Chapter VII

Insect Lipid Metabolism: Insights Into Gene Expression Regulation

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Abstract

The class Insecta has more than 1 million different cataloged species living in almost all habitats throughout the world, and they encompass many different lifestyles. Understanding how these animals evolved in terms of their physiology and biochemistry is reason enough to perform research in the basic science field. However, hematophagous insects spread vector-borne diseases, such as malaria, dengue fever and Chagas' disease through human populations living in tropical regions. In addition, the herbivorous species can damage plantation fields, leading to both reduced production and economic losses. Moreover, insects can be easily raised in laboratory colonies, and some can be readily genetically manipulated. Finally, some similarities between insect and mammalian physiology make these invertebrates excellent models to study human problems; for example, lipid metabolism deregulation, which can be responsible for the development of diseases such as obesity, arteriosclerosis and diabetes, one of the major health problems concerning the Western population. For all the above reasons, research groups around the world are working with insect models to generate data that can be used to control disease vectors and agricultural pests and may even help to understand human metabolism. Here, we will review the findings achieved in insect lipid metabolism, particularly over the last 10 years, and we will focus on how genes involved in these pathways are regulated. We will discuss how different environmental signals or metabolites and hormones produced

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by insects can control the activity of transcription factors to change gene expression profiles. The gaps found in our current knowledge and possible future directions in this research field are also discussed.

1. Introduction

Insects are the most widespread Class in the kingdom Animalia. This Class has more than 1 million species described, and they are distributed all around the world [1]. These animals have important ecological functions, such as being responsible for the pollination of many types of plants. However, insects also affect human life directly in different manners. For instance, hematophagous insects can disseminate vector-borne diseases. Almost 2.5 billion people, especially in Africa, are at risk of being infected with malaria, which is caused by protozoans from the genus *Plasmodium*. Approximately 2 million people die from malaria infections per year, most of whom are children [2]. This disease is transmitted via mosquitoes from the genus *Anopheles* that are present all over the globe, including the United States, Central America, Brazil, sub-Saharan Africa, Europe, Western Russia, Middle East, India and Southeast Asia, indicating that malaria infection could spread even further [3]. Other mosquitoes, such as *Aedes aegypti*, are vectors for the Dengue virus, which is the etiologic agent of Dengue Fever. This disease affects an estimated 2.5 billion people in Southeast Asia, the Pacific, the Americas, Africa and the Eastern Mediterranean [4,5].

Another important vector-borne disease is Chagas' Disease. Fifteen million people are infected with this disease and 90 million are at risk in the Americas, from the Southern United States to Southern Argentina and Chile [6]. Chagas' Disease is transmitted by kissing bugs from the Subfamily Triatominae; however, the species *Rhodnius prolixus* and *Triatoma infestans* are uniquely adapted to live in human dwellings and are considered the most important vectors of this disease [7]. Furthermore, approximately 500 million insect species feed on plants. If these agricultural pests cannot be controlled, they can lead to crop destruction and economic losses [8]. Indeed, over one thousand species of arthropods are associated with tea plant culture and can cause production yield loss of up to 50% [9].

The potential medical and economic impacts that may be caused by the insects noted above stimulate different lines of investigation in several research groups. In addition, many insect models can be easily genetically manipulated, which makes them extremely important tools for research studies [10]. Likewise, the similarity between mammals and insects at various points of metabolism makes these models interesting alternatives to study certain human diseases, including diabetes and metabolic syndromes, in addition to other problems associated with lipid metabolism [11-13]. Thus, the study of lipid metabolism may reveal differences between insects and mammals that can be explored to develop new ways to control insect pests and vectors. Alternatively, similarities could be found that may help to better understand various human diseases.

This chapter will first briefly review the current knowledge of lipid metabolism in insects with a focus on what has been discovered in the last decade. Second, we will discuss the transcription factors and the regulation of gene expression in these animals. Third, the main signals, including the hormones, metabolites and environmental signals, that are implicated in the regulation of genes involved in lipid metabolism will be discussed. Finally, the issues that still require clarification and possible future directions will be noted.

2. An Overview of Insect Lipid Metabolism

The last reviews describing insect lipid metabolism were published more than ten years ago [14, 15]. Arrese and colleagues [14] wrote that "the general reader will also be surprised to learn that we know little" and, unfortunately, this phrase still describes the overall knowledge of this subject. Although the last decade has been marked by the genomic revolution, with the genomes of more than 50 insect species sequenced, studies on the physiology and biochemistry of lipid metabolism have not progressed at the same rate. The interplay between genomics and physiology was also ignored until recently. Many of the questions raised by Arrese [14], Canavoso [15] and their collaborators have not been answered or even investigated. This chapter presents a short review on insect lipid metabolism updated with the major recent discoveries, with a special focus on the regulation of the genes involved in the pathways related to fat metabolism. Readers who want more extensive information are referred to the papers formerly cited [14, 15].
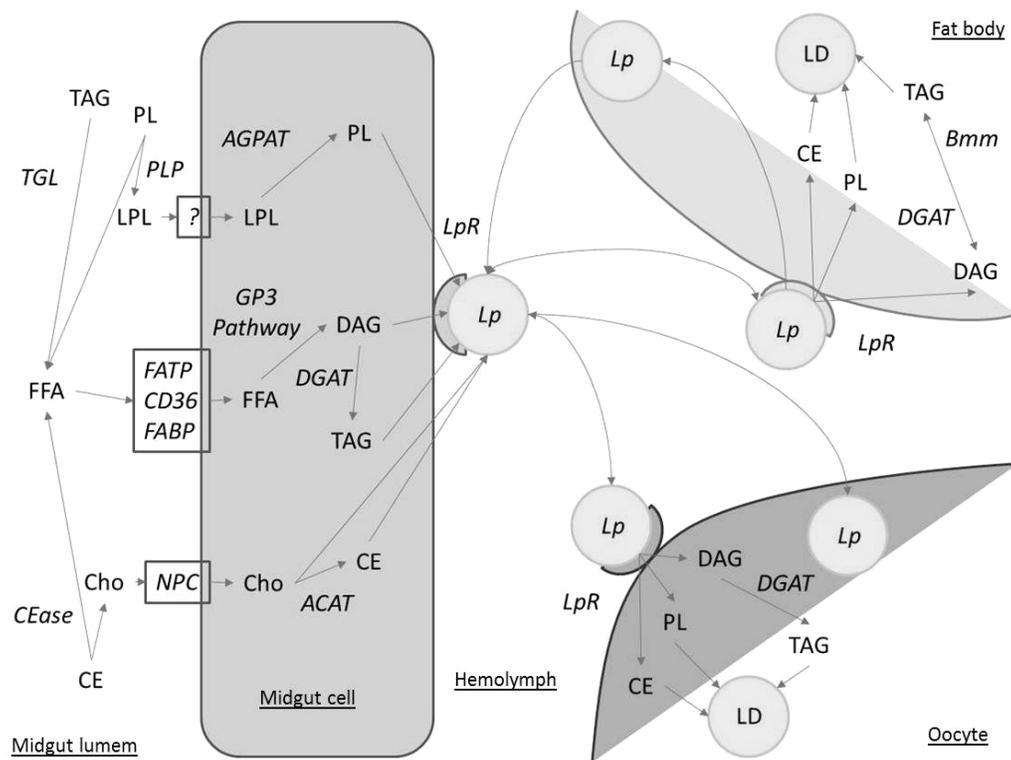

Figure 1. Overview of lipid metabolism in insects. Lipids are digested at the midgut lumen and absorbed and metabolized by midgut cells. Then, they are transported in the hemolymph by lipophorin to fat body and oocytes, where they are stored. Abbreviations: ACAT: acyl-CoA cholesterol acyltranferase; AGPAT: Lysophopholipid acyltransferase; Bmm: Brummer lipase; CE: Cholesteryl ester; CEase: Cholesterol esterase; Cho: Cholesterol; DAG: diacylglycerol; DGAT: Diacylglycerol acyltransferase; FABP: Fatty acid-binding protein; FATP: Fatty acid transport protein; FFA: Free fatty acid; LD: Lipid droplet; Lp: Lipophorin; LPL: Lysophospholipid; LpR: Lipophorin receptor; PL: Phospholipid; PLP: Phospholipase; TAG: triacylglycerol; TGL: Triacylglycerol lipase.

Insects, like other animals, receive their nutrients through food. Triglyceride (TAG) is commonly the main lipid component of the diet; although phospholipids (PL) and sterols are also present [16]. The midgut is the principal site of digestion and absorption of lipids in insects, and the activity of several enzymes, such as TAG lipases, phospholipases and cholesterol esterases, has been described in different species [17-23]. The main products generated by this digestion, such as free fatty acids (FFA), non-esterified cholesterol and lysophospholipids, are then absorbed by the intestinal epithelial cells [19, 24-28]. However, the way in which each different compound is incorporated by the midgut is mostly unclear.

Intestinal absorption of FFA is not completely understood, even in mammals. In vertebrate models, three proteins, namely plasma membrane-associated fatty acid-binding protein (FABPpm), fatty acid transport protein 4 (FATP4) and the fatty acid transporter (CD36), having high affinity to FFA, were found in enterocytes and are involved, in some way, with the incorporation of these lipids. Furthermore, the role of passive diffusion cannot be discarded [29]. In the tobacco hornworm *Manduca sexta*, the presence of two FABPs has been shown; however, no information about their relationship with the absorption of fatty acids is available [30]. The expression of a FATP was described in the midgut of the silkworm *Bombyx mori*, but its role in the absorption of fatty acids from the diet was not investigated [31]. Additionally, this insect also expresses a midgut protein related to CD36, and it is involved in the absorption of lutein and probably other lipid molecules, as well [32]. However, how the absorption of FFA by intestinal epithelial cells occurs remains mostly unknown.

