: 879–883. https://doi.org/10.1126/science.1071124
Donkor, J., M. Sariahmetoglu, J. Dewald, D. N. Brindley, and K. Reue, 2007 Three mammalian lipins act as phosphatidate phosphatases with distinct tissue expression patterns. J. Biol. Chem. 282: 3450–3457. https://doi.org/10.1074/jbc.M610745200
Downer, R., and H. Chino, 1985 Turnover of protein and diacylglycerol components of lipophorin in insect haemolymph. Insect Biochem. 15: 627–630. https://doi.org/10.1016/0020-1790(85)90124-6
Eastmond, P. J., 2006 SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating Arabidopsis seeds. Plant Cell 18: 665–675. https://doi.org/10.1105/tpc.105.040543
Fernando-Warnakulasuriya, G. J. P., and M. A. Wells, 1988 Isolation and characterization of lipophorin from Drosophila melanogaster larvae. Arch. Insect Biochem. Physiol. 8: 243–248. https://doi.org/10.1002/arch.940080405
Finck, B. N., M. C. Gropler, Z. Chen, T. C. Leone, M. A. Croce *et al.*, 2006 Lipin 1 is an inducible amplifier of the hepatic PGC-1alpha/PPARalpha regulatory pathway. Cell Metab. 4: 199–210. https://doi.org/10.1016/j.cmet.2006.08.005
Flatt, T., M.-P. Tu, and M. Tatar, 2005 Hormonal pleiotropy and the juvenile hormone regulation of Drosophila development and life history. BioEssays 27: 999–1010. https://doi.org/10.1002/bies.20290
Francis, V. A., A. Zorzano, and A. A. Teleman, 2010 dDOR is an EcR coactivator that forms a feed-forward loop connecting insulin and ecdysone signaling. Curr. Biol. 20: 1799–1808. https://doi.org/10.1016/j.cub.2010.08.055
Gäde, G., and L. Auerswald, 2003 Mode of action of neuropeptides from the adipokinetic hormone family. Gen. Comp. Endocrinol. 132: 10–20. https://doi.org/10.1016/S0016-6480(03)00159-X
Gáliková, M., M. Diesner, P. Klepsatel, P. Hehlert, Y. Xu *et al.*, 2015 Energy homeostasis control in Drosophila adipokinetic hormone mutants. Genetics 201: 665–683. https://doi.org/10.1534/genetics.115.178897
Gáliková, M., P. Klepsatel, J. Münch, and R. P. Kühnlein, 2017 Spastic paraplegia-linked phospholipase PAPLA1 is necessary for development, reproduction, and energy metabolism in Drosophila. Sci Rep. 7: 46516. https://doi.org/10.1038/srep46516
Garrido, D., T. Rubin, M. Poidevin, B. Maroni, A. Le Rouzic *et al.*, 2015 Fatty acid synthase cooperates with glyoxalase 1 to protect against sugar toxicity. PLoS Genet. 11: e1004995. https://doi.org/10.1371/journal.pgen.1004995
Géminard, C., E. J. Rulifson, and P. Léopold, 2009 Remote control of insulin secretion by fat cells in Drosophila. Cell Metab. 10: 199–207. https://doi.org/10.1016/j.cmet.2009.08.002
Grillet, M., B. Dominguez Gonzalez, A. Sicart, M. Pöttler, A. Cascalho *et al.*, 2016 Torsins are essential regulators of cellular lipid metabolism. Dev. Cell 38: 235–247. https://doi.org/10.1016/j.devcel.2016.06.017
Grippa, A., L. Buxó, G. Mora, C. Funaya, F.-Z. Idrissi *et al.*, 2015 The seipin complex Fld1/Ldb16 stabilizes ER-lipid droplet contact sites. J. Cell Biol. 211: 829–844. https://doi.org/10.1083/jcb.201502070
Grönke, S., M. Beller, S. Fellert, H. Ramakrishnan, H. Jäckle *et al.*, 2003 Control of fat storage by a Drosophila PAT domain protein. Curr. Biol. 13: 603–606. https://doi.org/10.1016/S0960-9822(03)00175-1
Grönke, S., A. Mildner, S. Fellert, N. Tennagels, S. Petry *et al.*, 2005 Brummer lipase is an evolutionary conserved fat storage

regulator in Drosophila. Cell Metab. 1: 323–330. https://doi.org/10.1016/j.cmet.2005.04.003

Grönke, S., G. Müller, J. Hirsch, S. Fellert, A. Andreou *et al.*, 2007 Dual lipolytic control of body fat storage and mobilization in Drosophila. PLoS Biol. 5: e137. https://doi.org/10.1371/journal.pbio.0050137

Grönke, S., D.-F. Clarke, S. Broughton, T. D. Andrews, and L. Partridge, 2010 Molecular evolution and functional characterization of Drosophila insulin-like peptides. PLoS Genet. 6: e1000857. https://doi.org/10.1371/journal.pgen.1000857

Guan, X. L., G. Cestra, G. Shui, A. Kuhrs, R. B. Schittenhelm *et al.*, 2013 Biochemical membrane lipidomics during Drosophila development. Dev. Cell 24: 98–111. https://doi.org/10.1016/j.devcel.2012.11.012

Guo, Y., T. C. Walther, M. Rao, N. Stuurman, G. Goshima *et al.*, 2008 Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. Nature 453: 657–661. https://doi.org/10.1038/nature06928

Gutierrez, E., D. Wiggins, B. Fielding, and A. P. Gould, 2006 Specialized hepatocyte-like cells regulate Drosophila lipid metabolism. Nature 445: 275–280. https://doi.org/10.1038/nature05382

Haemmerle, G., R. Zimmermann, M. Hayn, C. Theussl, G. Waeg *et al.*, 2002 Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. J. Biol. Chem. 277: 4806–4815. https://doi.org/10.1074/jbc.M110355200

Han, G.-S., W.-I. Wu, and G. M. Carman, 2006 The Saccharomyces cerevisiae Lipin homolog is a Mg2+-dependent phosphatidate phosphatase enzyme. J. Biol. Chem. 281: 9210–9218. https://doi.org/10.1074/jbc.M600425200

