# Regulation of Carbohydrate Energy Metabolism in *Drosophila melanogaster*

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**ABSTRACT** Carbohydrate metabolism is essential for cellular energy balance as well as for the biosynthesis of new cellular building blocks. As animal nutrient intake displays temporal fluctuations and each cell type within the animal possesses specific metabolic needs, elaborate regulatory systems are needed to coordinate carbohydrate metabolism in time and space. Carbohydrate metabolism is regulated locally through gene regulatory networks and signaling pathways, which receive inputs from nutrient sensors as well as other pathways, such as developmental signals. Superimposed on cell-intrinsic control, hormonal signaling mediates intertissue information to maintain organismal homeostasis. Misregulation of carbohydrate metabolism is causative for many human diseases, such as diabetes and cancer. Recent work in *Drosophila melanogaster* has uncovered new regulators of carbohydrate metabolism and introduced novel physiological roles for previously known pathways. Moreover, genetically tractable *Drosophila* models to study carbohydrate metabolism-related human diseases have provided new insight into the mechanisms of pathogenesis. Due to the high degree of conservation of relevant regulatory pathways, as well as vast possibilities for the analysis of gene–nutrient interactions and tissue-specific gene function, *Drosophila* is emerging as an important model system for research on carbohydrate metabolism.

**KEYWORDS** metabolism; insulin; glucose; gene regulation; nutrient sensing

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## Preface

CARBOHYDRATE metabolism is essential for all life, having profound implications for growth, reproduction, and organismal maintenance. As multicellular animals eat periodically and experience times of starvation, carbohydrate intake can undergo extreme fluctuations. Moreover, different cell types and developmental stages have their own metabolic requirements, which, together with the changing nutrient intake, pose the need for constant regulation of carbohydrate metabolism. Therefore, complex regulatory systems have evolved to integrate these functions. In recent years, *Drosophila melanogaster* (hereafter *Drosophila*) has been increasingly utilized to study the regulation of carbohydrate metabolism and new research fields have emerged around this topic. New insight has been gained into the regulatory pathways that respond to changes in carbohydrate intake and regulate metabolism to maintain homeostasis. These include gene regulatory networks and signaling pathways, which act locally in metabolically active peripheral tissues, as well as hormonal signals, which maintain organismal homeostasis through interorgan communication. Interesting cross-talk between carbohydrate metabolism and other physiological processes, such as circadian regulation and developmental transitions, have also been uncovered. Moreover, powerful *Drosophila* models to study carbohydrate metabolism-related human diseases have been established. The success of *Drosophila* research on providing new insights into carbohydrate metabolism has its foundation in the strengths of this model system. These include a high degree of conservation of the pathways controlling carbohydrate metabolism, the ease of using simple dietary schemes, which allow studies on interactions between genes and individual nutrients, as well as a powerful genetic toolkit, which is particularly advantageous in studies that address hormonal signaling between tissues. Here, we have highlighted the recent advances in *Drosophila* research on carbohydrate energy metabolism. For the sake of focus, we have excluded or only touched minimally upon some related themes, such as gustatory responses, the regulation of feeding behavior, lipid metabolism, and growth control.

## Part I

### *Homeostatic control of carbohydrate metabolism through intracellular nutrient sensing*

***Carbohydrate-responsive gene regulation and signaling:*** Fluctuations in nutrient intake pose constant requirements for homeostatic control of carbohydrate metabolism. Such regulation requires that cells are able to detect the levels of key carbohydrate-derived metabolites and consequently adjust the activity of regulatory pathways. An important layer of local regulation of carbohydrate homeostasis is mediated through so-called intracellular sugar sensing by a heterodimer of conserved basic helix-loop-helix transcription factors Mondo and Max-like protein X (Mlx, Bigmax) (Havula and Hietakangas 2012). In *Drosophila* larvae, Mondo-Mlx control the majority of the strongly sugar-responsive genes (Mattila *et al.* 2015).

Vertebrates have two Mondo paralogs, called MondoA (MLXIP) and ChREBP (Carbohydrate Response Element-Binding Protein, also known as MondoB, MLXIPL), both of which dimerize with Mlx (Havula and Hietakangas 2012). Studies in mammals have shown that the nuclear translocation and transcriptional activity of ChREBP/MondoA-Mlx are induced by glucose. The N-terminus of ChREBP and MondoA

contains a so-called Glucose-Sensing-Module (GSM), which includes the low glucose inhibitory domain (LID) and the Glucose-Response Activation Conserved Element (GRACE), both of which are required for glucose sensing (Havula and Hietakangas 2012). It has been proposed that the GSM of the Mondo proteins contains a conserved motif, which resembles the glucose-6-phosphate (G-6-P)-binding site of metabolic enzymes. The binding of G-6-P to the GSM would prevent the intramolecular inhibition of GRACE imposed by LID (McFerrin and Atchley 2012). However, direct structural evidence about the interaction of G-6-P (and possibly other phosphorylated hexoses) with MondoA/ChREBP is still missing. The intracellular glucose sensing appears to be highly conserved. For example, the domain structure, glucose responsiveness, and the heterodimerization with Mlx are conserved in *Drosophila* Mondo (Li *et al.* 2006; Havula and Hietakangas 2012). Moreover, *Drosophila* Mondo contains a conserved LxxLL nuclear receptor box signature, which likely allows Mondo to interact with nuclear receptors (McFerrin and Atchley 2012). In mammals, the activity of ChREBP is further regulated through post-translational modifications, such as phosphorylation and *O*-GlcNAc (N-acetylglucosamine) modification, but the role of these modifications in *Drosophila* remains unclear [reviewed in Havula and Hietakangas (2012)].

The physiological importance of intracellular sugar sensing is reflected by the fact that *Drosophila* larvae deficient of Mondo-Mlx display lethality on any diet containing high levels of sucrose, glucose, or fructose (Havula *et al.* 2013). The sugar intolerance of *mlx* mutants manifests in a physiologically relevant range of dietary sugars, as *mlx* mutants are unable to develop on red grapes, which are naturally rich in sugars. Interestingly, mice lacking ChREBP also display impaired survival on a diet rich in simple carbohydrates (Iizuka *et al.* 2004). In *Drosophila* larvae, Mondo and Mlx display highest expression levels in the fat body, intestine, and Malpighian tubules (Havula *et al.* 2013). Moreover, both genes are upregulated upon sugar feeding (Zinke *et al.* 2002; Mattila *et al.* 2015). The sugar intolerance phenotype of *mlx* mutants can be rescued by fat body-specific transgenic expression. In addition to sugar tolerance, Mondo-Mlx also affects feeding behavior; knockdown of Mondo in the fat body decreases (Sassu *et al.* 2012), while neuronal knockdown increases feeding (Docherty *et al.* 2015). However, the underlying mechanisms of how Mondo-Mlx controls *Drosophila* feeding behavior remain unknown.

Mondo-Mlx regulates its target genes by binding to the so-called carbohydrate response element (ChoRE), which is composed of two imperfect E-boxes divided by five bases and is well-conserved in *Drosophila* (Shih *et al.* 1995; Jeong *et al.* 2011; Bartok *et al.* 2015; Mattila *et al.* 2015). In addition to direct regulation of metabolic target genes, Mondo-Mlx controls the expression of other transcription factors, namely Cabut and Sugarbabe (Bartok *et al.* 2015; Mattila *et al.* 2015) (Figure 1). Cabut is an ortholog of mammalian Krüppel-like factors 10 and 11 and is a transcriptional repressor with many physiological roles, including growth control as well as developmental, metabolic, and circadian regulation (Rodriguez 2011; Bartok *et al.* 2015; Ruiz-Romero *et al.* 2015). Mondo-Mlx binds directly to the promoter of the *cabut* gene and *cabut* expression is strongly downregulated in *mlx* mutants (Havula *et al.* 2013; Bartok *et al.* 2015). While Cabut is essential for development, partial knockdown of Cabut allows larval development on a low-sugar diet, although the larvae become sugar intolerant. Sugarbabe is also a direct target of Mondo-Mlx (Mattila *et al.* 2015). It is a homolog of mammalian Gli-similar transcription factors and it has been long known as one of the most strongly sugar-responsive genes in *Drosophila* (Zinke *et al.* 2002). In addition to transcriptional control, Sugarbabe is regulated by a nutrient-dependent miRNA, miR-14 (Varghese *et al.* 2010). Sugarbabe-deficient larvae display some intolerance toward a high-sugar diet, albeit to a lesser extent than *mlx* mutants (Mattila *et al.* 2015).