The absorption of sterols was further investigated recently. In mammals, the Niemann-Pick C1-like 1 protein plays an essential role in cholesterol absorption [33]. In the fruit fly *Drosophila melanogaster*, a protein with a similar function has been identified. Flies containing the mutant *npc1b* gene have poor intestinal absorption of cholesterol, and the larvae die during the early stages of development [34]. However, double mutants for the genes *npc1a* and *npc1b* have normal cholesterol absorption, which indicates the existence of an alternative pathway for sterol absorption when the intracellular traffic is deficient [34]. In addition, intracellular proteins able to bind cholesterol may also have an important role in the intestinal absorption of sterols. Inhibiting the gene expression of sterol carrier protein 2/3-oxoacyl-CoA thiolase (*scpx*) by RNA interference (RNAi) caused a reduction in the cholesterol concentration in the hemolymph of the cotton leafworm *Spodoptera litura* [24]. Furthermore, the larvae had a developmental delay until pupation [24]. In cultured cells of the mosquito *A. aegypti*, the overexpression of the *sterol carrier protein-2* (*Aescp-2*) gene causes an increase in the incorporation of cholesterol [35]. These results indicate that these proteins are somehow related to cholesterol absorption.

Finally, there is no information about the mechanisms by which lysophospholipids are absorbed by the midgut cells in insects.

After being absorbed, these pre-digested lipid molecules are used as the precursors of the synthesis of more complex structures that will be transported to the other organs of the insect. FFA are used for the synthesis of diacylglycerol (DAG), PL and TAG. In mammals, the major route of TAG synthesis in the intestine is the monoacylglycerol (MAG) pathway [36]. In insects, however, some results indicate that the activity of this pathway is negligible and that glycerolipids are synthesized via the glycerol-3-phosphate (G3P) pathway [19, 25]. The G3P pathway essentially consists of the sequential acylations of G3P until TAG synthesis via the production of phosphatidic acid (PA) and DAG, among other intermediaries [37]. As

might be expected, several enzymes are involved in these processes, but only the phosphatidic acid phosphatase (PAP, which catalyzes the production of DAG from the dephosphorylation of PA) has been studied in the insect midgut. PAP activity was measured in the midgut of the kissing bug *Panstrongylus megistus*, and this activity increases after feeding [25]. This result indicates that the G3P pathway may be more active when the lumen of the midgut is filled, which may accelerate the absorption of fatty acids. PAP expression was also described in the midgut of *D. melanogaster*, although its biochemical activity was not analyzed [38]. Furthermore, it is known that midgut cells are able to use the FFA obtained from the meal to synthesize PL [19, 25]; however, the steps involved in this process have not been investigated. Although it is likely that intestinal cells are able to synthesize fatty acids from sugars or amino acids and that these cells can use lipids obtained from feeding to acquire energy through β-oxidation, these processes were not studied further.

To be transported to other organs of the insect, the lipids produced in the gut are loaded onto lipophorin (Lp), which is the main lipoprotein present in the hemolymph (insect blood). In mammals, the chylomicrons are synthesized by enterocytes, the lipids are associated with the chylomicrons intracellularly, and the lipoproteins are secreted into the lymphatic system [39]. This process does not occur in insects. Lp is not synthesized by the gut, but rather by the fat body, an organ that accumulates the functions of the adipose tissue and liver in these animals [40]. The lipoprotein produced by the fat body circulates in the hemolymph and reaches the midgut, where it binds to specific receptors in the cell membrane [41, 42]. The lipids are then loaded into Lp by a process that remains unclear. A second lipoprotein, lipid transfer particle (LTP), appears to be necessary, but its role has not been determined [43, 44]. In most insects, DAG and PL are the major lipids transported, although the lipid composition may vary between species [45-50]. If readers are interested in more information about this protein and the unique lipid transport system in insects, more specific reviews are available [51, 52].

Loaded with the lipids obtained from the midgut, Lp is then directed to the organs responsible for the storage of fat, such as the fat body. Apparently, this lipoprotein can deliver the transported lipids in two different manners depending on the insect species. First, Lp may be associated with specific membrane receptors on the cells of the fat body in a process that, in principle, is similar to what occurs in the gut [53]. The LTP also appears to play a role in this transfer [54]. Alternatively, Lp can be endocytosed by the cells of the fat body [55], similar to what occurs with the low density lipoprotein (LDL) in mammalian cells [56]. However, unlike LDL, which is degraded by the endosomes in mammals, Lp is recycled together with its receptor and released back into the hemolymph [57]. Thus, Lp is unloaded in one target organ and can return to another to be supplied again, acting as a reusable shuttle [58].

Some of the lipids received are stored in the form of TAG in lipid droplets similar to those in the adipocytes of mammals [59-66]. In addition to the lipids supplied by Lp, the fat body is able to synthesize fatty acids from acetate and amino acids [67-69]. Furthermore, the gene expression and enzymatic activity of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), both involved in *de novo* fatty acid synthesis, were shown in the fat body of different species of insects [68-71]. The fatty acids produced by *de novo* synthesis are also esterified and stored in TAGs; thus, the G3P pathway must play an important role. Indeed, the PAP enzyme is expressed in the fat body and participates in organ development and in the process of lipid accumulation [38, 72]. Moreover, the enzyme DAG acyltransferase (DGAT,

which catalyzes the synthesis of TAG through DAG acylation) is involved in the accumulation of fat in the larval fat body of *D. melanogaster* [73]. Stocks of TAG are mobilized by the insect in times of energy need, such as during flight or during the development of eggs in females [67, 74, 75]. The way to use these reserves is similar to that observed in the adipose tissue of mammals [76]. Two enzymes with TAG-lipase activity were described in the fat body of insects: the triglyceride lipase (TGL) [77, 78] and the Brummer lipase [79]. Moreover, these two activities appear to be activated at different times by different metabolic signals that allow the insect to finely adjust lipolysis [80]. It is known that TGL activity is regulated by phosphorylation; however, this is not at the lipase level but at the level of the proteins present in lipid droplets [81-83]. The DAG generated in the process of lipolysis is then delivered to Lp, but the way in which this happens is unclear. Lp then distributes the lipids to the organs with the highest demand for energy, depending on the physiological moment experienced by the insect. The metabolism of lipids in the fat body is perhaps the most studied topic in the last decade in the physiology of lipids in insects. Readers especially interested in this subject should read the recent review published by Arrese and Soulages [40].

In females, the process of egg production (oogenesis) requires a large amount of lipids that are stored as yolk and used as the source of energy during embryo development. Although ACC and FAS expression were described in the ovaries of *A. aegypti* [69], it is known that the ability of *de novo* fatty acid synthesis by oocytes is minor, and thus, the stored lipids are obtained from other organs [84]. These lipids are delivered by Lp present in the hemolymph, but the mechanisms of lipid transfer may be different between species [45, 47, 85-87]. In *D. melanogaster* and in the kissing bug *R. prolixus*, Lp is not endocytosed, and lipid transfer occurs via the binding of the lipoprotein to its receptor [85, 88]. However, in *P. megistus*, in the cowpea weevil *Callosobruchus maculatus* and in the mosquito *A. gambiae*, Lp is found within the oocytes, and the transfer of lipids may occur in the cytoplasm [45, 47, 87]. The DAG received from Lp is converted to TAG and stored in the oocytes [86]. DGAT is responsible for catalyzing this reaction, and it has been shown that DGAT plays an essential role in egg development [89]. DGAT is also involved in lipid accumulation in embryonic *Drosophila* cells in culture [90].

This brief review of the current knowledge in the area of lipid metabolism in insects was necessary to facilitate the development of the next sections in this chapter, where both the transcription factors and the metabolic signals capable of regulating the expression of genes involved in these processes will be discussed.

3. Insect Transcription Factors and Gene Expression Regulation

In insects, the expression of genes involved in lipid metabolism is regulated by a wide variety of transcription factors that are regulated in different manners and by signals that may be distinct. In the next sections, the operation and regulation of the most studied transcription factors in fat metabolism will be briefly discussed.

3.1. Forkhead Box Class O (FoxO)

Transcription factors of the forkhead family contain a highly conserved DNA-binding domain that gives the "fork head" name to this class of proteins [91]. The first gene of the family was identified in *D. melanogaster* mutants that had problems in the development of the terminal segments, and was called *fork head* (*fkh*) [92]. At that time, the identified protein did not have any known DNA-binding domain observed in other transcription factors. The identification, a year later, of a domain highly conserved in the fruit fly *Drosophila fkh* gene and in the mouse hepatocyte nuclear factor 3 beta (HNF3β) gene, led to the discovery of a new DNA-binding domain [93]. Today, this family of transcription factors has more than 2,000 known proteins that are organized into 18 classes or subfamilies named from A to S [94].

The forkhead box protein family is involved in a wide variety of metabolic processes and also in embryonic development, acting as the ultimate and active targets of signaling pathways such as the insulin/insulin growth factor pathway, the mitogen-activated protein kinase pathway and the Wnt/β-catenin pathway [95]. Among the family members, the class O has been highly investigated due to their close relationship with insulin signaling and the consequent influence on metabolism, including lipid metabolism. In this context, the study of these proteins in insects has a possible advantage. While mammals have several genes encoding FoxO proteins, fruit flies encode only one ortholog (*dfoxo*) [96-98]. The presence of only one gene allows a more straightforward analysis in experimental mutant models because it reduces the possibility of functional redundancy among similar genes. It is likely that all insects have only one ortholog of FoxO, similar to what is observed for *D. melanogaster* and the mosquito *Culex pipiens* [99]; however, there is no systematic study that has addressed this issue.

The systemic overexpression of dFoxO in *D. melanogaster* causes a reduction in the size of the fly due to a reduction in cell size and number [97]. However, tissue-specific overexpression (e.g., in the eyes or wings) causes only a reduction in cell number [98]. In addition, the expression of dFoxO in the fat body increases the lifetime of flies [100, 101]. Moreover, flies without the *dfoxo* gene are more sensitive to oxidative stress and are not able to increase gene expression levels of the insulin receptor (*Insr*) when fasted [102, 103]. These results provide evidence that FoxO is intimately involved in the insulin signaling pathway and in nutrient sensing mechanisms [91]. However, in *C. pipiens*, the inhibition of FoxO expression by RNAi causes a reduction in the amount of stored lipids, in the number of cells in the fat body and in life expectancy [99]. These contradictory results may be based on the difference in lifestyle between the fruit fly and the mosquito. In the study using *C. pipiens* as a model [99], the authors analyzed the role of FoxO in mosquitoes during diapause, which are subjected to different hormonal stimuli, including juvenile hormone (JH). A possible interaction between genes stimulated by this hormone and regulated by FoxO activity may explain the differences. However, further studies on this aspect need to be performed.