Harbison, S. T., S. Chang, K. P. Kamdar, and T. F. C. Mackay, 2005 Quantitative genomics of starvation stress resistance in Drosophila. Genome Biol. 6: R36. https://doi.org/10.1186/gb-2005-6-4-r36

Harris, T. E., T. A. Huffman, A. Chi, J. Shabanowitz, D. F. Hunt *et al.*, 2007 Insulin controls subcellular localization and multisite phosphorylation of the phosphatidic acid phosphatase, lipin 1. J. Biol. Chem. 282: 277–286. https://doi.org/10.1074/jbc.M609537200

Havula, E., M. Teesalu, T. Hyötyläinen, H. Seppälä, K. Hasygar *et al.*, 2013 Mondo/ChREBP-Mlx-regulated transcriptional network is essential for dietary sugar tolerance in Drosophila. *PLoS Genet* 9: e1003438. https://doi.org/10.1371/journal.pgen.1003438

Horne, I., V. S. Haritos, and J. G. Oakeshott, 2009 Comparative and functional genomics of lipases in holometabolous insects. Insect Biochem. Mol. Biol. 39: 547–567. https://doi.org/10.1016/j.ibmb.2009.06.002

Horner, M. A., K. Pardee, S. Liu, K. King-Jones, G. Lajoie *et al.*, 2009 The Drosophila DHR96 nuclear receptor binds cholesterol and regulates cholesterol homeostasis. Genes Dev. 23: 2711–2716. https://doi.org/10.1101/gad.1833609

Horton, J. D., 2002 Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis. Biochem. Soc. Trans. 30: 1091–1095. https://doi.org/10.1042/bst0301091

Jindra, M., M. Uhlirova, J.-P. Charles, V. Smýkal, and R. J. Hill, 2015 Genetic evidence for function of the bHLH-PAS protein Gce/Met as a juvenile hormone receptor. PLoS Genet. 11: e1005394. https://doi.org/10.1371/journal.pgen.1005394

Jünger, M. A., F. Rintelen, H. Stocker, J. D. Wasserman, M. Végh *et al.*, 2003 The Drosophila forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. J. Biol. 2: 20. https://doi.org/10.1186/1475-4924-2-20

Kamoshida, Y., S. Fujiyama-Nakamura, S. Kimura, E. Suzuki, J. Lim *et al.*, 2012 Ecdysone receptor (EcR) suppresses lipid accumulation in the Drosophila fat body via transcription control. Biochem. Biophys. Res. Commun. 421: 203–207. https://doi.org/10.1016/j.bbrc.2012.03.135

Kang, P., K. Chang, Y. Liu, M. Bouska, A. Birnbaum *et al.*, 2017 Drosophila Kruppel homolog 1 represses lipolysis through interaction with dFOXO. Sci. Rep. 7: 16369. https://doi.org/10.1038/s41598-017-16638-1

Karpac, J., B. Biteau, and H. Jasper, 2013 Misregulation of an adaptive metabolic response contributes to the age-related disruption of lipid homeostasis in Drosophila. Cell Rep. 4: 1250–1261. https://doi.org/10.1016/j.celrep.2013.08.004

Katewa, S. D., F. Demontis, M. Kolipinski, A. Hubbard, M. S. Gill *et al.*, 2012 Intramyocellular fatty-acid metabolism plays a critical role in mediating responses to dietary restriction in Drosophila melanogaster. Cell Metab. 16: 97–103. https://doi.org/10.1016/j.cmet.2012.06.005

Kawooya, J. K., D. J. van der Horst, M. C. van Heusden, B. L. Brigot, R. van Antwerpen *et al.*, 1991 Lipophorin structure analyzed by in vitro treatment with lipases. J. Lipid Res. 32: 1781–1788.

Kim, J., and T. P. Neufeld, 2015 Dietary sugar promotes systemic TOR activation in Drosophila through AKH-dependent selective secretion of Dilp3. Nat. Commun. 6: 1–10.

Kimmel, A. R., and C. Sztalryd, 2016 The perilipins: major cytosolic lipid droplet-associated proteins and their roles in cellular lipid storage, mobilization, and systemic homeostasis. Annu. Rev. Nutr. 36: 471–509. https://doi.org/10.1146/annurev-nutr-071813-105410

Kishita, Y., M. Tsuda, and T. Aigaki, 2012 Impaired fatty acid oxidation in a Drosophila model of mitochondrial trifunctional protein (MTP) deficiency. Biochem. Biophys. Res. Commun. 419: 344–349. https://doi.org/10.1016/j.bbrc.2012.02.026

Koelle, M. R., W. S. Talbot, W. A. Segraves, M. T. Bender, P. Cherbas *et al.*, 1991 The Drosophila EcR gene encodes an ecdysone receptor, a new member of the steroid receptor superfamily. Cell 67: 59–77. https://doi.org/10.1016/0092-8674(91)90572-G

Kohyama-Koganeya, A., T. Nabetani, M. Miura, and Y. Hirabayashi, 2011 Glucosylceramide synthase in the fat body controls energy metabolism in Drosophila. J. Lipid Res. 52: 1392–1399. https://doi.org/10.1194/jlr.M014266

Krahmer, N., Y. Guo, F. Wilfling, M. Hilger, S. Lingrell *et al.*, 2011 Phosphatidylcholine synthesis for lipid droplet expansion is mediated by localized activation of CTP:phosphocholine cytidylyltransferase. Cell Metab. 14: 504–515. https://doi.org/10.1016/j.cmet.2011.07.013

Krahmer, N., M. Hilger, N. Kory, F. Wilfling, G. Stoehr *et al.*, 2013 Protein correlation profiles identify lipid droplet proteins with high confidence. Mol. Cell. Proteomics 12: 1115–1126. https://doi.org/10.1074/mcp.M112.020230

Kretzschmar, D., G. Hasan, S. Sharma, M. Heisenberg, and S. Benzer, 1997 The swiss cheese mutant causes glial hyperwrapping and brain degeneration in Drosophila. J. Neurosci. 17: 7425–7432. https://doi.org/10.1523/JNEUROSCI.17-19-07425.1997