In addition to transcription factors, Mondo-Mlx controls other types of regulatory proteins, including protein kinase SIK3 (Salt-inducible kinase 3). SIK3 belongs to the family of AMP-activated protein kinase (AMPK)-related kinases and has been recently implicated in metabolic regulation downstream of insulin and glucagon signaling (Wang *et al.* 2011; Choi *et al.* 2015; Hirabayashi and Cagan 2015). Thus, SIK3 integrates signals from intracellular sugar sensing as well as hormonal control. *SIK3*-null mutants were originally identified as larval lethal, but recent data shows that on a low-sugar diet some pupae emerge, indicating that SIK3 loss-of-function leads to prominent sugar intolerance (Wang *et al.* 2011; Teesalu *et al.* 2017). Intracellular glucose sensing by Mondo-Mlx is also coupled with the systemic control of metabolism. Namely, sugar-inducible transforming growth factor β (TGF-β)/Activin ligand Dawdle (Daw) is a direct target of Mondo-Mlx. Daw signals through the Babo receptor and transcription factor SMAD2/Smox and is expressed in several peripheral tissues of larvae, displaying highest expression levels in the fat body (Mattila *et al.* 2015; Upadhyay *et al.* 2017). Similar to other regulatory genes that act downstream of Mondo-Mlx, *dawdle* is essential for sugar tolerance (Ghosh and O'Connor 2014; Mattila *et al.* 2015). Interestingly, Daw and Sugarbabe belong to a common regulatory pathway, since depletion of Daw and its downstream effector SMAD2/Smox prevents full activation of *sugarbabe* upon sugar feeding (Mattila *et al.* 2015). How Daw-dependent Activin signaling cooperates with Mondo-Mlx to control Sugarbabe expression is an interesting question for future studies. A more detailed description of TGF-β/Activin signaling will be presented later in this review.

Along with Mondo-Mlx and its downstream targets, other regulatory genes have been shown to be essential for sugar tolerance. One of them is a conserved nuclear receptor HNF4 (Hepatocyte nuclear factor 4), which can be activated in response to long-chain fatty acids (Palanker *et al.* 2009) (Figure 1). Mutants of *HNF4* display normal larval development, but they fail to eclose on standard laboratory food. However,

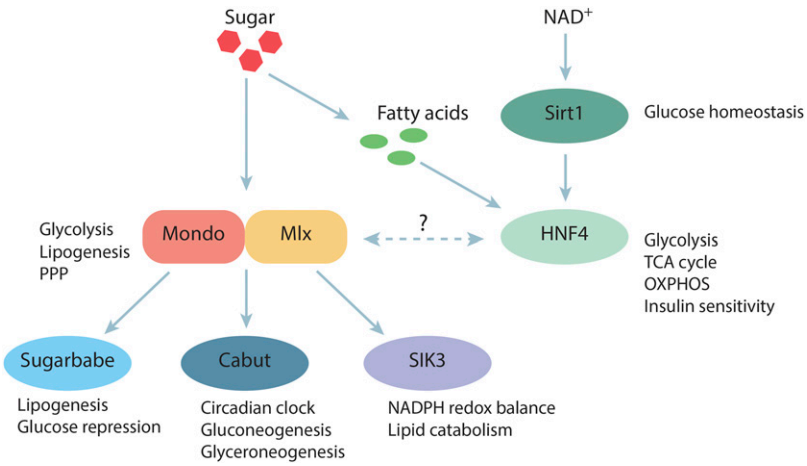


**Figure 1** Intracellular sugar-responsive gene regulatory network. The main regulators of sugar-responsive gene expression are the heterodimeric bHLH-Zip transcription factors Mondo and Mlx. Mondo-Mlx has a direct role in regulating gene expression programs, which are essential in glucose and fatty acid metabolism. In addition, Mondo-Mlx activates the transcription of a second tier of transcriptional regulators, including Sugarbabe and Cabut as well as other regulatory proteins such as protein kinase SIK3. In parallel, glucose regulates indirectly, through the generation of fatty acids and $NAD^+$, transcription factor HNF4 and deacetylase Sirt1, respectively. The transcriptome regulated by Mondo-Mlx and HNF4 are partially overlapping. However, how these factors interact is yet unknown. bHLH, basic helix-loop-helix; FA, fatty acid; HNF4, hepatocyte nuclear factor 4; Mlx, Max-like protein X; $NAD^+$, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; OXPHOS, oxidative phosphorylation; PPP, pentose phosphate pathway; SIK3, salt-inducible kinase 3; Sirt1, Sirtuin 1; TCA, tricarboxylic acid cycle.

reduction of dietary sugar rescues the development of the majority of mutants into adulthood. Thus, the sugar intolerance of *HNF4* mutants manifests at a later stage than that of Mondo-Mlx-deficient animals (Barry and Thummel 2016). Similar to *mlx* mutants, *HNF4* mutants also display highly elevated circulating glucose and trehalose levels, and the circulating glucose responds strongly to the sugar content of the diet. HNF4 maintains glucose homeostasis both in the fat body as well as in the insulin-producing cells (IPCs). Considering the cooperative function between ChREBP and HNF4 in mammals (Adamson *et al.* 2006), and the conserved nuclear receptor box in *Drosophila* Mondo (McFerrin and Atchley 2012), studies examining the possible interplay between HNF4 and Mondo-Mlx in *Drosophila* are warranted.

Another nutrient sensor critical for carbohydrate homeostasis is the $NAD^+$-dependent deacetylase Sirtuin 1 (Sirt1, Sir2). Loss of *Sirt1* in the adult fat body leads to hyperglycemia, obesity, and insulin resistance, which are all age-progressive (Banerjee *et al.* 2012; Palu and Thummel 2016). Through the control of systemic free fatty acid levels and insulin signaling, the fat body-specific activity of Sirt1 is further reflected in other tissues, for example by affecting mitochondrial function in the muscle (Banerjee *et al.* 2013). Similar to adults, fat body Sirt1 negatively regulates triglyceride accumulation in larvae (Reis *et al.* 2010). Interestingly, *Sirt1* mutants share many phenotypic features with the *HNF4* mutants and display deregulation of an overlapping set of genes (Palu and Thummel 2016). Moreover, *Sirt1* mutants display an age progressive reduction in HNF4 expression along with increased HNF4 acetylation, suggesting that Sirt1 maintains HNF4 stability through deacetylation (Palu and Thummel 2016). Since Sirt1 activity depends on the availability of the cofactor Nicotinamide adenine dinucleotide ($NAD^+$), which in turn depends on carbohydrate metabolism, it is conceivable that the glucose-dependent cellular metabolic status is reflected in the regulation of HNF4 through Sirt1 (Figure 1).

***O-GlcNAcylation, a link between carbohydrate metabolism and signaling:*** In addition to direct glucose sensing through Mondo-Mlx, cells have evolved additional mechanisms to convey information about intracellular metabolic status to the regulation of cell physiology. One such mechanism is the post-translational modification of proteins through *O*-linked GlcNAcylation, where a nitrogen-containing nucleotide sugar GlcNAc is reversibly added to serine and/or threonine residues, altering target protein activity, stability, specificity, or localization. Protein *O*-GlcNAcylation has been shown to target several key regulators important for cellular energetics and growth. These include, for example, c-Myc, p53, calcium/calmodulin-dependent kinase IV (CamKIV), casein kinase 2 (CK2), AMPK, the cAMP response element-binding protein (CREB), Forkhead box subgroup O (FOXO1), and AKT [reviewed by Hardivillé and Hart (2014)]. Accordingly, a wealth of data indicate that deregulated *O*-linked GlcNAcylation is associated with metabolic disorders such as insulin resistance and cancer [reviewed by Bond and Hanover (2015); Ferrer *et al.* (2016)].