FoxO activity is highly regulated by the insulin signaling pathway. In mammals, insulin regulates the activity of FoxO by binding to insulin receptor that activates a phosphorylation cascade of different targets that ends in the activation of protein kinase B (PKB or AKT) [104]. AKT, in turn, targets FoxO for phosphorylation, which causes FoxO retention in the cytoplasm, thereby preventing translocation to the nucleus and activation of its target genes [105].

Another mechanism of regulation is FoxO acetylation catalyzed by the histone acetyltransferases P300 and CBP. This acetylation causes inhibition of FoxO [106, 107]. This modification reduces the affinity of FoxO for DNA and increases the levels of AKT-mediated phosphorylation, potentiating the inhibition of the protein [107]. In addition, FoxO is activated by the deacetylation promoted by deacetylase SIRT1 in response to fast and consequent reduction in insulin signaling [108-110]. This activation of the FoxO signaling pathway appears to be highly conserved in insects based on data obtained from *D. melanogaster*. Recently, this was elegantly demonstrated by Wang and colleagues [111]. The authors demonstrate that fasting causes a reduction in the phosphorylation levels of insulin signaling pathway proteins, such as AKT and FoxO, in addition to salt inducible kinase 3 (SIK3), a kinase member of the AMP-activated protein kinase family. Instead, during feeding, AKT phosphorylates SIK3, thereby increasing its activity. The activated SIK3 targets a class IIa histone deacetylase called HDAC4 that, when phosphorylated, is inhibited and translocates from the nucleus to the cytoplasm. It is interesting to note that HDAC4 is able to deacetylate and activate FoxO. Thus, SIK3 inhibits FoxO transcriptional activity through a mechanism dependent on HDAC4 deacetylase. Accordingly, insulin regulates this transcription factor via AKT, which acts in two forms that complement each other: (1) direct phosphorylation and inhibition of FoxO, and (2) activation of SIK3, which phosphorylates and inhibits HDAC4 causing an indirect inhibition of FoxO.

Also in *D. melanogaster*, FoxO can be phosphorylated by c-Jun N-terminal kinase (JNK) in response to stress caused by high levels of reactive oxygen species (ROS) [112]. However, in this case, the transcriptional activity of FoxO is activated, leading to increased expression of its target genes [113]. FoxO can then be involved in the regulation of genes involved in stress response [105].

Both the transcriptome response and the role of FoxO in *D. melanogaster* that was fed different nutrients were also studied [114]. Gershman *et al*. were able to regulate the release of insulin-like peptides (ILPs) based on the protein content of the food available and, therefore, the activity of insulin and FoxO pathway were modulated. To identify potential gene targets of FoxO regulation in this study, the results were compared with those obtained using *Drosophila* S2 cells expressing a constitutively active form of FoxO (dFoxO-A3) [98]. Almost 1,000 possible targets of FoxO were identified, some of which were involved in lipid metabolism, as expected [114]. FoxO is involved in the activation of genes involved in β-oxidation, such as carnitine palmitoyltransferase (CPT) and acyl-CoA synthetase. Furthermore, genes involved in the synthesis of lipids, such as citrate lyase, glucose-6-phosphate dehydrogenase, ACC, FAS, β-ketoacyl-ACP synthase, ACP-S-malonyltransferase and serine-palmitoyltransferase, all had increased expression levels [114].

Notably, for genes involved in lipid metabolism, the direct action of FoxO has been shown only a few times. It was demonstrated in *D. melanogaster* that FoxO activates the expression of *dLip4* [115]. This gene encodes a TAG lipase expressed at moderate levels throughout the life of the fly, particularly in the fat body of the larvae [116, 117]. While it may be speculated that dLip4 is involved in the mobilization of lipid reserves, there are no biochemical data to confirm this hypothesis. Nevertheless, it was demonstrated both *in vitro* and *in vivo* that FoxO binds to the *dLip4* promoter in a region between 0.6 kb and 1.3 kb upstream of the transcription start site [115]. Furthermore, FoxO is important for the expression of *dLip4* during fasting [115]. The regulation of the expression of the lipase *brummer* and the mitochondrial acyl-CoA synthetase *pudgy*, which is involved in the

oxidation of fatty acids, are two more examples of FoxO directly interacting with the promoter of genes involved in lipid metabolism [111, 118]. Recently, Alic and colleagues [119] used whole-genome ChIP-chip analysis to identify more than 350 genes directly regulated by FoxO in adult females of *D. melanogaster*.

3.2. Ecdysone Receptor (EcR) and its Early Genes

20-hydroxyecdysone (20-HE) (i.e., ecdysone) is possibly the most studied hormone in insect endocrinology. Its name derives from its essential role in the molting process (ecdysis) during the development of immature insects to adulthood. However, this hormone has several other regulatory roles that were neglected until recently in both the larvae (or nymphs) and adult insects [120]. For example, 20-HE is required for the induction of expression of the *vitellogenin* (*Vg*) gene in the fat body of the adult female of the mosquito *A. aegypti* after the blood meal [121].

20-HE binds to its intracellular receptor, the ecdysone receptor (EcR), which is a transcription factor of the nuclear receptor superfamily [122]. The binding of 20-HE causes the dimerization of the receptor with another nuclear receptor called Ultraspiracle (USP) [123-125]. This heterodimer is an active transcription factor that regulates the expression of a number of genes, most of which encode for other transcription factors, such as the receptor itself (EcR and USP), and HR3, HR4, HR78, E75, E78, Broad and FTZ-F1 [126]. These transcription factors, called "early genes", regulate a range of other genes (i.e., "late genes") and control the metamorphosis of the immature insects as a whole. This signaling pathway controls moulting and is conserved in different insects, such as the fruit fly *D. melanogaster*, *A. aegypti*, the tobacco hornworm *M. sexta*, the cockroach *Blattella germanica* and the red flour beetle *Tribolium castaneum* [127-130]. However, in addition to controlling metamorphosis, the early genes of the ecdysone cascade may also regulate different parts of the insect's physiology. Recently, numerous studies have shown that several of these transcription factors may be involved in lipid metabolism.

One such early gene is *e75*. The protein encoded by this gene contains one molecule of heme as a prosthetic group, and its transcriptional activity is regulated by binding gases such as carbon monoxide or nitric oxide to the heme group [131, 132]. The gases abolish the ability of E75 to act as a transcriptional repressor and inhibit the activity of HR3 (to which E75 dimerizes), another early gene that is induced by ecdysone [131, 132]. One interesting study investigated the roles of nitric oxide, and consequently the activity of E75, in *D. melanogaster* larvae by using RNAi to inhibit the expression of nitric oxide synthase (NOS) present in the prothoracic gland [133]. The larvae developed a large accumulation of lipids in the fat body cells, as indicated by the increased number of lipid droplets and the amount of TAG. Based on the results reported by Colombani and colleagues [134], showing that the activity of the EcR is related to the accumulation of lipids in fat body cells, the authors believe that the effects caused by the inhibition of NOS are associated with E75 activity. These results were confirmed recently when it was shown that the inhibition of EcR expression by RNAi caused an accumulation of TAG in the fat body [135]. Other results support the idea that E75 has a role in lipid metabolism in insects. For example, *e75* null fruit fly larvae display a fat accumulation phenotype [136]. Furthermore, RNAi targeting the *e75* gene generates an increased amount of TAG in the fat body of *Drosophila* larvae [135].

An analysis of the available insect genomes reveals that these animals do not encode an ortholog of peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor known to be responsible for the regulation of lipogenesis in vertebrates [137]. Although the e75 gene is orthologous to the *Rev-erb* gene in mammals [131], the comparison of the primary sequences of E75 and PPARγ show significant conservation of the DNA-binding domain but reveal that the ligand binding domains are relatively different [138]. This may indicate that these transcription factors regulate similar promoters but are activated by different signals. All the evidence above seems to indicate that the E75 protein may have assumed the function of regulating lipogenesis in insects and that this gene is actually an analog of the PPARγ in vertebrates.

Other early genes in the ecdysone cascade are involved in regulating genes involved in lipid metabolism. In *D. melanogaster*, the *broad* gene, which encodes a transcription factor from the zinc finger family [139], regulates the expression of *npc1*, which is involved in cholesterol absorption [140]. In addition, several genes involved in the synthesis of ecdysone have predicted binding sites for Broad in their promoters and are regulated by Broad transcriptional activity [140]. These results show that ecdysone signaling can generate a positive feedback loop by activating the expression of Broad and consequently increasing the transcription of genes required for its own synthesis. Moreover, Cuifo *et al.* [141] speculated that a gene coding for the enzyme desmosterol reductase, involved in sterol metabolism in the silkworm *B. mori,* could be regulated by the activity of Broad; however, this hypothesis is based solely on the presence of predicted binding sites for the transcription factor in the promoter of desmosterol reductase gene without any concerted information about the relationship between this gene and Broad. Broad may also regulate other aspects of lipid metabolism through its genetic interaction with other transcription factors. For example, Broad inhibits the expression of *fkh* in *D. melanogaster* [142, 143], and this protein is involved in the expression of genes related to different aspects of lipid metabolism, such as the transport of fatty acid, β-oxidation and lipolysis, and lipid synthesis [144]. Thus, Broad could indirectly regulate metabolism.

In *A. aegypti*, *AeSCP-2* gene expression is regulated by the HR3 and βFTZ-F1 transcription factors [145], both representatives of the family of nuclear receptors [137]. It was determined that βFTZ-F1 binds directly to the *AeSCP-2* promoter and represses the expression of this gene, while HR3 is capable of inducing *AeSCP-2* transcription [145].