Krycer, J. R., L. J. Sharpe, W. Luu, and A. J. Brown, 2010 The Akt-SREBP nexus: cell signaling meets lipid metabolism. Trends Endocrinol. Metab. 21: 268–276. https://doi.org/10.1016/j.tem.2010.01.001

Kühnlein, R. P., 2011 The contribution of the Drosophila model to lipid droplet research. Prog. Lipid Res. 50: 348–356. https://doi.org/10.1016/j.plipres.2011.04.001

Kunduri, G., C. Yuan, V. Parthibane, K. M. Nyswaner, R. Kanwar *et al.*, 2014 Phosphatidic acid phospholipase A1 mediates ER–Golgi transit of a family of G protein–coupled receptors. J. Cell Biol. 206: 79–95. https://doi.org/10.1083/jcb.201405020

Kunte, A. S., K. A. Matthews, and R. B. Rawson, 2006 Fatty acid auxotrophy in Drosophila larvae lacking SREBP. Cell Metab. 3: 439–448. https://doi.org/10.1016/j.cmet.2006.04.011

Kurat, C. F., K. Natter, J. Petschnigg, H. Wolinski, K. Scheuringer *et al.*, 2005 Obese yeast: triglyceride lipolysis is functionally

conserved from mammals to yeast. J. Biol. Chem. 281: 491–500. https://doi.org/10.1074/jbc.M508414200

Lee, P.-T., G. Lin, W.-W. Lin, F. Diao, B. H. White *et al.*, 2018 A kinase-dependent feedforward loop affects CREBB stability and long term memory formation. Elife 7: e33007. https://doi.org/10.7554/eLife.33007

Lee, S., H. Bao, Z. Ishikawa, W. Wang, and H.-Y. Lim, 2017 Cardiomyocyte regulation of systemic lipid metabolism by the apolipoprotein B-containing lipoproteins in Drosophila. PLoS Genet. 13: e1006555. https://doi.org/10.1371/journal.pgen.1006555

Leevers, S. J., D. Weinkove, L. K. MacDougall, E. Hafen, and M. D. Waterfield, 1996 The Drosophila phosphoinositide 3-kinase Dp110 promotes cell growth. EMBO J. 15: 6584–6594. https://doi.org/10.1002/j.1460-2075.1996.tb01049.x

Lehmann, M., 2018 Endocrine and physiological regulation of neutral fat storage in Drosophila. Mol. Cell. Endocrinol. 461: 165–177. https://doi.org/10.1016/j.mce.2017.09.008

Lemaitre, B., and I. Miguel-Aliaga, 2013 The digestive tract of Drosophila melanogaster. Annu. Rev. Genet. 47: 377–404. https://doi.org/10.1146/annurev-genet-111212-133343

Lessing, D., and N. M. Bonini, 2009 Maintaining the brain: insight into human neurodegeneration from Drosophila melanogaster mutants. Nat. Rev. Genet. 10: 359–370. https://doi.org/10.1038/nrg2563

Lim, H. Y., W. Wang, R. J. Wessells, K. Ocorr, and R. Bodmer, 2011 Phospholipid homeostasis regulates lipid metabolism and cardiac function through SREBP signaling in Drosophila. Genes Dev. 25: 189–200. https://doi.org/10.1101/gad.1992411

Liu, L., K. Zhang, H. Sandoval, S. Yamamoto, M. Jaiswal *et al.*, 2015 Glial lipid droplets and ROS induced by mitochondrial defects promote neurodegeneration. Cell 160: 177–190. https://doi.org/10.1016/j.cell.2014.12.019

Liu, L., K. R. MacKenzie, N. Putluri, M. Maletić-Savatić, and H. J. Bellen, 2017 The glia-neuron lactate shuttle and elevated ROS promote lipid synthesis in neurons and lipid droplet accumulation in glia via APOE/D. Cell Metab. 26: 719–737.e6. https://doi.org/10.1016/j.cmet.2017.08.024

Liu, Q., R. M. P. Siloto, R. Lehner, S. J. Stone, and R. J. Weselake, 2012 Acyl-CoA:diacylglycerol acyltransferase: molecular biology, biochemistry and biotechnology. Prog. Lipid Res. 51: 350–377. https://doi.org/10.1016/j.plipres.2012.06.001

Liu, Y., W. Wang, G. Shui, and X. Huang, 2014 CDP-diacylglycerol synthetase coordinates cell growth and fat storage through phosphatidylinositol metabolism and the insulin pathway. PLoS Genet. 10: e1004172. https://doi.org/10.1371/journal.pgen.1004172

Magré, J., M. Delépine, E. Khallouf, T. Gedde-Dahl, L. Van Maldergem *et al.*, 2001 Identification of the gene altered in Berardinelli–Seip congenital lipodystrophy on chromosome 11q13. Nat. Genet. 28: 365–370. https://doi.org/10.1038/ng585

Makki, R., E. Cinnamon, and A. P. Gould, 2014 The development and functions of oenocytes. Annu. Rev. Entomol. 59: 405–425. https://doi.org/10.1146/annurev-ento-011613-162056

Matthews, K. A., C. Ozdemir, and R. B. Rawson, 2010 Activation of sterol regulatory element binding proteins in the absence of Scap in Drosophila melanogaster. Genetics 185: 189–198. https://doi.org/10.1534/genetics.110.114975

Mattila, J., and V. Hietakangas, 2017 Regulation of carbohydrate energy metabolism in Drosophila melanogaster. Genetics 207: 1231–1253.