The substrate for *O*-linked GlcNAcylation, UDP-GlcNAc, is the end product of the hexosamine biosynthesis pathway (HBP), which integrates inputs from glucose (fructose-6-phosphate), amino acid (glutamine), fatty acid [acetyl-coenzyme A (CoA)], and nucleotide/energy (UDP) metabolism (Figure 2). The regulation of the HBP flux is not fully understood, but fructose-6-phosphate availability and negative feedback inhibition by UDP-GlcNAc likely play major roles (McKnight *et al.* 1992). The members of the HBP are well-conserved in *Drosophila*, with two rate-limiting enzymes, glucose-fructose amidotransferases (Gfat1/2) and the orthologs of glucosamine-phosphate N-acetyltransferase (CG1969), phosphoacetylglucosamine mutase (nst) and UDP-N-acetylglucosamine diphosphorylase (mmy) (Figure 2). The activity of the HBP is essential for fly development, since mutants for *mmy* and *nst* are lethal with various developmental defects. Notably, UDP-GlcNAc is also a substrate for the N-linked protein glycosylation necessary for

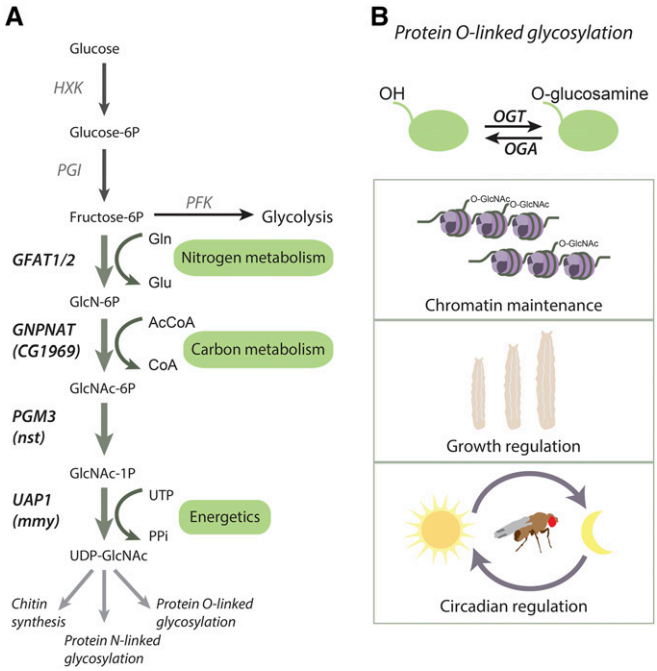


**Figure 2** The role of the HBP and protein *O*-linked GlcNAc conjugation in the regulation of *Drosophila* physiology. Schematic presentation of the HBP (A) and the known processes regulated by protein *O*-linked GlcNAc conjugation (B). (A) HBP competes for F-6-P with PFK, the rate-limiting enzyme of glycolysis. In the first and rate-limiting step of HBP, GFAT conjugates an amine group from glutamine to the F-6-P yielding GlcN-6-P and glutamate. In the following step, GNPNAT conjugates the acetyl group from acetyl-CoA to yield GlcNAc-6-P, which is then isomerized to GlcNAc-1-P by PGM3. Finally, UDP is conjugated to the GlcNAc-1-P by UAP1 to yield UDP-GlcNAc, which is a substrate for macromolecule glycosylation and chitin biosynthesis. Hence, HBP integrates inputs from glucose (G-6-P), amino acid (glutamine), fatty acid (acetyl-CoA) and energy (UDP) metabolism, making it a sensor of cellular nutrient and energy metabolism. N-linked glycosylation, covalent attachment of an oligosaccharide to asparagine residues, is a mechanism of protein maturation and trafficking between cell compartments. The UDP-GlcNAc polymer, also known as chitin, is the key component of *Drosophila* exoskeleton. (B) *O*-linked GlcNAcylation is a transient protein post-translational modification mechanism, which targets threonine and serine residues. *O*-linked GlcNAcylation is mediated by the activities of OGT and OGA to conjugate and deconjugate glucosamine, respectively. *O*-linked GlcNAcylation can compete, enhance, or attenuate protein phosphorylation, making it an important mechanism to regulate protein activity. In *Drosophila*, the activity of OGT is known to be involved in maintenance of chromatin state, in regulating larval growth through insulin signaling, and in regulating the maintenance of circadian rhythm. F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; GFAT1/2, glutamine fructose-6-phosphate amidotransferase 1/2; GlcN-6-P, glucosamine-6-phosphate; GlcNAc, N-acetylglucosamine; GlcNAc-1-P, N-acetyl-D-glucosamine-1-phosphate; GlcNAc-6-P, N-acetyl-D-glucosamine-6-phosphate; GNPNAT, glucosamine-phosphate N-acetyltransferase; HBP, hexosamine biosynthesis pathway; HXK, hexokinase; OGA, *O*-GlcNAcase; OGT, *O*-GlcNAc transferase; PFK, phosphofructokinase; PGI, phosphoglucose isomerase; PGM3, phosphoglucomutase 3; UAP1, UDP-N-acetylglucosamine pyrophosphorylase; UTP, uridine triphosphate.

appropriate protein folding, maturation, and membrane targeting, as well as chitin biosynthesis necessary for the production of apical extracellular matrices (Schimmelpfeng *et al.* 2006; Tonning *et al.* 2006). Therefore, the phenotypes of HBP pathway mutants may reflect defects in these functions.

The dynamic regulation of *O*-linked GlcNAc conjugation/deconjugation is mediated by the activity of a conserved pair of enzymes, *O*-GlcNAc transferase (Ogt, encoded by the *super sex combs* gene) and *O*-GlcNAcase (Oga), which catalyze the addition and removal of *O*-GlcNAc, respectively. In *Drosophila*, Ogt was first identified as belonging to the Polycomb group genes (PcG) due to the characteristic homeotic transformations of the mutant animal (Ingham 1984). It was later shown that Ogt can modify other PcG proteins located at the Polycomb Repressor Element (PRE) loci, thereby affecting transcriptional repression during embryonic development (Gambetta *et al.* 2009; Sinclair *et al.* 2009). The role of Ogt as a transcriptional regulator was further extended by the finding that *O*-GlcNAc-modified proteins can bind to sites throughout the genome, and not only at loci containing PREs (Liu *et al.* 2017). However, it is not known how these modifications correlate with gene expression and which genomic loci are affected by changes in *O*-GlcNAcylation. Interestingly, in a recent study by Selvan *et al.* (2017), a catalytically inactive OGA mutant was used as a substrate trap to enrich *O*-GlcNAcylated proteins from *Drosophila* embryos. By this approach, > 2000 proteins were identified as substrates for *O*-GlcNAcylation, suggesting it to be a major mechanism of protein modification during development (Selvan *et al.* 2017).

Modulation of *O*-linked GlcNAc conjugation by inhibiting Ogt and Oga during larval stages attenuates or enhances larval growth, respectively (Park *et al.* 2011). In addition, *Ogt* knockdown during larval development leads to an increase in autophagy (Park *et al.* 2015). These results, together with the findings that AKT and FOXO are regulated through *O*-GlcNAcylation, have led to the proposal that nutritional status and Ogt activity contribute to the regulation of the insulin signaling pathway during larval growth (Park *et al.* 2011, 2015). However, the exact mechanism of this regulation remains to be elucidated. One possibility is that *O*-linked GlcNAcylation affects growth cell nonautonomously, through the regulation of insulin-like peptide (dILP) synthesis and release from the IPCs. Indeed, targeted RNA interference (RNAi) knockdown of *Ogt* and *Oga* in the IPCs has been shown to attenuate and elevate *dilp* expression, respectively (Sekine *et al.* 2010). Interestingly, mice lacking *Ogt* in pancreatic β-cells develop diabetes through ER stress-induced β-cell apoptosis (Alejandro *et al.* 2015). These findings suggest that the regulation of insulin synthesis through *O*-linked GlcNAc cycling is conserved through evolution.

In conclusion, *O*-linked GlcNAc modification is emerging as an important regulator of *Drosophila* growth, metabolism, and physiology. Furthermore, it is tempting to speculate that the role of the *O*-GlcNAcylation is emphasized in cells involved in nutrient sensing, as suggested by the data on *O*-GlcNAcylation in the IPCs. Further studies are needed to uncover the role of this post-translational modification on the major peripheral nutrient-sensing tissues, the fat body and the intestine.

***Regulation of glycolysis and lipid metabolism upon sugar feeding:*** Activation of glycolysis is critical for the elimination of excess glucose entering the circulating hemolymph and sugar feeding strongly activates the expression of genes encoding glycolytic enzymes (Mattila *et al.* 2015) (Figure 3). A key mediator of glycolytic gene activation is Mondo-Mlx, as sugar feeding fails to activate the expression of glycolytic genes in *mlx* mutant larvae. HNF4, which also controls glucose homeostasis upon high-sugar feeding, promotes the expression of the Glucokinase homolog *Hexokinase C* (Barry and Thummel 2016). Genetic inhibition of glycolytic gene activation prevents the clearance of circulating glucose and reduces survival on a high-sugar diet, possibly reflecting the toxicity of high circulating free glucose (Havula *et al.* 2013; Garrido *et al.* 2015).