3.3. Sterol Regulatory Element Binding Protein (SREBP)

SREBPs are transcription factors from helix-loop-helix leucine zipper family and are conserved throughout evolution, from fungi to mammals [146]. These proteins are involved in controlling the expression of genes responsible for the synthesis of cholesterol and fatty acids [147]. The transcriptional activity of the SREBP pathway is directed by a complex process that comprises translocations between organelles and protease cleavage at specific points on SREBP. Importantly, this pathway is conserved among mammals and insects [148, 149]. The signal that triggers the SREBP activation in mammals is the level of cholesterol present in the cell [150]. However, insects are unable to perform *de novo* synthesis of cholesterol [151], and thus, the regulation of SREBP activity by cholesterol would not make sense. The study authored by Dobrosotskaya and colleagues [152] clarified this issue by demonstrating that the

fruit fly *D. melanogaster* SREBP is not regulated by cholesterol levels, but rather by the levels of phosphatidylethanolamine (PE). When the levels of PE are reduced, the precursor form of SREBP is translocated from the endoplasmic reticulum to the Golgi apparatus, where the protein is cleaved by two different proteases. This processing releases the N-terminal region of the protein from the membrane of the organelle, and the mature form migrates to the nucleus where it functions as a transcription factor [149]. SREBP is carried to the Golgi complex by SREBP cleavage-activating protein (SCAP). In mammals, the interaction between SCAP and SREBP is regulated by the protein encoded by *insulin induced gene* (INSIG). INSIG is the sterol sensor of the SREBP pathway. When cholesterol levels are reduced, INSIG releases SCAP leading to SREBP cleavage [149]. All proteins that participate in the pathway are present in *Drosophila*, with the exception of INSIG [153]. Thus, how SCAP is regulated in insects remains unknown.

The importance of SREBP in insects has only been studied in *D. melanogaster*. Mutant flies for this gene do not survive beyond the second instar, indicating that SREBP is essential [154]. The lethality phenotype can be reversed by expressing SREBP in the midgut and fat body or by providing the larvae soybean oil in the medium [154]. One result is striking: the feeding of the mutant larvae with TAG containing only palmitate (tripalmitin) reduces mortality by 29%. However, feeding with free palmitate results in 52% recovery [154]. This seems to indicate that the larvae have a problem with the digestion of lipids, although this hypothesis has not yet been investigated. A lipase encoded by the *CG6295* gene is regulated by SREBP [154-156]. This gene is only expressed during the larvae and adults stage [117] and only in the midgut [116], thus indicating that this lipase may be involved in the digestion of TAG. The relationship of SREBP with insect digestion needs to be further studied. Furthermore, the silencing of SREBP by RNAi causes a reduction in the size of both cells and organs in *D. melanogaster* [157].

The activation of the target of rapamycin (TOR) pathway also regulates the activity of SREBP, although the details are not known [157]. It was demonstrated that silencing the insulin signaling pathway by RNAi reduces the expression of SREBP target genes. Moreover, the activation of the same pathway via the inhibition of *dp110* gene expression, an insulin pathway repressor, causes an increase in the expression of same genes [157]. These results indicate a possible interaction between insulin signaling and the activity of SREBP, thus leading to increased expression of genes related to lipogenesis, similar to what occurs in mammals [146].

The activity of SREBP can also be regulated by post-translational modification, such as acetylation or deacetylation. Deacetylation of SREBP both in *Caenorhabditis elegans* and in mice reduces the stability of the protein and leads to its degradation via the proteasome [158]. Something similar can also occur in *D. melanogaster*. Mutant flies for the gene *silent information regulator 2* (*sir2*), a histone deacetylase, display a deregulation in the expression of genes involved in lipid synthesis [136]. Several target genes of SREBP, such as acetyl-CoA synthetase, acyl-CoA synthetase, ACC and FAS, have increased expression levels [136]. However, a direct relationship between SREBP and Sir2 has not yet been demonstrated in *Drosophila*.

It is important to mention that recent studies have shown that the activation of SREBP does not occur via a single mechanism, and alternative forms of activation were identified. Flies mutant for the *dsp2* gene, encoding the second protease in the SREBP cleavage pathway, are viable and capable of cleaving and activating SREBP [155]. These results were

surprising considering that all SREBP functions are dependent on SREBP cleavage by SP2. The explanation was given by Amarneh *et al.* [159]. *Drosophila* SREBP could be activated through cleavage by the caspase Drice at one point near to the cleavage site catalyzed by SP2. Furthermore, the double mutant, *drice/dsp2*, had the same lethal phenotype that occurs in the *dsrebp* mutant. Similarly, dSCAP is not essential for the activation of SREBP in *D. melanogaster* [156]. SREBP continues to be processed in *dscap* mutant flies. However, this mutant did not depend on Drice to activate SREBP because the *drice/dscap* double mutant is as viable as the mutants for each individual gene. Furthermore, SREBP is cleaved by SP1 and SP2 in the absence of SCAP, indicating that SREBP is transported to the Golgi apparatus. However, in the absence of SCAP, SP2 is still required for the cleavage of SREBP because the *dscap/dsp2* double mutant flies have the same phenotype as *dsrebp* mutant flies [160]. It is still unclear how the insects are able to continue processing the cleavage of SREBP in the absence of SCAP; however, research into this question will yield interesting information on the activation pathway of this transcription factor, including for mammals.

Thus, studies in *Drosophila* described three different modes of activation and cleavage of SREBP: (1) the "classical" mode, as described for mammals, but triggered by the levels of PE [152, 153]; (2) SP2-independent cleavage and SP2-dependent cleavage by Drice [155, 159]; and (3) cleavage independent of SCAP [156, 160].

3.4. Hormone Receptor-Like in 96 (HR96)

Although insects are unable to synthesize cholesterol [151], these animals encode several genes involved in the absorption, metabolism and transport of sterols that need to be regulated. As discussed above, SREBP is not involved in this regulation [153]. In 2009, Horner and colleagues [161] showed that the HR96 nuclear receptor binds cholesterol and is responsible for the transcriptional response of several genes to changes in cholesterol levels in the diet in larvae of the fruit fly *D. melanogaster*. Mutant flies for HR96 have a curious phenotype. All insects are auxotrophic for cholesterol, but the *dhr96* mutant larvae are extremely sensitive to low levels of this sterol in the diet. Moreover, when a diet rich in cholesterol is provided, these same larvae display sterol accumulation. These phenotypes were caused by the deregulation of the expression of the NPC1 sterol transporter, indicating that HR96 plays a role in regulating cholesterol uptake from the diet. These results were confirmed by Bujold and colleagues in 2010 [162]. Interestingly, feeding wild-type *D. melanogaster* larvae with high levels of cholesterol triggers a transcriptional response very similar to that observed in the *dhr96* mutant animals [162]. Additionally, cholesterol itself represses the expression of HR96. These results led to the proposition of a model where cholesterol binds to HR96 and represses its transcriptional activity. At low cholesterol concentrations, HR96 is active and its target genes are transcribed; however, at high cholesterol concentrations, HR96 is inhibited. As HR96 own promoter is subject to its regulation, cholesterol has an enhanced action because it is capable of inhibiting the transcription of the receptor itself [162].

dhr96 mutant flies are also sensitive to starvation and die earlier than the wild-type flies [163]. Moreover, the *dhr96* mutants are resistant to obesity induced by a high-calorie diet. These mutants are resistant to treatment with inhibitors of intestinal lipase activity and display a reduction in lipase activity in the midgut. HR96 controls the expression of a digestive lipase

named *magro* [163, 164]. This lipase has both TAG lipase and cholesterol esterase activity. Thus, magro hydrolyzes cholesterol ester and TAG in the midgut of flies and is responsible for maintaining lipid metabolism homeostasis. The function of magro is so important that its expression in *dhr96* mutant flies is able to partially rescue the cholesterol accumulation and the lack of TAG reserves phenotypes [164].

The HR96 nuclear receptor is also involved in *D. melanogaster* response to xenobiotics [165]. The *dhr96* mutant flies are more sensitive to insecticides such as DDT, and the transcriptional response to the presence of these xenobiotics is perturbed.

Finally, HR96 is involved in the reproductive process in the red flour beetle *T. castaneum*. The inhibition of HR96 expression using RNAi reduces the beetle oviposition by 40%; however, the eggs are not viable because in the absence of HR96, embryo development is interrupted during the mid-stage of germ band growth [166].

HR96 is involved in a wide variety of metabolic processes and appears to be the main regulator of intestinal lipid metabolism. This nuclear receptor is present in the genomes of all insects analyzed so far [137]. Thus, the study of this gene in other models having lifestyles different from *D. melanogaster* will bring more information about how lipid metabolism is regulated in the gut of insects.

3.5. Hepatocyte Nuclear Factor 4 (HNF4)

The HNF4 transcription factor belongs to the nuclear receptor family and is present in the genomes of all insects analyzed thus far [137]. The HNF4 receptor subfamily has undergone a major expansion in *C. elegans*. Indeed, this worm has 269 different paralogs in this group [167]. However, insects have only one such gene, making them good models for studying the roles of this protein [137]. The fruit fly was used as a model for investigation of this gene's function in an elegant publication by Palanker and colleagues [168].

Flies mutant for this gene are sensitive to prolonged fasting and have difficulty to regulate lipid metabolism, presenting reduced mobilization of fat reserves from the fat body and containing an excess of FFA. Consistent with these observations, the larvae had reduced expression of genes involved in lipolysis and β-oxidation. The authors also showed that the transcriptional activity of HNF4 is activated during fasting and by supplementation with fatty acids. These results led to a model to explain the activation of the nuclear receptor. During fasting, the lipolysis of stored TAG generates FFA that activate HNF4. This nuclear receptor then increases the expression of genes participating during all stages of lipid catabolism, including lipolysis, the activation of fatty acids, the transport of these acyl-CoAs to the mitochondria and β-oxidation. Therefore, HNF4 regulates lipid mobilization during fasting [168].

The ability to activate β-oxidation genes and the characteristic of being regulated by fatty acid levels suggest that the insect HNF4 is similar to the PPARα of mammals [169]. Because insects do not encode genes orthologous to PPAR [137], HNF4 may have assumed a similar function and may be an analog of PPARα [168].

In the mosquito *C. pipiens*, HNF4 increases its expression along with genes involved in fatty acid oxidation during diapause, which may indicate that this nuclear receptor also plays a role in the regulation of lipid mobilization in this model [170]. In the red flour beetle *T. castaneum*, HNF4 is important for reproduction because RNAi silencing of this gene reduces

egg laying by 40%, and only 50% of laid eggs hatch. However, the relationship between HNF4 and reproduction or embryogenesis remains to be fully investigated [166].