Mattila, J., E. Havula, E. Suominen, M. Teesalu, I. Surakka *et al.*, 2015 Mondo-mlx mediates organismal sugar sensing through the gli-similar transcription factor sugarbabe. Cell Rep. 13: 350–364. https://doi.org/10.1016/j.celrep.2015.08.081

Mayer, R. J., and D. J. Candy, 1969 Control of haemolymph lipid concentration during locust flight: an adipokinetic hormone from the corpora cardiaca. J. Insect Physiol. 15: 611–620. https://doi.org/10.1016/0022-1910(69)90259-5

McKay, R. M., J. P. McKay, L. Avery, and J. M. Graff, 2003 C elegans: a model for exploring the genetics of fat storage. Dev. Cell 4: 131–142. https://doi.org/10.1016/S1534-5807(02)00411-2

Meltzer, S., J. A. Bagley, G. L. Perez, C. E. O'Brien, L. DeVault *et al.*, 2017 Phospholipid homeostasis regulates dendrite morphogenesis in Drosophila sensory neurons. Cell Rep. 21: 859–866. https://doi.org/10.1016/j.celrep.2017.09.089

Min, K. T., and S. Benzer, 1999 Preventing neurodegeneration in the Drosophila mutant bubblegum. Science 284: 1985–1988. https://doi.org/10.1126/science.284.5422.1985

Moessinger, C., K. Klizaite, A. Steinhagen, J. Philippou-Massier, A. Shevchenko *et al.*, 2014 Two different pathways of phosphatidylcholine synthesis, the Kennedy Pathway and the Lands Cycle, differentially regulate cellular triacylglycerol storage. BMC Cell Biol. 15: 43. https://doi.org/10.1186/s12860-014-0043-3

Moraru, A., G. Cakan-Akdogan, K. Strassburger, M. Males, S. Mueller *et al.*, 2017 THADA regulates the organismal balance between energy storage and heat production. Dev. Cell 41: 72–81.e6 (erratum: Dev. Cell 41: 450). https://doi.org/10.1016/j.devcel.2017.03.016

Musselman, L. P., and R. P. Kühnlein, 2018 Drosophila as a model to study obesity and metabolic disease. J. Exp. Biol. 221: jeb163881. https://doi.org/10.1242/jeb.163881

Musselman, L. P., J. L. Fink, P. V. Ramachandran, B. W. Patterson, A. L. Okunade *et al.*, 2013 Role of fat body lipogenesis in protection against the effects of caloric overload in Drosophila. J. Biol. Chem. 288: 8028–8042. https://doi.org/10.1074/jbc.M112.371047

Nässel, D. R., and J. Vanden Broeck, 2015 Insulin/IGF signaling in Drosophila and other insects: factors that regulate production, release and post-release action of the insulin-like peptides. Cell. Mol. Life Sci. 73: 271–290. https://doi.org/10.1007/s00018-015-2063-3

Nässel, D. R., Y. Liu, and J. Luo, 2015 Insulin/IGF signaling and its regulation in Drosophila. Gen. Comp. Endocrinol. 221: 255–266. https://doi.org/10.1016/j.ygcen.2014.11.021

Nirala, N. K., M. Rahman, S. M. Walls, A. Singh, L. J. Zhu *et al.*, 2013 Survival response to increased ceramide involves metabolic adaptation through novel regulators of glycolysis and lipolysis. PLoS Genet. 9: e1003556. https://doi.org/10.1371/journal.pgen.1003556

Oldham, S., H. Stocker, M. Laffargue, F. Wittwer, M. Wymann *et al.*, 2002 The Drosophila insulin/IGF receptor controls growth and size by modulating PtdInsP(3) levels. Development 129: 4103–4109.

Osborne, T. F., and P. J. Espenshade, 2009 Evolutionary conservation and adaptation in the mechanism that regulates SREBP action: what a long, strange tRIP it's been. Genes Dev. 23: 2578–2591. https://doi.org/10.1101/gad.1854309

Öst, A., A. Lempradl, E. Casas, M. Weigert, T. Tiko *et al.*, 2014 Paternal diet defines offspring chromatin state and intergenerational obesity. Cell 159: 1352–1364. https://doi.org/10.1016/j.cell.2014.11.005

Owusu-Ansah, E., and N. Perrimon, 2014 Modeling metabolic homeostasis and nutrient sensing in Drosophila: implications for aging and metabolic diseases. Dis. Model. Mech. 7: 343–350. https://doi.org/10.1242/dmm.012989

Pagac, M., D. E. Cooper, Y. Qi, I. E. Lukmantara, H. Y. Mak *et al.*, 2016 SEIPIN regulates lipid droplet expansion and adipocyte development by modulating the activity of glycerol-3-phosphate acyltransferase. Cell Rep. 17: 1546–1559. https://doi.org/10.1016/j.celrep.2016.10.037

Palanker, L., J. M. Tennessen, G. Lam, and C. S. Thummel, 2009 Drosophila HNF4 regulates lipid mobilization and beta-oxidation. Cell Metab. 9: 228–239. https://doi.org/10.1016/j.cmet.2009.01.009

Palm, W., J. L. Sampaio, M. Brankatschk, M. Carvalho, A. Mahmoud *et al.*, 2012 Lipoproteins in Drosophila melanogaster–assembly, function, and influence on tissue lipid composition. PLoS Genet. 8: e1002828. https://doi.org/10.1371/journal.pgen.1002828

Palu, R. A. S., S. A. Praggastis, and C. S. Thummel, 2017 Parental obesity leads to metabolic changes in the F2 generation in Drosophila. Mol. Metab. 6: 631–639. https://doi.org/10.1016/j.molmet.2017.03.012

Panáková, D., H. Sprong, E. Marois, C. Thiele, and S. Eaton, 2005 Lipoprotein particles are required for Hedgehog and Wingless signalling. Nature 435: 58–65. https://doi.org/10.1038/nature03504

Papadopoulou, D., M. W. Bianchi, and M. Bourouis, 2004 Functional studies of shaggy/glycogen synthase kinase 3 phosphorylation sites in Drosophila melanogaster. Mol. Cell. Biol. 24: 4909–4919. https://doi.org/10.1128/MCB.24.11.4909-4919.2004

Park, Y., Y.-J. Kim, and M. E. Adams, 2002 Identification of G protein-coupled receptors for Drosophila PRXamide peptides, CCAP, corazonin, and AKH supports a theory of ligand-receptor coevolution. Proc. Natl. Acad. Sci. USA 99: 11423–11428 (erratum: Proc. Natl. Acad. Sci. USA 99: 13961). https://doi.org/10.1073/pnas.162276199