Downstream of the glycolytic pathway, pyruvate needs to be transferred into mitochondria to be catabolized further in the tricarboxylic acid (TCA) cycle. Sugar feeding modestly activates the expression of the *Drosophila* mitochondrial pyruvate carrier (Mpc1) (Mattila *et al.* 2015). Inhibiting mitochondrial transport of pyruvate in *Mpc1* mutants causes increased levels of glycolytic intermediates and high circulating glucose and trehalose. *Mpc1* mutants also survive poorly on a carbohydrate-only diet (Bricker *et al.* 2012). HNF4 promotes the expression of genes encoding components of the TCA cycle and oxidative phosphorylation (OXPHOS) pathway. Strikingly, nearly all transcripts of the mitochondrial genome are significantly downregulated in *HNF4* mutants and chromatin immunoprecipitation has revealed specific HNF4 enrichment in the mitochondrial DNA control region, which regulates the transcription of both mtDNA strands (Barry and Thummel 2016). Impaired OXPHOS leads to poor survival on a high-sugar diet, as evidenced by the mutant phenotype of the *technical knockout* (*tko*) gene (Kemppainen *et al.* 2016). Consistent with the gene expression changes, *HNF4* mutants show elevated levels of G-6-P and dihydroxyacetone phosphate (Barry and Thummel 2016). Moreover, intermediates of the polyol pathway, namely sorbitol and fructose, are elevated in *HNF4* mutants. This pathway is activated when normal homeostatic clearance of circulating glucose is impaired, such as in the case of untreated diabetes (Luo *et al.* 2016). Interestingly, elevated levels of sorbitol are also present in the *mlx* mutants, underlining the similarities of the metabolic phenotypes of *HNF4* and *mlx* mutants (Teesalu *et al.* 2017). It will be interesting to learn whether the oxidative and osmotic stress caused by sorbitol synthesis and accumulation contributes to the sugar intolerance phenotype.

In addition to activating gene expression that promotes glucose catabolism, the counteracting flux of carbon from the TCA cycle toward gluco- and glyceroneogenesis needs to be inhibited. To this end, sugar feeding downregulates the expression of both cytoplasmic and mitochondrial isoforms of phosphoenolpyruvate carboxykinase (PEPCK) in adults (Bartok *et al.* 2015). The Mondo-Mlx target Cabut is upregulated upon sugar feeding and it directly binds to the promoter of the *Pepck* gene, repressing its activity.

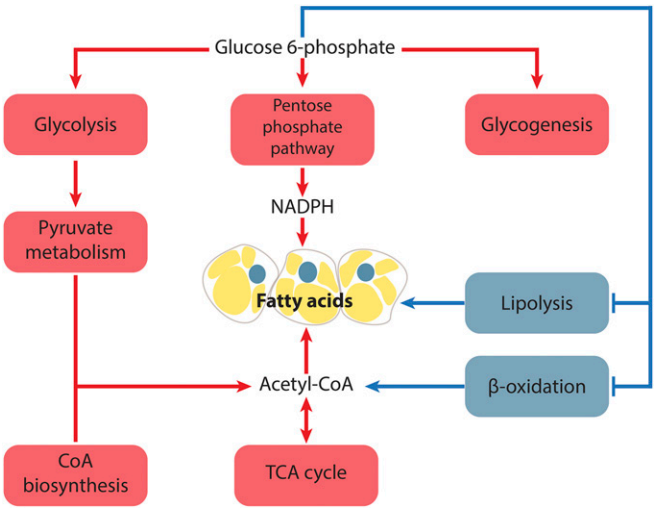


**Figure 3** The *de novo* synthesis of fatty acids and glycogenesis is coordinated in response to dietary sugars. The increase in cellular G-6-P levels leads to the orchestrated regulation of several metabolic processes important in the synthesis of fatty acids and glycogen and, as a result, clearance of intracellular sugars. The majority of G-6-P is channeled through glycolysis, resulting in elevated pyruvate and the production of acetyl-CoA. The process is coordinated with increased levels of CoA biosynthesis. Acetyl-CoA is utilized by the TCA cycle to produce intermediates of amino acid metabolism, ATP, NADH, and citrate. Citrate is further channeled to the fatty acid biosynthesis. The process of fatty acid synthesis is accompanied with the activity of the pentose phosphate pathway yielding the necessary reductive power in the form of NADPH. Parallel to the fatty acid synthesis, elevated levels of G-6-P shut down the process of lipid catabolism through lipolysis and generation of acetyl-CoA through β-oxidation. CoA, coenzyme A; G-6-P, glucose-6-phosphate; NADPH, nicotinamide adenine dinucleotide phosphate; TCA, tricarboxylic acid cycle.

Concomitantly, sugar feeding strongly activates the expression of lipogenic enzymes: ATP citrate lyase, acetyl-CoA carboxylase (ACC), and Fatty acid synthase 1 (FASN1), which convert citrate derived from the TCA cycle into fatty acids (Zinke *et al.* 2002; Sassu *et al.* 2012; Musselman *et al.* 2013; Mattila *et al.* 2015) (Figure 3). This response also depends on Mondo-Mlx, which directly binds to the promoters of at least FASN1 and ACC (Mattila *et al.* 2015). This highlights the evolutionary conservation of intracellular sugar sensing as *FAS* and *ACC* are well-established direct targets of mammalian ChREBP (Ishii *et al.* 2004). In *Drosophila*, lipogenic gene expression is also positively regulated by Sugarbabe, which constitutes a positive feed-forward loop to drive lipogenic gene expression during sustained sugar feeding (Mattila *et al.* 2015). Impaired fatty acid synthesis results in reduced survival on a high-sugar diet (Musselman *et al.* 2013; Garrido *et al.* 2015). Genetic inhibition of lipogenesis in mutants with defective *FASN1* and *FASN2* leads to developmental lethality, which can be partially rescued by dietary lipids. Under these lipid-rescued conditions, addition of sugar into the diet of *FASN* mutants causes lethality and an accumulation of advanced glycation end products (AGEs) (Garrido *et al.* 2015). The harmful effects of sugar feeding can be rescued by the overexpression of glyoxalase-1, which counteracts the toxicity of methyglyoxal, suggesting a causal link between sugar-induced toxicity and

AGEs in this setting. Notably, the role of fatty acid synthesis on sugar tolerance is likely to depend on the other diet components, as on a yeast-based diet addition of sugar can give a growth advantage to larvae with reduced *FASN1* expression (Havula *et al.* 2013). High lipogenesis in response to high dietary sugar poses an elevated need for CoA, which is a cosubstrate for FAS. Sugar feeding leads to strong activation of genes involved in CoA biosynthesis to compensate for the increased need for CoA (Palanker Musselman *et al.* 2016). On the other hand, genes encoding lipid catabolic enzymes, such as triacylglycerol lipases and acyl-CoA dehydrogenases, are downregulated by dietary sugar (Zinke *et al.* 2002; Mattila *et al.* 2015). Perilipin expression is also increased under these conditions, possibly suppressing basal levels of lipolysis (Beller *et al.* 2010; Mattila *et al.* 2015). In sum, high-sugar feeding promotes lipid biosynthesis and inhibits lipid catabolism to channel excess carbon derived from sugars into triacylglycerols (Figure 3).

Fatty acid biosynthesis requires the reductive power of NADPH. A key mechanism to reduce $NADP^+$ into NADPH is via the activity of the oxidative branch of the pentose phosphate pathway (PPP). Sugar feeding strongly activates the expression of genes encoding PPP components, including the rate-limiting enzyme glucose-6-phosphate dehydrogenase [G-6-PD, encoded by the *Zwischenferment* (*Zw*) gene in *Drosophila*] (Zinke *et al.* 2002; Mattila *et al.* 2015). PPP gene expression is fully dependent on Mondo-Mlx (Mattila *et al.* 2015). In fact, the PPP constitutes one of the most highly enriched pathways among Mondo-Mlx targets. Moreover, HNF4 contributes to the expression of a subset of PPP genes (Barry and Thummel 2016). The PPP is also post-translationally activated through phosphorylation of G-6-PD by protein kinase SIK3 (Teesalu *et al.* 2017). Impaired activation of PPP in *SIK3* and *mlx* mutants leads to elevated oxidative stress, which contributes to the sugar intolerance.

An important aspect of sugar-induced metabolism is the simultaneous regulation and coordination of multiple metabolic pathways (Figure 3). This encompasses the activation of glycolysis, lipogenesis, PPP, CoA synthesis, and lipid desaturation, along with inhibition of the counteracting catabolic routes. It is worth noting that sugar feeding also controls other metabolic pathways including the synthesis of nonessential amino acids serine and glutamine (Mattila *et al.* 2015). While the role of these pathways in sugar homeostasis is unclear, genetic inhibition of their activity is detrimental for survival and growth on a high-sugar diet, implying their physiological importance (Mattila *et al.* 2015).