3.6. NF-κB Like Transcription Factors

Insects lack an adaptive immune system and rely solely on an innate response to fight pathogens [171]. The innate immune system can be divided into three modes of action (i.e., melanization, phagocytosis and the expression of antimicrobial peptides) [172]. The expression of peptides is regulated by signaling pathways triggered by the recognition of a pathogenic structure, which leads to the activation of membrane receptors. The Toll receptor pathway is an example of a pathway involved in this process [173].

After receptor activation, a signaling cascade is triggered and leads to the activation of two transcription factors that are orthologous to mammalian NF-κB. These are Dorsal and DIF in the fruit fly *D. melanogaster* [174]. When inactivated, Dorsal and DIF are retained in the cytoplasm and are associated with their inhibitor, Cactus [175, 176]. During the activation of Dorsal/DIF, Cactus is phosphorylated and degraded via the proteasome [177]. This frees Dorsal/DIF, which then translocates to the nucleus as a homodimer and activates the transcription of its target genes, including antimicrobial peptides [178-180].

In addition to regulating the innate immune system, the transcription factors of the NF-κB family are involved with other cellular processes such as embryonic development [173]. Lipid metabolism can also be controlled by Dorsal/DIF, perhaps as an integral part of the immune system [181].

In the mosquito *A. aegypti*, infection by different pathogens such as Gram (+) bacteria, fungi or *Plasmodium*, causes an increase in expression of Lp and its receptor. This effect has the direct participation of both the Toll pathway and the transcription factor REL1, which is the mosquito gene orthologous to the *Drosophila dorsal* gene [181]. These results indicate that the transcription factors regulated by the pathways of the immune system of insects may have important roles in lipid metabolism. Future studies may provide useful information for the physiology of lipids and the control of insect vectors and the diseases that they transmit.

4. Regulation of Genes by Insect Hormones

The study of the endocrinology of insects is an important area of entomology. The hormones produced by these animals indicate different physiological situations, such as nutritional status, the need for mobilization of reserves and the moulting of immature forms, among others. Although some of the effects of hormones are fast and involve only changes in enzyme activity, others depend on the regulation of gene expression. In this section, the influence of some hormones on the expression of genes involved in lipid metabolism will be discussed.

4.1. Insulin-Like Peptides (ILPs)

The ILPs are produced by specialized cells in the brain of insects [182] and, similar to mammalian insulin, are able to regulate the circulating levels of carbohydrates in the hemolymph [183]. Because the insulin signaling pathway appears to be highly conserved in insects, it is expected that the ILPs act similarly to insulin [96]. Indeed, it was possible to generate insects with symptoms of type II diabetes using only a high-sugar diet and no genomic modifications [184]. Feeding the fruit fly *D. melanogaster* larvae with this diet caused hyperglycemia in the animals, with various metabolic signals indicative of insulin resistance, obesity and a deregulation of the activation profile of FoxO [184]. These results clearly show that the insulin signaling pathway is highly conserved in animals and that insects may be good models to study diabetes.
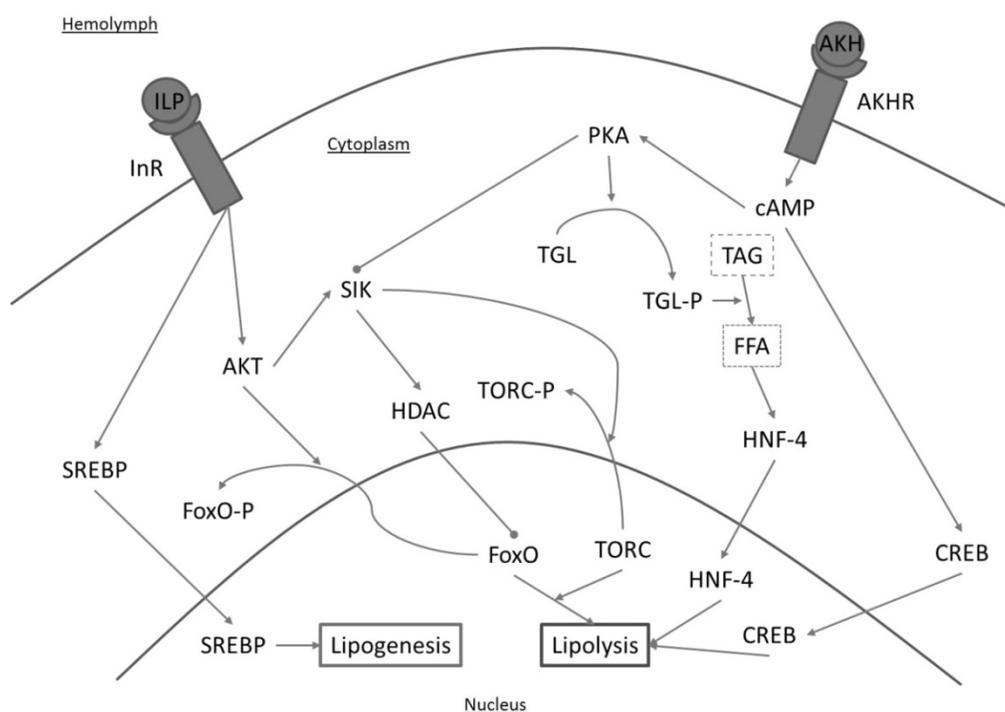

Figure 2. Effects of ILP and AKH on lipid metabolism. ILP activates lipogenesis through SREBP and inhibits lipolysis through phosphorylation of FoxO and TORC. On the other hand, AKH activates CREB and HNF-4 and inhibits SIK activity, increasing lipolysis. Abbreviations: CREB: cAMP response element-binding protein; FFA: Free fatty acid; FoxO-P: Phosphorylated FoxO; HDAC: Histone deacetylase; HNF-4: Hepatocyte nuclear factor 4; ILP: Insulin-like peptide; InR: Insulin receptor; SIK: Salt inducible kinase; SREBP: Sterol regulatory element binding protein; TAG: triacylglycerol; TGL: Triglyceride lipase; TGL-P: Phosphorylated TGL; TORC: Transducer of regulated CREB activity; TORC-P: Phosphorylated TORC;

The transcription factors FoxO and SREBP regulate genes of opposing functions. FoxO activates genes for β-oxidation, and SREBP induces the expression of genes involved in lipid synthesis [114, 153]. The presence of insulin inhibits the activity of FoxO and activates SREBP, leading to the accumulation of reserves. *Brummer* lipase, *Lip3*, *Lip4*, CPT and acyl-

CoA synthetase are genes repressed by the action of ILPs via FoxO [111, 114, 115]. Interestingly, *Lip3* and CPT are also regulated by HNF4, but in opposite directions [168], confirming that FoxO and HNF4 finely control lipid metabolism.

The ILPs can also regulate gene expression via other transcription factors, including the cAMP response element-binding protein (CREB) through the modulation of the activity of its coactivator, transducer of regulated CREB activity (TORC). In mammals, this coactivator acts together with FoxO in the regulation of genes during fasting [185-187]. In *D. melanogaster*, the signaling triggered by ILPs inhibits TORC activity in a phosphorylation-dependent process mediated by SIK2 [188]. This leads to the phosphorylation and degradation of the coactivator, and to a reduction in the expression of its target genes, including an acyl-CoA-binding protein (ACBP) [188]. This family of proteins is conserved throughout evolution, binds esters of acyl-CoA with high affinity and has functions relevant to various aspects of lipid metabolism [189].

However, the analysis of the effects of ILP signaling can be complicated if other signaling factors are acting simultaneously. For example, the worker honey bees (*Apis mellifera*) undergo a natural process of losing their lipid reserves during their development [190]. This process is greatly influenced by nutritional factors (and hence ILP signaling) in young bees, but it is also regulated by other signals, such as pheromones and the expression of Vg, especially in older bees [191]. A diet rich in lipids and proteins induces the expression of genes related to lipid storage in young bees, including genes such as lysophosphatidic acid acyltransferase (AGPAT), FAS and fatty acid desaturase (FAD) [191]. However, older nurse bees do not respond to this diet rich in lipids and proteins [191]. This may be explained by resistance to ILP signaling because these bees have high levels of expression of an ILP (*ilp2*) and an insulin receptor (*InR1*), but the expression of these genes is not regulated by changes in diet. The action of other factors, such as the pheromone of the queen of the hive, may be responsible for this regulation of the nutritional signal response. The same type of interaction between hormones occurs during diapause in the mosquito *C. pipiens* [99].

4.2. Adipokinetic Hormone (AKH)

The AKHs are a family of neuropeptides produced by cells present in the *corpus cardiacum*, an organ analogous to the pituitary gland of mammals. AKHs are involved in the mobilization of lipids and carbohydrates during the stimulation of flight in insects [192]. Although it has been thought that AKH's role was confined to this function, this hormone is also important for the homeostasis of circulating levels of carbohydrate in a very similar manner to glucagon produced by the pancreas of vertebrates [193]. Thus, insects have hormones that are analogous to insulin and glucagon in terms of their role in regulating metabolism. However, the importance of AKH as a regulator of gene expression has not been thoroughly explored, and its effects have always been credited to changes in enzyme activity profile.

AKH seems capable of activating two different signaling pathways via its binding to membrane receptors [194]. This hormone can activate a G_q protein with subsequent activation of phospholipase C and the generation of Ca^{2+} and inositol triphosphate as second messengers. In addition, AKH can increase adenylate cyclase activity, thereby increasing intracellular levels of cAMP and in turn activating cAMP activated protein kinase A (PKA).

Through these two pathways, AKH affects the metabolic activity of the target cell [194]. However, AKH may possibly have an effect on gene expression.

For example, the increase in cAMP levels and PKA activation may have a direct effect on transcription factors such as CREB [195]. In fact, there is a direct relationship between AKH signaling and the activation of CREB-responsive promoters in the fruit fly *D. melanogaster* [196]. In addition, the activation of PKA can lead to the phosphorylation and the inhibition of proteins from the SIK family [197], thereby participating in the signaling pathway of the ILPs. Thus, AKH may act as an ILP antagonistic hormone by releasing FoxO and TORC from their inhibition and leading to the activation of genes involved in lipolysis and fatty acid oxidation. This aspect has been neglected, and there are no results yet to support this hypothesis.