Parra-Peralbo, E., and J. Culi, 2011 Drosophila lipophorin receptors mediate the uptake of neutral lipids in oocytes and imaginal disc cells by an endocytosis-independent mechanism. *PLoS Genet* 7: e1001297. https://doi.org/10.1371/journal.pgen.1001297

Parvy, J.-P., L. Napal, T. Rubin, M. Poidevin, L. Perrin *et al.*, 2012 Drosophila melanogaster Acetyl-CoA-carboxylase sustains a fatty acid-dependent remote signal to waterproof the respiratory system. PLoS Genet. 8: e1002925. https://doi.org/10.1371/journal.pgen.1002925

Patel, R. T., J. L. Soulages, B. Hariharasundaram, and E. L. Arrese, 2005 Activation of the lipid droplet controls the rate of lipolysis of triglycerides in the insect fat body. J. Biol. Chem. 280: 22624–22631. https://doi.org/10.1074/jbc.M413128200

Péterfy, M., J. Phan, P. Xu, and K. Reue, 2001 Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. Nat. Genet. 27: 121–124. https://doi.org/10.1038/83685

Peterson, T. R., S. S. Sengupta, T. E. Harris, A. E. Carmack, S. A. Kang *et al.*, 2011 mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell 146: 408–420. https://doi.org/10.1016/j.cell.2011.06.034

Porstmann, T., C. R. Santos, B. Griffiths, M. Cully, M. Wu *et al.*, 2008 SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab. 8: 224–236. https://doi.org/10.1016/j.cmet.2008.07.007

Pospisilik, J. A., D. Schramek, H. Schnidar, S. J. F. Cronin, N. T. Nehme *et al.*, 2010 Drosophila genome-wide obesity screen reveals hedgehog as a determinant of Brown *vs.* White adipose cell fate. Cell 140: 148–160. https://doi.org/10.1016/j.cell.2009.12.027

Prasad, S. V., G. J. Fernando-Warnakulasuriya, M. Sumida, J. H. Law, and M. A. Wells, 1986 Lipoprotein biosynthesis in the larvae of the tobacco hornworm, Manduca sexta. J. Biol. Chem. 261: 17174–17176.

Puig, O., M. T. Marr, M. L. Ruhf, and R. Tijan, 2003 Control of cell number by Drosophila FOXO: downstream and feedback regulation of the insulin receptor pathway. Genes Dev. 17: 2006–2020.

Rajan, A., and N. Perrimon, 2012 Drosophila cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. Cell 151: 123–137 [corrigenda: Cell 152: 1197 (2013)]. https://doi.org/10.1016/j.cell.2012.08.019

Rajan, A., and N. Perrimon, 2013 Of flies and men: insights on organismal metabolism from fruit flies. BMC Biol. 11: 38. https://doi.org/10.1186/1741-7007-11-38

Rajan, A., B. E. Housden, F. Wirtz-Peitz, L. Holderbaum, and N. Perrimon, 2017 A mechanism coupling systemic energy sensing to adipokine secretion. Dev. Cell 43: 83–98.e6. https://doi.org/10.1016/j.devcel.2017.09.007

Rava, P., and M. M. Hussain, 2007 Acquisition of triacylglycerol transfer activity by microsomal triglyceride transfer protein during evolution. Biochemistry 46: 12263–12274. https://doi.org/10.1021/bi700762z

Rawson, R. B., 2003 The SREBP pathway--insights from Insigs and insects. Nat. Rev. Mol. Cell Biol. 4: 631–640. https://doi.org/10.1038/nrm1174

Reiff, T., J. Jacobson, P. Cognigni, Z. Antonello, E. Ballesta *et al.*, 2015 Endocrine remodelling of the adult intestine sustains reproduction in Drosophila. Elife 4: e06930. https://doi.org/10.7554/eLife.06930

Reis, T., M. R. Van Gilst, and I. K. Hariharan, 2010 A buoyancy-based screen of Drosophila larvae for fat-storage mutants reveals a role for Sir2 in coupling fat storage to nutrient availability. PLoS Genet. 6: e1001206. https://doi.org/10.1371/journal.pgen.1001206

Rodríguez-Vázquez, M., D. Vaquero, E. Parra-Peralbo, J. E. Mejía-Morales, and J. Culi, 2015 Drosophila lipophorin receptors recruit the lipoprotein LTP to the plasma membrane to mediate lipid uptake. PLoS Genet. 11: e1005356. https://doi.org/10.1371/journal.pgen.1005356

Rusten, T. E., K. Lindmo, G. Juhasz, M. Sass, P. O. Seglen *et al.*, 2004 Programmed autophagy in the Drosophila fat body is induced by ecdysone through regulation of the PI3K pathway. Dev. Cell 7: 179–192. https://doi.org/10.1016/j.devcel.2004.07.005

Ryan, R. O., N. H. Haunerland, W. S. Bowers, and J. H. Law, 1988 Insect lipid transfer particle catalyzes diacylglycerol exchange between high-density and very-high-density lipoproteins. Biochim. Biophys. Acta 962: 143–148. https://doi.org/10.1016/0005-2760(88)90105-1

Sajwan, S., R. Sidorov, T. Stašková, A. Žaloudíková, Y. Takasu *et al.*, 2015 Targeted mutagenesis and functional analysis of adipokinetic hormone-encoding gene in Drosophila. Insect Biochem. Mol. Biol. 61: 79–86. https://doi.org/10.1016/j.ibmb.2015.01.011

Schmitt, S., R. Ugrankar, S. E. Greene, M. Prajapati, and M. Lehmann, 2015 Drosophila Lipin interacts with insulin and TOR signaling pathways in the control of growth and lipid metabolism. J. Cell Sci. 128: 4395–4406. https://doi.org/10.1242/jcs.173740

Schulz, J. G., A. Laranjeira, L. Van Huffel, A. Gärtner, S. Vilain *et al.*, 2015 Glial β-oxidation regulates Drosophila energy metabolism. Sci. Rep. 5: 7805.