***Regulation of trehalose metabolism:*** Trehalose is the most abundant circulating carbohydrate in insects. It is a disaccharide composed of two α-glucose molecules linked in a 1,1-glycosidic bond. Due to its nonreductive nature, it is nontoxic and therefore tolerated at high circulating levels (~2000 mg/dl in third-instar larvae) (Ugrankar *et al.* 2015). This is in striking contrast with circulating glucose levels, which are typically maintained within a range of 5–30 mg/dl in larvae (Ugrankar *et al.* 2015). Interestingly, adult haemolymph contains 10–20-fold more glucose, suggesting a profound difference in carbohydrate metabolism and glucose tolerance between the life cycle stages (Tennessen *et al.* 2014b) (for discussion about differences in glucose sensing between larval and adult stages see *Regulation of dILP expression and secretion by carbohydrates*). The availability of high circulating trehalose has been considered critical to provide sufficient energy for insect flight muscle (Becker *et al.* 1996). Moreover, trehalose provides the energy needed for brain function. The *Drosophila* nervous system is surrounded by layers of glial cells, which maintain the blood-brain barrier (BBB). These BBB glial cells take up trehalose and metabolize it through the glycolytic pathway to secrete lactate and alanine to fuel neurons (Volkenhoff *et al.* 2015). Failures in BBB glial trehalose metabolism will lead to neuronal cell death. Trehalose was also suggested to contribute to the maintenance of neuroepithelial stem cells of the optic lobe (Chen *et al.* 2014), but this has been questioned in a recent study (Yasugi *et al.* 2017). In addition to its function as an energy source, trehalose has a role in protecting against environmental stresses, such as cold temperature and desiccation stress (Koštál *et al.* 2011; Thorat *et al.* 2016; Yoshida *et al.* 2016).

Trehalose is synthesized in the fat body from G-6-P and UDP-glucose by two enzymatic activities, Trehalose-6-phosphate synthase and Trehalose-6-phosphate phosphatase. These enzymatic activities are provided by the two catalytic domains of *Drosophila* Tps1 protein, both of which are essential for trehalose biosynthesis (Yoshida *et al.* 2016). Loss of *Tps1* leads to trehalose-deficient animals (Matsuda *et al.* 2015). Surprisingly, trehalose is dispensable for larval development, as *Tps1*-deficient animals display lethality only at the late pupal stage. However, the trehalose-deficient larvae are sensitive to nutrient limitation, displaying rapid lethality upon starvation. The *Drosophila* genome encodes two putative trehalose transporters (Tret1-1 and Tret1-2). Tret1-1 has been shown to transport trehalose across the plasma membrane (Kanamori *et al.* 2010). *Tret1-2* has emerged recently during evolution through a duplication event and is present only in *D. melanogaster* and its closest relatives (Volkenhoff *et al.* 2015). The *Tret1-1* expression pattern suggests that it releases trehalose from the fat body into circulation and mediates the uptake of trehalose by other tissues (Kanamori *et al.* 2010; Volkenhoff *et al.* 2015). Trehalose is catabolized by the Trehalase enzyme. The *Drosophila* genome encodes two genes with putative trehalose-hydrolyzing catalytic activity, of which *Treh* displays ubiquitous expression and *CG6262* is mainly expressed in the testis. Loss of trehalase activity prevents trehalose catabolism, leading to highly elevated circulating trehalose levels (Yoshida *et al.* 2016). Similarly to *Tps1* mutants, *Treh* mutants are viable as larvae, but display pupal lethality and starvation sensitivity (Yoshida *et al.* 2016). Moreover, circulating glucose levels are significantly downregulated in *Tps1* and *Treh* mutants, implying that trehalose turnover is needed to maintain systemic glucose levels (Matsuda *et al.* 2015; Yoshida *et al.* 2016).

In contrast to circulating glucose, trehalose levels do not respond strongly to dietary sugars (Ugrankar *et al.* 2015),

although *Tps1* expression is elevated by high-sugar feeding (Musselman *et al.* 2013). Moreover, many genes that impact circulating glucose levels do not affect trehalose levels, implying that glucose and trehalose levels are independently regulated (Ugrankar *et al.* 2015). However, trehalose levels display high variation during *Drosophila* development. Trehalose levels rise gradually during embryonic development and reach maximum levels in larvae (Matsuda *et al.* 2015). During metamorphosis, trehalose levels drop gradually, possibly reflecting high consumption or reduced synthesis of trehalose during pupal stages (Matsuda *et al.* 2015).

***Regulation of glycogen metabolism:*** Similar to other animals, glycogen is a key storage form of carbohydrates in *Drosophila* (Baker and Thummel 2007; Matsuda *et al.* 2015). In larvae, glycogen is predominantly stored in the body wall muscle while in the adult fly glycogen is abundant in the fat body and flight muscle (Wigglesworth 1949; Ruaud *et al.* 2011). Moreover, high levels of glycogen accumulate in the oocyte during late stages of oogenesis. Glycogen accumulation in oocytes occurs via remodeling of the electron transport chain into a respiratory quiescent mode, and is essential for the developmental competence of the oocyte (Sieber *et al.* 2016).

The expression of glycogen synthase (GlyS) is elevated by dietary sugar and depletion of GlyS from the larval fat body delays development on a high-sugar diet (Garrido *et al.* 2015). This suggests that glycogen synthesis needs to be dynamically controlled with respect to sugar intake. Two transcription factors, DHR38 and Mef2, have been demonstrated to regulate the expression of several glycogen biosynthesis genes (Ruaud *et al.* 2011; Clark *et al.* 2013). DHR38 is an orphan nuclear receptor homologous to the nuclear receptor 4A family in mammals. DHR38 is highly expressed in the larval body wall and gut, and its expression is induced by yeast feeding (Ruaud *et al.* 2011). *DHR38* mutants show significantly reduced levels of glycogen in the body wall muscle, which is consistent with strongly reduced expression of Phosphoglucomutase, a critical enzyme of glycogen biosynthesis (Ruaud *et al.* 2011). Mef2 promotes glycogen synthesis in adults by activating the expression of several glycogen biosynthetic genes (Clark *et al.* 2013). Mef2 serves as a switch between metabolic and immune gene regulation. Once phosphorylated by S6K, Mef2 promotes glycogen and lipid biosynthesis. However, this phosphorylation is lost upon infection and activation of biosynthetic pathways is reduced, while immune response genes are upregulated. Glycogen stores are also regulated by the sugar sensor Mondo-Mlx, since *mlx* mutants possess strongly elevated glycogen stores (Havula *et al.* 2013). Whether this is due to direct regulation of glycogen metabolism or is an indirect consequence of impaired lipid biosynthesis remains to be explored. Supporting the latter, it has been observed that inhibition of fatty acid biosynthesis leads to elevated glycogen levels in larvae (Garrido *et al.* 2015). Low oxygen availability (hypoxia) has a strong impact on carbohydrate metabolism, including increased mobilization of glycogen. This response is prevented by loss of hypoxia-inducible factor (HIF) activity, but the underlying mechanisms remain to be explored (Y. Li *et al.* 2013).

Glycogen breakdown occurs through two parallel mechanisms: glycogenolysis and glycogen autophagy (Zirin *et al.* 2013). Genetic experiments in the larval skeletal muscle have shown that simultaneous inhibition of both autophagy and glycogenolysis fully prevents glycogen catabolism during starvation, and both pathways are needed for maximal efficiency of glycogen breakdown. Interestingly, GlyS interacts with Atg8, raising the possibility that GlyS acts as an adaptor between glycogen metabolism and the autophagy machinery. Another line of evidence linking glycogen synthesis and autophagy comes from the analysis of Rack1, a conserved guanine nucleotide-binding scaffold protein with a WD40-repeat. Loss of Rack1 leads to an attenuated autophagic response upon starvation and a dramatic $>$ 10-fold reduction of glycogen stores in the larval fat body (Erdi *et al.* 2012). Furthermore, Rack1 colocalizes with glycogen particles as well as with Shaggy, the *Drosophila* ortholog of GlyS kinase 3β (GSK-3β) (Erdi *et al.* 2012), further suggesting that Rack1 might promote glycogen synthesis. Glycogen phosphorylase (GlyP), which catalyzes the rate-limiting step in glycogenolysis, is highly expressed in the carcass of the larva and in the fat body and carcass of the adult (FlyAtlas, Chintapalli *et al.* 2007). The expression of GlyP is elevated on a high-sugar diet, but the functional relevance of this regulation remains unknown (Musselman *et al.* 2011; Mattila *et al.* 2015). GlyP activity is essential to maintain *Drosophila* flight muscle function as *GlyP* mutants display severely reduced wing beat frequency (Eanes *et al.* 2006). Thus, glycogen autophagy is insufficient to compensate for the loss of glycogenolysis in the flight muscle.