Interesting results from the expression of *Brummer* lipase indicate that gene regulation can be more complicated. *Brummer* lipase expression is increased during fasting in *D. melanogaster* larvae [79, 80]. Because this gene is a direct target for regulation by FoxO [111] it was expected that AKH signaling would lead to the activation of FoxO and an increased expression of *Brummer*. Surprisingly, AKH receptor mutants or larvae that are unable to produce this hormone have greater activation of the expression of *Brummer*. Moreover, chronic AKH overexpression leads to the inhibition of lipase expression [80]. The mechanism by which this occurs is unknown and illustrates the need to better understand the action of AKH. The expression of PAP is also activated during fasting, but its regulatory mechanism is still unknown [72]. The same is true for the expression of the *glaz* gene, a lipocalin orthologous to apolipoprotein D, in the head of flies [198]. It would be interesting to investigate whether ILPs and AKH are involved in these gene regulations and if the same type of regulation observed in *Brummer* also occurs with PAP and *glaz*.

4.3. 20-Hydroxyecdysone (20-HE)

As discussed in section 3.2, 20-HE and the genes regulated by this hormone have functions at various stages during development and on the physiology of insects and may be involved in lipid metabolism. Some results show that ecdysone regulates different genes in lipid metabolism, and the investigation of the relationship between this hormone and this facet of physiology deserves more attention.

In the mosquito *A. aegypti*, the expression of Lp increases in the fat body during vitellogenesis [199]. During this process, the levels of circulating 20-HE in the hemolymph are elevated [127]. Moreover, incubating the fat body in culture in the presence of 20-HE induced expression of Lp [199]. The expression of this gene also increases during vitellogenesis in the mosquito *A. gambiae* [200]. The promoter of the Lp gene in this mosquito presents ecdysone responsive elements; however, the direct action of the hormone has not been tested [200].

In the fruit fly *D. melanogaster*, the expression of the *start1* gene, which encodes a mitochondrial transporter of cholesterol likely involved in steroid synthesis [201], is dependent on the presence of ecdysone [202]. In fact, this expression is dependent on the transcription factor Broad, which regulates, aside from *start1*, a series of genes involved in steroidogenesis, including *npc1*, *ecd*, *dib*, *woc*, *giant*, *shadow*, *shade*, *phantom*, *dare* and *mld* [140]. The presence of Broad-binding sites in the promoters of these genes indicates direct

regulation [140]. Thus, ecdysone should be able to regulate its own synthesis via the regulation of these key genes.

In the silkworm *B. mori*, five transporters from the ATP-binding cassette G (ABCG) family are expressed predominantly in the midgut and are positively regulated by 20-HE through its EcR/USP heterodimer receptor [203]. This mechanism appears to be conserved in insects because the *e23* gene of *D. melanogaster*, belonging to the same family, is also regulated by 20-HE [204]. In mammals, the transporters from the ABCG family are involved in the transport of xenobiotics and sterols through the plasma membrane of cells [205]. If the same is true for these genes in insects, it is tempting to speculate that 20-HE may play a role in sterol absorption and homeostasis in the gut of *B. mori*. In *A. aegypti*, the expression of *AeSCP-2* is also regulated by 20-HE, as well as by the ecdysone early genes, *hr3* and *ftz-f1* [145, 206]. The proteins encoded by this gene bind to both cholesterol and palmitic acid and may be involved in the intracellular transport of these lipids in the midgut (where they are mostly expressed) [145, 206]. Thus, ecdysone may also play a role in lipid absorption in the mosquito midgut. It is important to remember that in *D. melanogaster* 20-HE also regulates the expression of *npc1*, a cholesterol transporter essential for larvae survival [34], through the action of Broad [140].

Unlike the above results, 20-HE inhibits the expression of a lipase in the midgut of the cotton bollworm *Helicoverpa armigera* [207]. However, because no information is known about the metabolic function of this protein it is difficult to speculate which effect this inhibition contributes to caterpillars' digestion. In *B. mori*, 20-HE reduces the caterpillars' feeding, which then induces the lipolysis of TAG reserves in the fat body due to starvation. Consequently, the expression of *Brummer* lipase increases. Although the authors have shown that ecdysone does not act on the fat body cells through its receptor, an action of the hormone on the ILP signaling pathway and the activity of FoxO cannot be ruled out [134].

All of the above results indicate that 20-HE can play an important role in the regulation of lipid uptake in the midgut, its transport in the hemolymph, and in the synthesis of ecdysteroids. The interaction of ecdysone with lipid metabolism is an interesting topic for future investigations.

4.4. Juvenile Hormone (JH)

JH is a sesquiterpenoid produced by cells of the *corpora allata* that works in conjunction with ecdysone in controlling ecdysis in immature insects [208]. In addition to the molt, this hormone is involved in other physiological processes, such as the maturation of eggs during vitellogenesis. However, knowledge about its mechanism of action is very scarce compared to what is known about ecdysone. For instance, the JH receptor was not known yet, and two candidate genes, the nuclear receptor *usp* and the transcription factor *methoprene-tolerant* (*met*), competed for the role [208].

Many of the analyses of JH effects on gene expression indicate that it acts in an antagonistic manner to ecdysone. JH induces the expression of a lipase in the cotton bollworm *H. armigera*, while 20-HE causes its inhibition [207]. Similarly, in the mosquito *A. aegypti*, while *AeSCP-2L1* expression is stimulated by JH, *AeSCP-2L2* is insensitive to this hormone but is induced by 20-HE [206].

Some other results indicate that JH may be related to lipid metabolism. In *H. armigera*, the gene expression of an ACBP in the gut of caterpillars appears to be related to the levels of JH in the hemolymph, although there was no direct relationship between the two phenomena presented by the authors [209]. In the Jeffrey pine beetle *Dendroctonus jeffreyi*, JH treatment induces the expression of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in males [210]. This enzyme is part of the route of synthesis of this species' pheromone called frontalin [211]. However, HMG-CoA reductase is also important for the production of JH [212], indicating that this hormone can regulate its synthesis by means of positive feedback.

4.5. Serotonin

Serotonin acts as a neurohormone in several insects, particularly in the kissing bug *R. prolixus*, where it regulates various aspects of physiology during blood feeding, including saliva secretion and diuresis [213]. Recently, serotonin has been shown to be a signal capable of modulating gene expression. After feeding, the expression of *RpACBP-1* is induced in the posterior midgut, and this effect can be mimicked by the injection of serotonin [214]. Furthermore, injection of spiperone, a serotonin receptor antagonist, is capable of blocking the increase in *RpACBP-1* gene expression caused by feeding, illustrating that the serotonin released in the hemolymph is responsible for the variation of gene expression [214]. The effect of serotonin is mediated by activation of a G_s protein and the consequent increase in the levels of cAMP; however, the downstream signaling pathway remains unknown [214]. These results suggest that serotonin may also play a role in the regulation of lipid uptake in the midgut of hematophagous insects.

In the mosquito *A. aegypti*, the expression of several genes involved in lipid metabolism and absorption in the intestine is increased after blood feeding. This includes an FATP gene, genes involved in fatty acid synthesis (FAS and FAD) and in the synthesis of phosphatidic acid (G3P acyltransferase and AGPAT), several genes involved in β-oxidation and ketolysis, and an FABP gene [215]. It would be interesting to investigate whether serotonin or other compounds released into the hemolymph are involved with this regulation.

4.6. Diapause Hormone (DH)

To survive for long periods of time without food or in adverse weather conditions, such as the winter in colder regions of the globe, some insects undergo a group of metabolic changes called diapause [216]. Diapause is a process of metabolic depression triggered by environmental signals that are reflected by changes in the hormonal profile of the insect [216]. Although in some insects this hormonal regulation is orchestrated by general hormones, such as ILPs and JH [216], others, such as the silkworm *B. mori* and the cotton bollworm *H. armigera*, have a specific peptide called the diapause hormone (DH) to regulate the process of diapause [217, 218]. Interestingly, while DH induces diapause in *B. mori* [217], the same peptide acts on *H. armigera* to reactivate the metabolism of the pupae and end diapause [218, 219].

In *H. armigera*, DH also regulates the expression of genes involved in lipid metabolism. The expression of a gene encoding an ACBP increases when prothoracic glands are incubated with DH [220]. This may indicate that ACBP is necessary for the production of ecdysone in prothoracic gland, initiating the process through which diapause is broken.

5. Gene Regulation by Metabolites

Different products of cell metabolism are able to function as important signals indicative of various metabolic situations, such as the nutritional status of the organism. These metabolites may act by regulating the cellular transcriptional machinery, thus affecting metabolic functions. Lipid products generated from the cellular reactions may act by binding to nuclear receptors directly or indirectly by controlling transcription factors, resulting in changes to lipid metabolism. In this section, the function of some of these metabolites is discussed.

5.1. β-Glucosyl-O-Tyrosine

The glycosylated amino acid β-glucosyl-O-tyrosine was identified in several insects, including the fruit flies *Drosophila busckii* and *Ceratitis capitata*, the tobacco hornworm *M. sexta* and the silkworm *B. mori* [221-224]. This compound functions as a reserve of tyrosine to be used in the synthesis of quinones and diphenols, both of which are important substances for the hardening of the newly synthesized cuticle after ecdysis [221]. However, β-glucosyl-O-tyrosine also appears to be a signal that regulates the gene expression machinery. The injection of physiological amounts of β-glucosyl-O-tyrosine induces an increase in the gene expression of *pgACBP*, an ACBP expressed exclusively in the pheromone gland of *B. mori* [222, 225]. In addition, the expression of *pgACBP* follows the variations in the levels of the glycosylated amino acids in the hemolymph [222]. It is interesting to note that this protein is extremely important for the accumulation of TAG in the pheromone gland and for the production of the pheromone bombykol [226]. Although the receptor for β-glucosyl-O-tyrosine has not been identified, it is very specific because small changes in the structure of the glycosylated amino acid significantly reduce its ability to induce an increase in the expression of *pgACBP* [227]. For example, galactosyl-tyrosine has only 20% of the activity of the original compound [227]. The signaling pathway activated by β-glucosyl-O-tyrosine is still completely unknown.