Schüpbach, T., and E. Wieschaus, 1991 Female sterile mutations on the second chromosome of Drosophila melanogaster. II. Mutations blocking oogenesis or altering egg morphology. Genetics 129: 1119–1136.

Schwedes, C. C., and G. E. Carney, 2012 Ecdysone signaling in adult Drosophila melanogaster. J. Insect Physiol. 58: 293–302. https://doi.org/10.1016/j.jinsphys.2012.01.013

Seegmiller, A. C., I. Dobrosotskaya, J. L. Goldstein, Y. K. Ho, M. S. Brown *et al.*, 2002 The SREBP pathway in Drosophila: regulation by palmitate, not sterols. Dev. Cell 2: 229–238. https://doi.org/10.1016/S1534-5807(01)00119-8

Sellers, J. A., L. Hou, H. Athar, M. M. Hussain, and G. S. Shelness, 2003 A Drosophila microsomal triglyceride transfer protein homolog promotes the assembly and secretion of human apolipoprotein B. Implications for human and insect transport and metabolism. J. Biol. Chem. 278: 20367–20373. https://doi.org/10.1074/jbc.M300271200

Sieber, M. H., and A. C. Spradling, 2015 Steroid signaling establishes a female metabolic state and regulates SREBP to control

oocyte lipid accumulation. Curr. Biol. 25: 993–1004. https://doi.org/10.1016/j.cub.2015.02.019

Sieber, M. H., and C. S. Thummel, 2009 The DHR96 nuclear receptor controls triacylglycerol homeostasis in Drosophila. Cell Metab. 10: 481–490. https://doi.org/10.1016/j.cmet.2009.10.010

Sieber, M. H., and C. S. Thummel, 2012 Coordination of triacylglycerol and cholesterol homeostasis by DHR96 and the Drosophila LipA homolog magro. Cell Metab. 15: 122–127. https://doi.org/10.1016/j.cmet.2011.11.011

Sivachenko, A., H. B. Gordon, S. S. Kimball, E. J. Gavin, J. L. Bonkowsky *et al.*, 2016 Neurodegeneration in a Drosophila model of adrenoleukodystrophy: the roles of the Bubblegum and Double bubble acyl-CoA synthetases. Dis. Model. Mech. 9: 377–387. https://doi.org/10.1242/dmm.022244

Slack, C., C. Werz, D. Wieser, N. Alic, A. Foley *et al.*, 2010 Regulation of lifespan, metabolism, and stress responses by the Drosophila SH2B protein, Lnk. *PLoS Genet.* 6: e1000881.

Smith, S., A. Witkowski, and A. K. Joshi, 2003 Structural and functional organization of the animal fatty acid synthase. Prog. Lipid Res. 42: 289–317. https://doi.org/10.1016/S0163-7827(02)00067-X

Sociale, M., A.-L. Wulf, B. Breiden, K. Klee, M. Thielisch *et al.*, 2018 Ceramide synthase Schlank is a transcriptional regulator adapting gene expression to energy requirements. Cell Rep. 22: 967–978. https://doi.org/10.1016/j.celrep.2017.12.090

Song, W., D. Ren, W. Li, L. Jiang, K. W. Cho *et al.*, 2010 SH2B regulation of growth, metabolism, and longevity in both insects and mammals. Cell Metab. 11: 427–437. https://doi.org/10.1016/j.cmet.2010.04.002

Song, W., J. A. Veenstra, and N. Perrimon, 2014 Control of lipid metabolism by tachykinin in Drosophila. Cell Rep. 9: 40–47. https://doi.org/10.1016/j.celrep.2014.08.060

Song, W., D. Cheng, S. Hong, B. Sappe, Y. Hu *et al.*, 2017 Midgut-derived activin regulates glucagon-like action in the fat body and glycemic control. Cell Metab. 25: 386–399. https://doi.org/10.1016/j.cmet.2017.01.002

Staubli, F., T. J. D. Jorgensen, G. Cazzamali, M. Williamson, C. Lenz *et al.*, 2002 Molecular identification of the insect adipokinetic hormone receptors. Proc. Natl. Acad. Sci. USA 99: 3446–3451. https://doi.org/10.1073/pnas.052556499

Subramanian, M., S. K. Metya, S. Sadaf, S. Kumar, D. Schwudke *et al.*, 2013 Altered lipid homeostasis in Drosophila InsP3 receptor mutants leads to obesity and hyperphagia. Dis. Model. Mech. 6: 734–744. https://doi.org/10.1242/dmm.010017

Sundermeyer, K., J. K. Hendricks, S. V. Prasad, and M. A. Wells, 1996 The precursor protein of the structural apolipoproteins of lipophorin: cDNA and deduced amino acid sequence. Insect Biochem. Mol. Biol. 26: 735–738. https://doi.org/10.1016/S0965-1748(96)00060-4

Sztalryd, C., and D. L. Brasaemle, 2017 The perilipin family of lipid droplet proteins: gatekeepers of intracellular lipolysis. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1862: 1221–1232. https://doi.org/10.1016/j.bbalip.2017.07.009

Taurino, M., S. Costantini, S. De Domenico, F. Stefanelli, G. Ruano *et al.*, 2018 SEIPIN proteins mediate lipid droplet biogenesis to promote pollen transmission and reduce seed dormancy. Plant Physiol. 176: 1531–1546. https://doi.org/10.1104/pp.17.01430

Teleman, A. A., 2010 Molecular mechanisms of metabolic regulation by insulin in Drosophila. Biochem. J. 425: 13–26. https://doi.org/10.1042/BJ20091181

Teleman, A. A., S. Maitra, and S. M. Cohen, 2006 Drosophila lacking microRNA miR-278 are defective in energy homeostasis. Genes Dev. 20: 417–422. https://doi.org/10.1101/gad.374406

Tennessen, J. M., and C. S. Thummel, 2011 Coordinating growth and maturation - insights from Drosophila. Curr. Biol. 21: R750–R757. https://doi.org/10.1016/j.cub.2011.06.033