***Regulation of carbohydrate digestion through glucose repression:*** Homeostatic control of metabolism affects not only channeling of metabolites into various end products, but also the enzymes involved in nutrient breakdown within the intestine. Specifically, it was recognized several decades ago that the presence of glucose in the *Drosophila* diet inhibits the activities of enzymes needed for the breakdown of polymeric carbohydrates, such as starch and oligosaccharides (Hickey and Benkel 1982; Benkel and Hickey 1986, 1987). Subsequent studies have shown that both at the larval and adult stages, various forms of sugar (*i.e.* sucrose, glucose, fructose, and trehalose) have a profound repressive effect on the expression of genes encoding enzymes that possess glycoside hydrolase activities, including α-amylases, maltases, and α-mannosidases (Zinke *et al.* 2002; Chng *et al.* 2014; Mattila *et al.* 2015). Collectively, the phenomenon of repressing the expression of enzymes and the digestion of carbohydrate polymers in the presence of a readily utilizable monosaccharide is termed "glucose repression" (Chng *et al.* 2014). Glucose repression might be a physiological response to suppress overload of systemic glucose under conditions where glucose catabolic pathways are close to saturation.

The mechanisms underlying glucose repression have been shown to involve several transcription factors. The maintenance of amylase expression is attributed to the nuclear receptors HNF4 and DHR38, whereas the repression by glucose is achieved through Mondo-Mlx, Sugarbabe, and the TGF-β/Activin target SMAD2 (Ruaud *et al.* 2011; Chng *et al.* 2014; Mattila *et al.* 2015; Barry and Thummel 2016). Mondo-Mlx resides high in the hierarchy of the sugar-responsive transcriptional network, which directly activates the expression of Sugarbabe and the TGF-β/Activin ligand Daw (Mattila *et al.* 2015). Interestingly, Daw expression is highest in the fat body where it functions as a secreted ligand to repress amylase expression in intestinal enterocytes through SMAD2 (Chng *et al.* 2014). In the intestine, Mondo-Mlx and SMAD2 converge to regulate *sugarbabe* expression, which is necessary and sufficient to repress the expression of amylases (Mattila *et al.* 2015). However, the details of the combinatorial function of Mondo-Mlx and TGF-β/activin signaling, as well as the significance of overlapping cell autonomous and noncell autonomous mechanisms, remain to be elucidated. The prevailing model suggests that while Mondo-Mlx monitors sugar uptake directly in the intestine, TGF-β/Activin signaling is needed to transmit information about the carbohydrate status of the fat body. Such a mechanism would coordinate the expression of amylases, utilization of carbohydrate polymers, and glucose uptake in the intestine according to the metabolic status of the animal.

## Part II

### *Carbohydrate-responsive hormonal circuits: Insulin/glucagon axis and beyond*

***The dILP/glucagon circuit:*** The maintenance of metabolic homeostasis during feeding and fasting periods requires constant communication between nutrient-storing and nutrient-consuming tissues. Several hormonal regulators have evolved for intertissue communication, which orchestrate the allocation of nutrients between growth, maintenance functions, and energy generation. The main hormonal system for maintaining carbohydrate homeostasis in metazoan organisms is the insulin–glucagon circuit, which responds to the levels of circulating glucose. In mammals, glucagon is released from the pancreatic α-cells upon low glucose concentration, promoting lipid and glycogen catabolism and gluconeogenesis to release glucose into the bloodstream. The rise in circulating glucose after feeding triggers insulin secretion from the pancreatic β-cells promoting anabolic metabolism such as lipogenesis and glycogenesis to clear excess glucose from circulation. In addition to the insulin/glucagon axis, several other hormones contribute to carbohydrate homeostasis. Some of them act in parallel to trigger independent responses, whereas many of the hormones regulating carbohydrate metabolism are interconnected to insulin/glucagon signaling, for example by modulating the secretion of these hormones or by influencing the sensitivity of the response in the signal-receiving tissue (Padmanabha and Baker 2014).

The insulin/glucagon axis is well-conserved in *Drosophila*. The *Drosophila* genome encodes eight dILPs (Brogiolo *et al.* 2001; Grönke *et al.* 2010; Colombani *et al.* 2012), and a single glucagon-like peptide, adipokinetic hormone (dAKH) (Schaffer *et al.* 1990). The regulation of the dILP–dAKH circuit varies between the larval and adult stages, reflecting the profound differences between these life cycle phases in terms of feeding behavior, nutritional demands, and growth (Figure 4). While larvae feed and grow constantly until they reach a critical size for pupariation, the adult life consists of periods of feeding and fasting regulated by the circadian clock and the availability of food. Thus, the framework of metabolic regulation in larvae and adult animals are very different.

***dILPs:*** *Drosophila* ILPs vary in terms of temporal and spatial expression patterns, suggesting the evolution of unique physiological functions and specific modes of regulation [reviewed by Nässel *et al.* (2015)]. The current interpretation is that the various dILPs have adopted specialized roles in maintaining growth and metabolic homeostasis in different nutritional conditions and stages of the life cycle. However, analyses of individual *dILP* mutants have revealed that none of the dILPs are essential during development, suggesting that they can act in a redundant and compensatory manner (Grönke *et al.* 2010). The main site for dILP synthesis is a cluster of median neurosecretory cells referred to as the IPCs of the fly brain, where dILPs 1, 2, 3, and 5 are expressed (Brogiolo *et al.* 2001; Ikeya *et al.* 2002; Rulifson *et al.* 2002; Broughton *et al.* 2005; Colombani *et al.* 2012; Liu *et al.* 2016). Loss of the IPCs by targeted induction of cell death during early larval stages causes profound consequences to larval growth and metabolic homeostasis. The animals are developmentally delayed, have impaired growth, and possess elevated hemolymph glucose concentration. In addition, the total levels of lipids, trehalose, and glycogen are elevated in these animals (Rulifson *et al.* 2002; Broughton *et al.* 2005). These phenotypes can be reversed by the expression of dILP2 (Rulifson *et al.* 2002). Adult flies that develop from IPC-ablated larvae, or larvae deficient for dILPs 2, 3, and 5, contain high hemolymph glucose concentrations and elevated stored trehalose, glycogen, and lipid (Ikeya *et al.* 2002; Broughton *et al.* 2005; Grönke *et al.* 2010). Collectively, these findings show that the dILPs emanating from the IPCs are critical regulators of glucose metabolism, and that the physiological function of the IPCs resembles that of mammalian pancreatic β-cells.

***Regulation of dILP expression and secretion by carbohydrates:*** The insulin signal emanating from the IPCs is subjected to several layers of regulation. These include cell-intrinsic regulation of transcription, protein processing, and protein secretion, as well as extrinsic factors such as neurotransmitters and hormonal signals from peripheral tissues [reviewed by Nässel and Broeck (2016); Alfa and Kim (2016)]. Below, we will review the mechanisms of IPC regulation by carbohydrates in the order of (1) direct regulation of