5.2. Phospholipids and Sphingolipids

Phospholipids and sphingolipids are major structural lipids of cells as essential constituents of cell membranes. Both classes of lipids are also important for intracellular signaling. As discussed above, the levels of PE are responsible for regulating the translocation, processing and activation of SREBP [152]. In turn, the sphingolipids act as

secondary messengers by transducing signals between cellular compartments and regulating processes such as differentiation and apoptosis [228].

Aside from the effects related to SREBP activation, phospholipids may regulate the expression of digestive enzymes in a manner not yet known. In the lightbrown apple moth *Epiphyas postvittana*, the modulation of gene expression of several digestive lipases was studied in relation to the diet offered to the caterpillar. The reduction of phospholipid content in the diet appears to induce an increase in the expression of pancreatic-like lipases, but no effect was observed in the gastric-like lipases [229]. However, the different types of food given to the caterpillar did not allow for very precise conclusions.

In the fruit fly *D. melanogaster*, the *schlank* gene encodes a ceramide synthase [71]. *Schlank* mutants present, as one might predict, a reduction in the amount of ceramide, a sphingolipid, but also have a smaller amount of TAG in the fat body [71]. Bauer *et al.* determined that these mutants display increased expression of the lipases *dlip3* and *brummer*, indicating that the ceramides act as inhibitors of lipolysis [71]. The mechanism through which ceramide acts is unknown; however, one may speculate a possible interaction with the FoxO transcription factor. Furthermore, the absence of *schlank* causes a reduction in the levels of SREBP protein and SREBP translocation to the nucleus [71]. As a consequence, the gene expression of lipogenic enzymes, such as acetyl-CoA synthetase, acyl-CoA synthetase, FAS and ACC, is reduced [71]. This shows that sphingolipids can act both on the synthesis of lipids and on lipolysis. Further study and analyses in other models would be interesting.

5.3. Fatty Acids

The discovery of the mode of action of nuclear receptor HNF4 in insects marked fatty acids as important regulators of the oxidative process [168]. The expression of proteins such as FABP has been studied for its induction by the presence of fatty acids. In the mosquito *C. pipiens*, the expression of FABP and HNF4 seem to be related to the process of insect diapause [170]. Moreover, the transcriptional activity of the muscular FABP promoter of the migratory locust *Locusta migratoria* is increased in the presence of linoleic acid [230]. Furthermore, linoleic acid induces the binding of nuclear proteins to this promoter [230]. As might be expected, antibodies against PPAR were not able to reduce this binding. The regions in the FABP promoter that are responsive to fatty acids were identified [231], but the transcription factor responsible for the regulation of its gene expression is still unknown. However, the suggestion that HNF4 is involved is an attractive hypothesis.

A change in the intracellular levels of fatty acids may occur for various reasons, such as the interruption of the β-oxidation pathway, and this may have offsetting effects on gene expression. For example, mutating the *enigma* gene, which encodes an acyl-CoA dehydrogenase in the fruit fly *D. melanogaster*, causes an increase in *dLip3* gene expression and a reduction in expression of an enoyl-CoA hydratase [232]. As mentioned previously, *dLip3* is a target gene of HNF4 [168]. Thus, the reduction of the β-oxidation process caused by the absence of *enigma* may have increased the intracellular concentration of fatty acids, which resulted in HNF4 activation and the deregulation of gene expression.

The quantity of ingested fatty acids in the diet also has effects on gene expression, especially on the expression of proteins involved in the digestion and absorption of lipids. In a study on the gene expression of digestive lipases in the lightbrown apple moth *E.*

postvittana, larvae fed a diet without fatty acids displayed increased expression of both pancreatic-like lipases and gastric-like lipases [229]. In *D. melanogaster*, supplementation of the larvae medium with canola oil, which is rich in monounsaturated fatty acids, causes a large induction in the gene expression of the Lp receptor [162].

Similarly, feeding wax moth *Galleria mellonella* caterpillars with coconut oil, which is rich in saturated fatty acids, increases the expression of Lp receptor [233]. How fatty acids cause these effects remains unclear, but it is possible to hypothesize the involvement of HNF4 or SREBP because both of these proteins regulate the expression of a midgut lipase of unknown function [154, 168]. The HR96 nuclear receptor appears to play a central role in the regulation of lipid homeostasis in the midgut and is another strong candidate. Moreover, a high fat diet induced an increase in the expression of the *dlip2* lipase [234]. This type of diet also appears to regulate the activity of the TOR pathway and thus inhibits the expression of *Brummer* lipase and activates the expression of *FAS* [234]. These results may indicate that the TOR pathway is at least partly related to transcription factors such as FoxO, HNF4, and SREBP. When Chinese bees *Apis cerana* are fed conjugated linoleic acid, the gene expression of *AcLSD-1* is reduced. *AcLSD-1* codes for a structural protein of the lipid droplets [235]. Interestingly, the addition of rosiglitazone, a PPARγ agonist, in the diet induced the expression of *AcLSD-1* [235]. Because insects have no orthologous gene of PPARγ [137], it is quite surprising that this drug had any effect. It would be interesting to determine the pathway or proteins on which rosiglitazone is acting.

Fatty acids may have more indirect effects, such as in the ecological relationship between the plant *Morinda* and the fruit fly *Drosophila sechellia*. The fruit of the Rubiaceae *Morinda citrifolia* has several compounds that are toxic for different species of *Drosophila*, with the exception of *D. sechellia*.

This species has specialized in using these fruits to lay their eggs, and the larvae are resistant to the fruit toxins [236]. Furthermore, one of the *Morinda* toxins is a fatty acid, and *D. sechellia* flies are attracted to this toxin itself [236]. Interestingly, the presence of this fatty acid modulates gene expression in the flies, and it may be related to increased egg laying by *D. sechellia* in *Morinda* fruits [237]. A gene that encodes a phospholipase has its expression increased in the presence of *Morinda* fatty acids [237]. This phospholipase is expressed only in female ovaries [116, 117], which may indicate a direct relationship to oogenesis. Moreover, genes possibly involved in oxidation processes, such as an acyl-CoA ligase, which is also regulated by HR96 [165], and 3-hydroxyacyl-CoA dehydrogenase, have reduced expression [237].

These results indicate that the presence of fatty acids from *Morinda* fruit may render the fly metabolism less oxidative. It is important to remember that these fatty acids may have no direct effect on the activation of nuclear receptors. The fatty acids bind to odorant-binding proteins and activate olfactory receptors that trigger a cell signaling cascade [238]. However, the pathways or transcription factors involved in the described gene expression changes are still unknown.

Fatty acid derivatives, such as prostaglandins, can also regulate the expression of lipid metabolism genes; however, the few studies that exist are preliminary. For example, prostaglandins A_1 and E_1 reduce the gene expression of a retinoic acid-binding protein in cotton bollworm *Helicoverpa zea* cells in culture [239]; however, the mechanism is unknown.

5.4. Cholesterol

The discovery of the ability of the HR96 nuclear receptor to bind to and be regulated by cholesterol could explain the way insects are able to control the expression of genes involved in the metabolism of cholesterol without having the SREBP pathway activated, as it is in mammals [161, 162]. Because insects are unable to synthesize cholesterol *de novo* [151], the presence of this lipid in the diet is an essential and important signal to be detected.

In the fruit fly *D. melanogaster*, the presence of dietary cholesterol differentially regulates the expression of *npc1* and *npc2* [161, 162]. While *npc2d* and *npc1b* are repressed by cholesterol, *npc2e* and *npc2c* are induced [161, 162]. This opposite modulation indicates that these genes have different roles in the intestinal absorption of cholesterol [34, 240]. It is important to note that this transcriptional regulation is dependent on the HR96 nuclear receptor [161, 162]. Another gene regulated by the presence of cholesterol in the diet is the *magro* lipase, which is also essential in midgut lipid homeostasis [161-164]. Other genes involved in lipid metabolism, such as the enzyme acyl-CoA cholesterol acyltransferase, the ABCA1 transporter and the Lp receptor, are induced by the presence of cholesterol in the diet in an HR96-dependent manner [162].

5.5. Carbohydrates

The relationship between the levels of carbohydrates and the regulation of gene expression involved in lipid metabolism has not been well studied in insects.

In the fruit fly *D. melanogaster*, the effect of the presence of carbohydrates in the diet was analyzed using whole-genome microarrays [241]. When the larvae are fed carbohydrates, the expression of some genes are suppressed, including several lipases (including *dlip3*), an acyl-CoA ligase, CPT1 and a FATP. Moreover, expression of other genes, such as FAS, an acetyl-CoA thioesterhydrolase, an acetyl-CoA synthetase, an ATP-citrate lyase and ACC, are increased. These results indicate that a high sugar diet causes changes in the gene expression profile, leading to a reduction in the digestion and absorption of lipids; however, the synthesis of lipids is accelerated. These effects appear to be regulated by a specific transcription factor, *sugarbabe*, which belongs to the zinc finger family [241]. In addition to directly regulating the expression of genes, *sugarbabe* also participates in the production and release of the ILPs [242], being an important factor for controlling metabolism in insects.

These results illustrate that carbohydrates may be important signals for regulating the transcriptional activity of lipid metabolism genes, and this topic needs to be investigated in greater detail.

6. Effect of the Environment on Gene Expression

The environment around the insects will also strongly influence the expression of several metabolic genes, including those related to lipid metabolism. The expression of digestive genes in herbivore insects can be modulated by chemical compounds present in plants.

Moreover, insects need to defend themselves against pathogens and adapt to climate changes. These factors are discussed below.

6.1. Chemical Plant Defenses and Response to Insecticides

Most herbivorous insects are adapted to feed on certain species of plants, a clear phenomenon of co-evolution. Plants produce different toxic compounds, and only a few insects are able to adapt. One example is the Hessian fly *Mayetiola destructor*, which feeds on wheat crops [243]. Interestingly, some strains of wheat are resistant to this insect. The explanation may lie in the ability of the resistant wheat to modulate the expression of a gene required for the fly to feed. The larvae produce a lipase that may be important to the digestive process that is secreted into the saliva [244]. The expression of this gene is induced when the larvae are feeding on susceptible wheat; however, this increase is inhibited if the wheat is resistant to predation [244]. The mechanism by which this occurs is still unknown.