Tennessen, J. M., N. M. Bertagnolli, J. Evans, M. H. Sieber, J. Cox *et al.*, 2014 Coordinated metabolic transitions during Drosophila embryogenesis and the onset of aerobic glycolysis. G3 (Bethesda) 4: 839–850. https://doi.org/10.1534/g3.114.010652

Thiam, A. R., R. V. Farese, Jr., and T. C. Walther, 2013 The biophysics and cell biology of lipid droplets. Nat. Rev. Mol. Cell Biol. 14: 775–786. https://doi.org/10.1038/nrm3699

Thiel, K., C. Heier, V. Haberl, P. J. Thul, M. Oberer *et al.*, 2013 The evolutionarily conserved protein CG9186 is associated with lipid droplets, required for their positioning and for fat storage. J. Cell Sci. 126: 2198–2212. https://doi.org/10.1242/jcs.120493

Thomas, H. E., H. G. Stunnenberg, and A. F. Stewart, 1993 Heterodimerization of the Drosophila ecdysone receptor with retinoid X receptor and ultraspiracle. Nature 362: 471–475. https://doi.org/10.1038/362471a0

Thul, P. J., K. Tschapalda, P. Kolkhof, A. R. Thiam, M. Oberer *et al.*, 2017 Targeting of the Drosophila protein CG2254/Ldsdh1 to a subset of lipid droplets. J. Cell Sci. 130: 3141–3157. https://doi.org/10.1242/jcs.199661

Tian, Y., J. Bi, G. Shui, Z. Liu, Y. Xiang *et al.*, 2011 Tissue-autonomous function of Drosophila seipin in preventing ectopic lipid droplet formation. PLoS Genet. 7: e1001364. https://doi.org/10.1371/journal.pgen.1001364

Ugrankar, R., Y. Liu, J. Provaznik, S. Schmitt, and M. Lehmann, 2011 Lipin is a central regulator of adipose tissue development and function in Drosophila melanogaster. Mol. Cell. Biol. 31: 1646–1656. https://doi.org/10.1128/MCB.01335-10

Vagin, V. V., Y. Yu, A. Jankowska, Y. Luo, K. A. Wasik *et al.*, 2013 Minotaur is critical for primary piRNA biogenesis. RNA 19: 1064–1077. https://doi.org/10.1261/rna.039669.113

Valente, V., R. M. Maia, M. C. B. Vianna, and M. L. Paçó-Larson, 2010 Drosophila melanogaster lipins are tissue-regulated and developmentally regulated and present specific subcellular distributions. FEBS J. 277: 4775–4788. https://doi.org/10.1111/j.1742-4658.2010.07883.x

van Heusden, M. C., D. J. van der Horst, J. K. Kawooya, and J. H. Law, 1991 In vivo and in vitro loading of lipid by artificially lipid-depleted lipophorins: evidence for the role of lipophorin as a reusable lipid shuttle. J. Lipid Res. 32: 1789–1794.

Van Hoof, D., K. W. Rodenburg, and D. J. Van der Horst, 2002 Insect lipoprotein follows a transferrin-like recycling pathway that is mediated by the insect LDL receptor homologue. J. Cell Sci. 115: 4001–4012. https://doi.org/10.1242/jcs.00113

Van Hoof, D., K. W. Rodenburg, and D. J. van der Horst, 2003 Lipophorin receptor-mediated lipoprotein endocytosis in insect fat body cells. J. Lipid Res. 44: 1431–1440. https://doi.org/10.1194/jlr.M300022-JLR200

Varghese, J., S. F. Lim, and S. M. Cohen, 2010 Drosophila miR-14 regulates insulin production and metabolism through its target, sugarbabe. Genes Dev. 24: 2748–2753. https://doi.org/10.1101/gad.1995910

Verdu, J., M. A. Buratovich, E. L. Wilder, and M. J. Birnbaum, 1999 Cell-autonomous regulation of cell and organ growth in Drosophila by Akt/PKB. Nat. Cell Biol. 1: 500–506. https://doi.org/10.1038/70293

Vihervaara, T., and O. Puig, 2008 dFOXO regulates transcription of a Drosophila acid lipase. J. Mol. Biol. 376: 1215–1223. https://doi.org/10.1016/j.jmb.2007.12.042

Walker, A. K., R. L. Jacobs, J. L. Watts, V. Rottiers, K. Jiang *et al.*, 2011 A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. Cell 147: 840–852. https://doi.org/10.1016/j.cell.2011.09.045

Walls, S. M., Jr., S. J. Attle, G. B. Brulte, M. L. Walls, K. D. Finley *et al.*, 2013 Identification of sphingolipid metabolites that induce obesity via misregulation of appetite, caloric intake and fat

storage in Drosophila. *PLoS Genet* 9: e1003970. https://doi.org/10.1371/journal.pgen.1003970

Walls, S. M., A. Cammarato, D. A. Chatfield, K. Ocorr, G. L. Harris *et al.*, 2018 Ceramide-protein interactions modulate ceramide-associated lipotoxic cardiomyopathy. Cell Rep. 22: 2702–2715. https://doi.org/10.1016/j.celrep.2018.02.034

Walther, T. C., J. Chung, and R. V. Farese, 2017 Lipid droplet biogenesis. Annu. Rev. Cell Dev. Biol. 33: 491–510. https://doi.org/10.1146/annurev-cellbio-100616-060608

Wang, B., J. Goode, J. Best, J. Meltzer, P. E. Schilman *et al.*, 2008 The insulin-regulated CREB coactivator TORC promotes stress resistance in Drosophila. Cell Metab. 7: 434–444. https://doi.org/10.1016/j.cmet.2008.02.010

Wang, B., N. Moya, S. Niessen, H. Hoover, M. M. Mihaylova *et al.*, 2011 A hormone-dependent module regulating energy balance. Cell 145: 596–606. https://doi.org/10.1016/j.cell.2011.04.013

Wang, H., M. Becuwe, B. E. Housden, C. Chitraju, A. J. Porras *et al.*, 2016 Seipin is required for converting nascent to mature lipid droplets. Elife 5: e16582. https://doi.org/10.7554/eLife.16582

Wang, H., M. V. Airola, and K. Reue, 2017 How lipid droplets "TAG" along: glycerolipid synthetic enzymes and lipid storage. Biochim. Biophys. Acta 1862: 1131–1145. https://doi.org/10.1016/j.bbalip.2017.06.010

Wang, S., S. Liu, H. Liu, J. Wang, S. Zhou *et al.*, 2010 20-hydroxyecdysone reduces insect food consumption resulting in fat body lipolysis during molting and pupation. J. Mol. Cell Biol. 2: 128–138. https://doi.org/10.1093/jmcb/mjq006

Weers, P. M., D. J. van der Horst, W. J. van Marrewijk, M. van den Eijnden, J. M. van Doorn *et al.*, 1992 Biosynthesis and secretion of insect lipoprotein. J. Lipid Res. 33: 485–491.