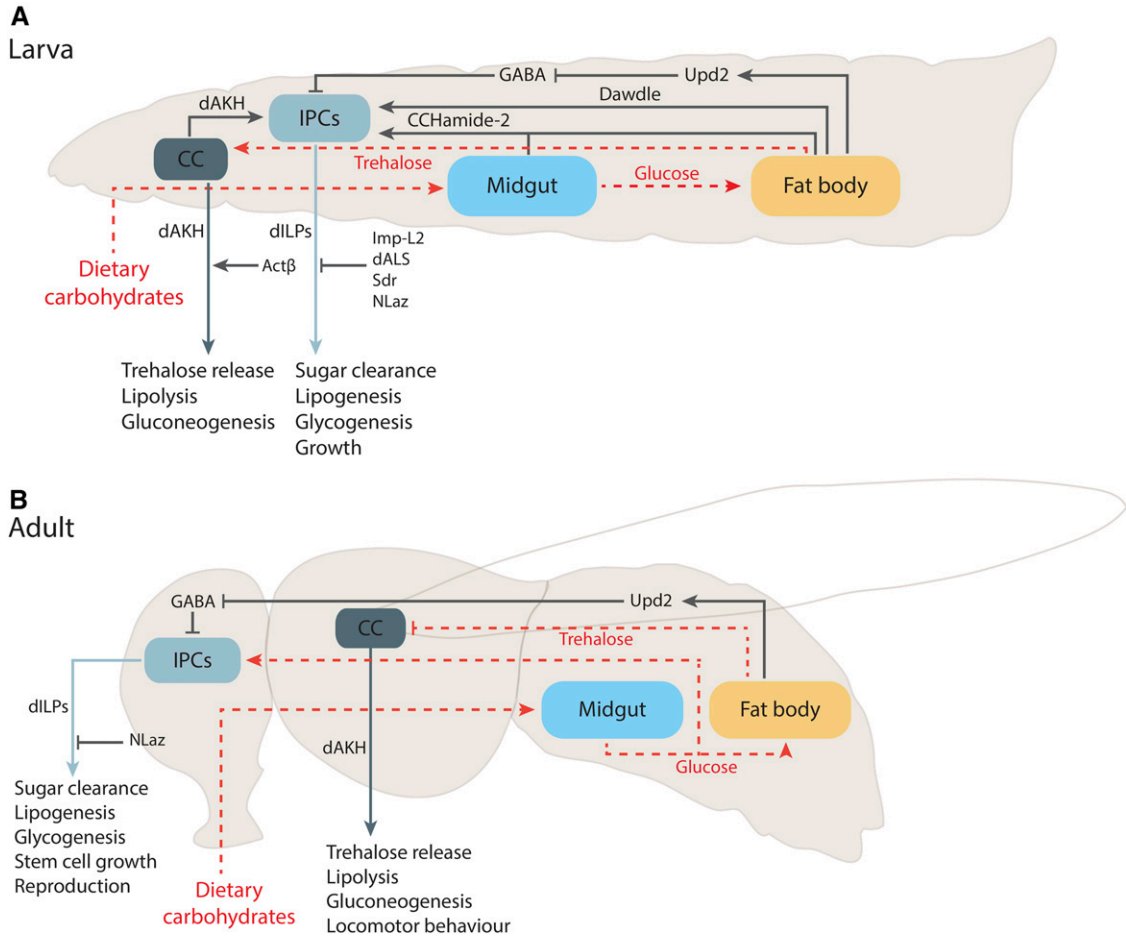


**Figure 4** Insulin-like peptide-glucagon circuit in *Drosophila*. Schematic presentation of larva (A) and adult fly (B), illustrating mechanisms that regulate the output of dILP and dAKH signaling in response to dietary carbohydrates. Dietary carbohydrates are digested in the midgut and glucose is taken up by the intestinal enterocytes. Glucose is converted into trehalose in the fat body and released into circulation. In the larva, trehalose has a biphasic effect to dAKH; low and high trehalose concentrations are shown to stimulate dAKH secretion. Whether such regulation also exists in adults is unknown. Larval IPCs are inherently insensitive to glucose, but carbohydrates regulate dILP secretion through remote mechanisms. These include dAKH from the CC as well as CCHamide-2, Dawdle, and Upd2 secreted from the fat body. Only Upd2 has been shown to function at the larval and adult stages. At the adult stage, glucose regulates IPCs directly by modulating the activity of $K^{ATP}$ channels and cell depolarization leading to dILP secretion. The output of dAKH and dILP signaling is regulated through humoral factors, such as Activinβ, Imp-L2, dALS, Sdr, and NLaZ. Only NLaz has been shown to function at larval and Activinβ adult stage. Actβ, Activinβ; CC, *corpora cardiaca*; dAKH, *Drosophila* adipokinetic hormone; dALS, *Drosophila* acid-labile subunit; dILP, *Drosophila* insulin-like peptide; Imp-L2, imaginal morphogenesis protein-late 2; IPC, insulin producing cells; NLaz, Neural Lazarillo; Sdr, secreted decoy of InR; Upd2, unpaired 2.

secretion, (2) indirect regulation of secretion through hormones, and (3) regulation of dILP expression.

An important regulatory mechanism of the IPCs is direct glucose sensing from hemolymph, which differs significantly between the larval and adult stages. In mammalian pancreatic islets, glucose depolarizes β-cell membrane potential by shutting down the ATP-sensitive potassium channels ($K^{ATP}$), leading to action potential firing and the opening of voltage-dependent $Ca^{2+}$ channels [reviewed by MacDonald *et al.* (2005)]. Interestingly, in contrast to the adult stage, larval IPCs lack the ability to respond to glucose directly, since they do not express the $K^{ATP}$ complex necessary for cell depolarization (Kim and Rulifson 2004). Instead, carbohydrates regulate larval IPCs indirectly, through dAKH secreted by the *corpora cardiaca* (CC), and through other hormonal signals originating from the fat body and intestine (Rajan and Perrimon 2012; Ghosh and O'Connor 2014; Kim and Neufeld 2015; Sano *et al.* 2015). The larval IPC and CC neurons send axonal projections to each other and it is therefore likely that these cells interact in the process of nutrient sensing (Rulifson *et al.* 2002; Kim and Rulifson 2004; Lee and Park 2004). As an additional mechanism for nutrient sensing, larval IPCs employ the hexosamine synthesis pathway and *O*-linked GlcNAc conjugation (Sekine *et al.* 2010). RNAi knockdown of Ogt or Oga in the larval IPCs either increases or decreases ILP secretion, respectively. However, the targets for *O*-GlcNAcylation in the IPCs remain unknown.

In contrast to the larval stage, fully developed IPCs in adult flies have been shown to respond to glucose and secrete dILPs in a manner similar to mammalian β-cells. Glucose-uptake

through GLUT1 stimulates mitochondrial ATP production, which shuts down $K^{ATP}$ channels, leading to cell depolarization, potassium influx, and dILP release through exocytosis (Kréneisz *et al.* 2010; Park *et al.* 2014). Blocking ATP production by inhibiting pyruvate transport to mitochondria prevents dILP secretion in the adult fly, suggesting that the regulation of mitochondrial metabolism is a key step in the larval to adult IPC maturation (McCommis *et al.* 2016). A major regulator of this transition is the *Drosophila* ortholog of the nuclear receptor HNF4 (Hepatocyte nuclear factor 4). Loss-of-function of *HNF4* in *Drosophila* has a profound impact on the regulation of glycolysis, mitochondrial respiration, and insulin signaling at the adult stage. In the IPCs, HNF4 coordinates gene expression to direct a metabolic switch toward OXPHOS and glucose-induced dILP secretion (Barry and Thummel 2016).

In addition to direct glucose sensing, the IPCs are subjected to nutrient regulation through multiple signals derived from the fat body and midgut (Figure 4). Here, we focus only on the mechanisms responding to carbohydrates, as the signals responding to amino acids are extensively reviewed elsewhere (Andersen *et al.* 2013; Droujinine and Perrimon 2016). The fat body conveys information about carbohydrates through at least three mechanisms. These include secretion of the cytokine Unpaired 2 (Upd2), secretion of the TGF-β/activin ligand Daw, and CCHamide-2 secretion (Rajan and Perrimon 2012; Ghosh and O'Connor 2014; Sano *et al.* 2015). *upd2* expression is upregulated in the fat body of adult flies upon high-sugar and high-fat diet feeding, and knockdown of *upd2* in the fat body leads to the hallmark phenotypes of IPC-deficient flies; small size and elevated hemolymph glucose concentration (Rajan and Perrimon 2012). Interestingly, Upd2 acts on IPCs indirectly, by silencing a set of intermediate γ-aminobutyric acid (GABA)ergic neurons. Knockdown of the JAK/STAT signaling components, the receptor Dome, or the transcription factor Stat92E, in the GABAergic neuron population inhibits dILP2 secretion and causes metabolic defects (Rajan and Perrimon 2012). The GABAergic neurons hence inhibit dILP release through synaptic firing to IPCs and JAK/STAT activation through Upd2 attenuates this effect. These results suggest that Upd2 functions analogously to the Leptin adipokine system in mammals. Indeed, overexpression of human Leptin in the fly fat body can rescue the phenotypes of *upd2* mutant flies. While the exact mechanism of Upd2 activation in the fat body is unknown, the TGF-β/Activin ligand Daw responds to dietary sugars in a Mondo-Mlx-dependent manner (Ghosh and O'Connor 2014; Mattila *et al.* 2015). Loss-of-function mutants of *daw* are larval lethal and possess metabolic defects reminiscent of attenuated insulin signaling. Daw signals directly to the IPCs through the TGF-β/activin receptor Baboon, regulating the secretion of dILPs 2 and 5 (Ghosh and O'Connor 2014). A third mechanism by which carbohydrates remotely promote dILP expression and secretion from the IPCs is through CCHamide-2, which is synthesized by the fat body and midgut enteroendocrine cells in a sugar-inducible manner (see below for a further discussion of CCHamides) (Sano *et al.* 2015).