Some leguminous plants produce protease inhibitors such as the Bowman-Birk inhibitor or the soybean trypsin inhibitor [245, 246]. However, these inhibitors also appear to affect lipid metabolism in insects. In the fruit fly *D. melanogaster*, the presence of these two inhibitors in the diet reduced the expression of *scpx* [247]. The mechanism is also unknown, but this result may indicate that the protease inhibitors also affect the metabolism of sterol in the midgut.

Exposure to insecticides also induces a response at the transcriptional level. For example, an insecticide-resistant strain of the mosquito *A. gambiae* has increased expression of genes involved in the synthesis of lipids, including FAS and acetyl-CoA synthetase [248]. Furthermore, these genes were further induced when the mosquitoes were exposed to the insecticide permethrin [248]. An FABP also had its expression increased after treatment with the insecticide [248]. It is unclear how the insecticide causes this effect on gene expression or what the relationship between compound resistance and lipid metabolism is.

6.2. Pathogen Infections

As described previously, insects do not have adaptive immunity and depend only on innate immunity to combat infections by different pathogens [171]. Some studies indicate that lipid metabolism is affected by the response to infection or may even be part of the immune response.

In the red flour beetle *T. castaneum*, the expression of phospholipase A_2 is possibly involved in the immune response, and it is induced via the Toll receptor pathway and the Imd pathway [249].

In the mosquito *A. aegypti*, the expression of the Lp gene and its receptor are induced by infecting the insects with Gram (+) bacteria or fungi [181]. Infection by avian malaria (*Plasmodium gallinaceum*) is also capable of inducing the expression of these genes [181, 250]. The activation of the Toll receptor pathway and the REL1 transcription factor are responsible for this regulation, and Cheon *et al.* were able to identify the binding sites for REL1 in the Lp receptor gene promoter [181]. Interestingly, the inhibition of Lp gene expression by RNAi prevents the development of the parasite *P. gallinaceum* [181, 251].

Although the parasite can use Lp as a nutritional source [252], the absence of the lipoprotein reduces the expression of Vg [251]. In some way Vg interferes with the immune response against *P. gallinaceum* in the mosquito, and the reduction in the amount of Vg in the hemolymph seems to facilitate the immune response against the parasite [251]. Although there are too few studies for any general conclusion to be made, the relationship between infection and the expression of Lp appears to be unique to mosquitoes. The infection of honey bees (*A. mellifera*) with bacteria was not able to induce an increase in the expression of the lipoprotein [253].

Infecting the mosquito *A. gambiae* with *Plasmodium berghei* causes increased expression of apolipophorin-III (ApoLp-III) in the midgut [254]. Moreover, this effect is dependent on the development of the parasite [254]. However, contrary to what was shown for the relationship between *A. aegypti* and *P. gallinaceum*, the inhibition of ApoLp-III expression by RNAi causes a large increase in the infection in mosquitoes [254]. This result indicates that ApoLp-III is related to the immune response in insects. Some other results support this assertion. An oral infection caused by bacteria results in the increased expression of ApoLp-III in the caterpillar of the cabbage semilooper *Trichoplusia ni* [255]. Similarly, the injection of *Escherichia coli* into the caterpillars of the fall webworm *Hyphantria cunea* induces the expression of ApoLp-III [256]. In addition, ApoLp-III has an effect on its own expression in a positive feedback system [256]. The injection of purified ApoLp-III is capable of activating the immune system of the caterpillar, and the co-injection of the lipoprotein with bacteria has a synergistic effect [256]. Interestingly, ApoLp-III is involved in the encapsulation of injected pathogens [256]. In *A. mellifera*, the bacterial infection causes a major inhibition of ApoLp-III expression [253].

Viral infections also affect the expression of lipid metabolism genes. *Drosophila* S2 cells infected with the Flock House RNA virus displayed an increase in the expression of the enzymes CTP:phosphocholine cytidylyltransferase 1 and 2 (CCT1 and CCT2), which are involved in the synthesis of phosphatidylcholine, an acyl-CoA dehydrogenase and *dlip4* [257]. This modulation is essential for viral replication. The infected cells display a greater level of phosphatidylcholine, which is important for the viral replication cycle [257]. Furthermore, inhibiting the expression of the CCTs by RNAi is capable of inhibiting the production of new viruses [257]. However, how the infection is capable of regulating the expression of these genes has not been investigated.

6.3. Temperature and Hydric Stress

The seasons bring changes in weather, including temperature and rainfall variations. Insects must adapt to these changes. The process of diapause is an example of what these animals do to survive harsh climatic conditions [216]. In the mosquito *C. pipiens*, *FAS* expression is induced during diapause, during which the animal seeks to store energy as fat [258]. In this case, temperature does not influence gene expression because diapause is triggered mainly by changes in the photoperiod [258].

However, in the fruit fly *D. melanogaster*, temperature regulates the expression of different lipid metabolism genes. Compared to flies maintained at 15 °C, flies incubated at 26°C have a higher expression of genes involved in fatty acid synthesis, such as acetyl-CoA synthetase and FAD, and in the metabolism of phospholipids, such as sphinganine-1-

phosphate lyase (*sply*), CDP-ethanolamine diglyceride transferase (*Cdpct*), phosphoethanolamine cytidylyltransferase (*Pect*) and phospholipase D (*Pld*) [259]. Moreover, the expression of SREBP is reduced in the flies maintained at 26 °C [259]. However, when the flies are kept at 26 °C and then transferred to an environment of 15 °C, the expression profile is altered, and the expression levels of *sply*, *Cdpct*, *Pld* and acetyl-CoA synthetase are increased [259]. This change seems to be involved in an adaptation process that alters cell membrane fluidity in response to temperature. It is interesting to note that FAD expression was also induced during pre-diapause in the embryos of the ground cricket *Allonemobius socius* [260]. The same occurs in the onion maggot *Delia antiqua* pupae acclimated to cold or during overwintering diapause [261].

Another example comes from an interesting model used to study the effects of humidity, the Antarctic midge *Belgium antarctica*. The only insect to inhabit the South Pole suffers from large variations in the amount of liquid water available and spends most of the year frozen [262]. Therefore, it must be well adapted to changes in humidity to survive. In one study [263], when these insects were subjected to dehydration, the gene expression of two FADs and a phospholipase A were increased. These results may support the idea that changes in gene expression aimed at modifying the properties of the cell membranes allow it to function at lower temperatures or at restrictive humidity levels.

Finally, in *D. melanogaster*, the expression of an ACBP present in neurons is regulated by changes in temperature and humidity [264]. Because ACBP is involved in regulating feeding activity [264, 265], it may represent an important link between environmental conditions and the search for food.

Conclusion

Based on the information discussed above, it is clear that most of what is known about the regulation of gene expression in relation to lipid metabolism is restricted to the fruit fly *D. melanogaster*. The advantages of using *D. melanogaster* as a model are obvious; however, insects have many different lifestyles, and the results obtained for this animal may not be representative of others. Therefore, the study of other insects is essential to better understand fat metabolism. In this context, a new technique published by Peng and colleagues [266] can facilitate the genetic manipulation of other models and help to obtain new results. The authors described a simple method for introducing extrachromosomal DNA into the embryos of the mosquito *A. aegypti*. The developing oocytes were able to endocytose genetic vectors injected into females on a vitellogenic stage and were genetically manipulated by these vectors. It is possible that this technique can be applied to additional insect species. If so, genetic manipulation similar to that available for *D. melanogaster* can be used in other insects. Before long it will be possible to determine if the observations described for the fruit fly are the rule or the exception among insects.

Other points discussed in this chapter that are especially interesting and should be targets of investigation in the coming years:

1. Insects lack genes that are orthologous to the mammalian PPARs [137]. The HNF4 nuclear receptor may be a functional analog of PPARα, thus controlling the oxidation

of fatty acids [168]. Some results appear to indicate that the E75 nuclear receptor is the analog for PPARγ [133, 135, 136, 138], but the results are not conclusive. Further studies on the relationship between E75 and lipid metabolism are needed, and the results may be important for correlating the similarities and differences between insects and mammals. Moreover, rosiglitazone seems to affect lipid metabolism in the Chinese bee *A. cerana* [235]. Identifying the target of this drug in insects would be extremely valuable.

2. SREBP is clearly involved in the regulation of lipid synthesis [152-156, 159, 160, 267]. However, this transcription factor may also participate in the digestive process. A non-characterized lipase expressed only in the intestine [116] is highly regulated by SREBP [154-156]. This may indicate a relationship between SREBP and lipid digestion; however, this topic needs to be investigated. The results obtained may even reveal the functions of SREBP in other animals, including mammals. This lipase is also regulated by HNF4 [168]. What is the function of this nuclear receptor in digestion? Additionally, SCAP is not essential for the activation of SREBP [156, 160], and insects do not have an ortholog to INSIG [153, 267]. How the levels of PE can regulate and trigger the activation of SREBP is a topic that is still far from being fully understood.

3. The HR96 nuclear receptor seems to be the main regulator of lipid homeostasis in the gut of *D. melanogaster* [161-164]. However, the results are restricted to the fruit fly. The analysis of the functions of this protein in other insects is essential and may reveal a new target to combat pest insects and vectors.

4. With respect to the hemolymph sugar homeostasis, AKH has the same function as glucagon [193]. However, its functions as a regulator of gene expression have not been investigated. The activity of the transcription factor CREB can be regulated by AKH [196], but this is the only information known. There is plenty of opportunity for research in this area.

5. Serotonin and β-glucosyl-O-tyrosine are newly discovered regulators of gene expression [214, 222]. Many questions can be asked and much remains to be studied about these regulators. For example, what other genes are regulated by these factors? In which insects do they also have this function? And, perhaps most importantly, what other factors present in the hemolymph regulate lipid metabolism through gene expression and are still unknown?

Acknowledgments

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