Welte, M. A., 2015 Expanding roles for lipid droplets. Curr. Biol. 25: R470–R481. https://doi.org/10.1016/j.cub.2015.04.004

Welte, M. A., and A. P. Gould, 2017 Lipid droplet functions beyond energy storage. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1862: 1260–1272. https://doi.org/10.1016/j.bbalip.2017.07.006

Welte, M. A., S. Cermelli, J. Griner, A. Viera, Y. Guo *et al.*, 2005 Regulation of lipid-droplet transport by the perilipin homolog LSD2. Curr. Biol. 15: 1266–1275. https://doi.org/10.1016/j.cub.2005.06.062

Werz, C., K. Köhler, E. Hafen, and H. Stocker, 2009 The Drosophila SH2B family adaptor Lnk acts in parallel to chico in the insulin signaling pathway. *PLoS Genet.* 5: e1000596. https://doi.org/10.1371/journal.pgen.1000596

Wicker-Thomas, C., D. Garrido, G. Bontonou, L. Napal, N. Mazuras *et al.*, 2015 Flexible origin of hydrocarbon/pheromone precursors in Drosophila melanogaster. J. Lipid Res. 56: 2094–2101. https://doi.org/10.1194/jlr.M060368

Wilfling, F., H. Wang, J. T. Haas, N. Krahmer, T. J. Gould *et al.*, 2013 Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocalizing from the ER to lipid droplets. Dev. Cell 24: 384–399. https://doi.org/10.1016/j.devcel.2013.01.013

Wilfling, F., A. R. Thiam, M.-J. Olarte, J. Wang, R. Beck *et al.*, 2014 Arf1/COPI machinery acts directly on lipid droplets and enables their connection to the ER for protein targeting. Elife 3: e01607. https://doi.org/10.7554/eLife.01607

Xu, P., S. Y. Vernooy, M. Guo, and B. A. Hay, 2003 The Drosophila MicroRNA Mir-14 suppresses cell death and is required for normal fat metabolism. Curr. Biol. 13: 790–795. https://doi.org/10.1016/S0960-9822(03)00250-1

Xu, X., P. Gopalacharyulu, T. Seppänen-Laakso, A.-L. Ruskeepää, C. C. Aye *et al.*, 2012 Insulin signaling regulates fatty acid catabolism at the level of CoA activation. PLoS Genet. 8: e1002478. https://doi.org/10.1371/journal.pgen.1002478

Yan, Y., H. Wang, H. Chen, A. Lindström-Battle, and R. Jiao, 2015 Ecdysone and insulin signaling play essential roles in readjusting the altered body size caused by the dGPAT4 mutation in Drosophila. J. Genet. Genomics 42: 487–494. https://doi.org/10.1016/j.jgg.2015.06.008

Yan, Y., H. Wang, M. Hu, L. Jiang, Y. Wang *et al.*, 2017 HDAC6 suppresses age-dependent ectopic fat accumulation by maintaining the proteostasis of PLIN2 in Drosophila. Dev. Cell 43: 99–111.e5. https://doi.org/10.1016/j.devcel.2017.09.001

Yao, T. P., W. A. Segraves, A. E. Oro, M. McKeown, and R. M. Evans, 1992 Drosophila ultraspiracle modulates ecdysone receptor function via heterodimer formation. Cell 71: 63–72. https://doi.org/10.1016/0092-8674(92)90266-F

Yao, T. P., B. M. Forman, Z. Jiang, L. Cherbas, J. D. Chen *et al.*, 1993 Functional ecdysone receptor is the product of EcR and Ultraspiracle genes. Nature 366: 476–479. https://doi.org/10.1038/366476a0

Zechner, R., F. Madeo, and D. Kratky, 2017 Cytosolic lipolysis and lipophagy: two sides of the same coin. Nat. Rev. Mol. Cell Biol. 18: 671–684. https://doi.org/10.1038/nrm.2017.76

Zhang, P., and K. Reue, 2017 Lipin proteins and glycerolipid metabolism: roles at the ER membrane and beyond. Biochim. Biophys. Acta 1859: 1583–1595. https://doi.org/10.1016/j.bbamem.2017.04.007

Zhao, X., and J. Karpac, 2017 Muscle directs diurnal energy homeostasis through a myokine-dependent hormone module in Drosophila. Curr. Biol. 27: 1941–1955.e6. https://doi.org/10.1016/j.cub.2017.06.004

Ziegler, A. B., C. Thiele, F. Tenedini, M. Richard, P. Leyendecker *et al.*, 2017 Cell-autonomous control of neuronal dendrite expansion via the fatty acid synthesis regulator SREBP. Cell Rep. 21: 3346–3353. https://doi.org/10.1016/j.celrep.2017.11.069

Zimmermann, R., J. G. Strauss, G. Haemmerle, G. Schoiswohl, R. Birner-Gruenberger *et al.*, 2004 Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 306: 1383–1386. https://doi.org/10.1126/science.1100747

Zinke, I., C. S. Schütz, J. D. Katzenberger, M. Bauer, and M. J. Pankratz, 2002 Nutrient control of gene expression in Drosophila: microarray analysis of starvation and sugar-dependent response. EMBO J. 21: 6162–6173. https://doi.org/10.1093/emboj/cdf600

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