As discussed above, glucose sensing by the IPCs is accompanied by other nutrient-derived hormonal cues. However, the significance of these different nutritional signals and how they are integrated in the IPCs to elicit a physiological dILP response is still not fully understood. For example, a detailed study of dILP expression in the adult fly across a panel of isocaloric diets differing in their protein-to-carbohydrate ratios has shown that the expression of dILPs 2, 3, and 5 peak in response to different diets (Post and Tatar 2016). *dilp2* mRNA is highest in response to diets low in protein whereas *dilp5* expression peaks in response to high dietary protein content. On the other hand, *dilp3* expression is enhanced in animals on a low-calorie diet, with a protein-to-carbohydrate ratio of 1:8. This suggests that the dILPs expressed in the IPCs are subjected to differential nutritional regulation. The expression of dILPs 3 and 5 respond to nutrient levels during larval stages, whereas dILP2 does not (Ikeya *et al.* 2002). Interestingly, only a few transcription factors are known to be involved in the nutrient-regulated dILP expressions. For example, these include Sugarbabe, which was shown to repress the expression of dILPs 3 and 5 (Varghese *et al.* 2010). Furthermore, it is not known how the differences in dILP expression correlate with circulating dILP proteins and signaling in the peripheral tissues. In fact, direct dILP visualization in the IPCs through immunofluorescence suggests that protein secretion is the key regulatory point regarding peripheral insulin signaling (Géminard *et al.* 2009). Under low nutritional conditions, dILPs accumulate in the neurosecretory cells and are rapidly released upon nutritional stimulus. The recent development of ELISA immunoassays that allow direct measurement of circulating dILP levels is likely to uncover the impact of dILP transcription and secretion more precisely (Pasco and Léopold 2012; Park *et al.* 2014; Post and Tatar 2016).

***Regulation of insulin sensitivity and carbohydrate metabolism within insulin target tissues:*** The insulin-induced signaling pathway (IIS) is an ancient signaling system to control animal growth, metabolism, and differentiation. In humans, the IIS is diversified into two branches, the insulin and insulin-like growth factor pathways, which control metabolism and growth, respectively (Saltiel and Kahn 2001; Chitnis *et al.* 2008). *Drosophila* ILPs signal through a sole ortholog of Insulin-like receptor (dInR) meaning that the regulation of growth and metabolism is achieved by the same downstream signaling events (Fernandez *et al.* 1995; Shingleton *et al.* 2005). An exception to this is the relaxin-like hormone dILP8, which acts through the leucine-rich repeat-containing G protein-coupled receptor (GPCR) 3 (Lgr3) to coordinate organ growth with the timing of larval maturation (Colombani *et al.* 2015; Garelli *et al.* 2015). The dInR signals through a well-known and conserved insulin receptor substrate (IRS)/phosphoinositide 3-kinase (PI3K)/AKT pathway (Teleman 2009; Nässel *et al.* 2015). Upon ligand binding, dInR is

autophosphorylated and binds to the IRS Chico and to the SH2B family adapter protein Lnk (Böhni *et al.* 1999; Werz *et al.* 2009). Activation of the dInR leads to the phosphorylation of Chico, providing a binding site for the lipid kinase PI3K. Elevated levels of phosphatidylinositol-(3,4,5)-triphosphates causes recruitment of the AKT and PDK1 kinases at the plasma membrane via their lipid-binding pleckstrin homology domains. AKT is then phosphorylated and activated by PDK1 and TORC2 (Rintelen *et al.* 2001; Yang *et al.* 2006; Hietakangas and Cohen 2007). Activation of AKT is central to the growth-promoting and metabolic effects of IIS, having a large number of identified phosphorylation targets. These include, for example, the inhibitory regulation of GSK-3β (Shaggy), FOXO (dFOXO), and Tuberous sclerosis complex 2 (TSC2, Gigas) (Potter *et al.* 2002; Puig *et al.* 2003; Buttrick *et al.* 2008; Sieber *et al.* 2016). The best-known transcriptional mediator of IIS is dFOXO (Puig *et al.* 2003; Teleman *et al.* 2008; Alic *et al.* 2011). Upon activation, AKT phosphorylates dFOXO at three conserved sites (T44, S190, and S259), which leads to its cytoplasmic retention and inactivation (Puig *et al.* 2003). Hence, during low IIS, dFOXO is nuclear and binds to the promoter of genes involved in macromolecular catabolism, stress resistance, growth, apoptosis, and innate immunity (Gershman *et al.* 2007; Alic *et al.* 2011). In addition, dFOXO activates a second tier of transcriptional regulators through dMyc and the ETS-family transcription factors Anterior open (Aop) and Pointed (Pnt) (Teleman *et al.* 2008; Alic *et al.* 2014). Overexpression of a constitutively nuclear mutant of dFOXO in S2 cells, or in the wing imaginal disc, leads to a strong reduction of cell proliferation, partly through the transcriptional activation of d4EBP (Jünger *et al.* 2003; Puig *et al.* 2003). The role of dFOXO in glucose metabolism is less well understood. Transcriptional profiling experiments suggest that dFOXO has a major role in mitochondrial biogenesis through repression of PGC-1 (encoded by the *spargel* gene in *Drosophila*) and mitochondrial ribosome proteins (Gershman *et al.* 2007). In addition, dFOXO regulates gluconeogenesis through the activation of PEPCK expression, which could explain the increased hemolymph glucose levels observed in IPC-deficient larvae and adult flies (Rulifson *et al.* 2002; Broughton *et al.* 2005; Harvey *et al.* 2008). However, it is clear that additional players are involved in IIS-mediated carbohydrate homeostasis. This is exemplified by the finding that a direct target of dILPs, an α glucosidase encoded by the *tobi* gene, is regulated independently of dFOXO (Buch *et al.* 2008). One such factor could be dMyc, which is positively regulated by IIS through the TOR signaling branch and shares common transcriptional targets with dFOXO (Teleman *et al.* 2008; Li *et al.* 2010).

Perhaps the most prominent metabolic feature of IIS activation is the accumulation of stored triglyceride reserves manifested by the increase in lipid droplet number within fat body cells (Britton *et al.* 2002). One possible mechanism for IIS-induced lipogenesis is the AKT-mediated activation of the Sterol response element-binding protein (Porstmann *et al.* 2008). Yet surprisingly little is known about the direct mechanisms of IIS-induced lipogenesis *in vivo*. The IIS-induced transcriptional response has been studied by measuring gene expression from PI3K-overexpressing larvae (Li *et al.* 2010). Interestingly, only a few genes directly associated to the regulation of carbohydrate and fatty acid metabolism where found regulated in this data set. These included, for example, Hexokinase A (hex-A) and a long-chain fatty acid-CoA ligase (bgm) as well as the transcription factor Sugarbabe. These results suggest that the transcriptional program downstream of IIS is tissue-specific, and a more refined experimental strategy is needed to reveal the full spectrum of metabolic regulation by insulin-like signaling in the fly.

The physiological response to the activation of the IIS cascade is tightly regulated through negative feedback mechanisms, such as the inhibition of dAKT by TOR complex 1 (Kockel *et al.* 2010). Perturbations in these mechanisms might lead to insulin resistance, where even elevated levels of insulin are unable to activate the IIS cascade. For example, a high-sugar diet has been shown to lead to insulin resistance (Musselman *et al.* 2011; Pasco and Léopold 2012). This happens via a lipocalin-like protein Neural Lazarillo (NLaz) secreted from the fat body in a JNK-dependent manner (Hull-Thompson *et al.* 2009; Pasco and Léopold 2012). Mutant animals for *NLaz* contain less glucose, glycogen, and triglycerides, whereas overexpression of NLaz protein in the fat body results in higher glucose titers compared to control animals. In addition, mutant *NLaz* fat body cells have higher PI3K activity (Hull-Thompson *et al.* 2009). Taken together, the results suggest a model where upon organismal stress, such as high circulating sugars, *NLaz* expression is activated in the fat body through JNK, which leads to dampening of IIS cascade sensitivity. In addition, *Drosophila* IIS is modulated by secreted hormonal cues directly interacting with circulating dILPs. Some of these factors resemble the mammalian IGF-binding proteins (IGFBPs), which have several functions, ranging from carrier proteins to modulators of signaling activity (Duan and Xu 2005). An ortholog of the IGFBPs in *Drosophila* is the Imp-L2 protein, which is expressed in the fat body, CC, and IPCs. Imp-L2 binds to dILP2 and prevents downstream signaling under nutrient-deprived conditions (Honegger *et al.* 2008). Interestingly, the Imp-L2/dILP2 complex includes another IGFBP member, namely the *Drosophila* ortholog of Acid-labile subunit (ALS), dALS (Arquier *et al.* 2008). dALS is expressed in the fat body and IPCs, and its expression is strongly suppressed upon amino acid starvation. Overexpression or knockdown of *dALS* in the larval fat body reduces or increases adult body size, respectively. Furthermore, *dALS* overexpression in the fat body antagonizes the metabolic defects of dILP2 overexpression, including lowered circulating trehalose and higher total triglyceride levels. Interestingly, whereas dALS acts specifically on the Imp-L2/dILP2 complex, a glia-derived factor, referred to as Secreted Decoy of InR (SDR), binds with highest affinity to dILP3, independent of Imp-L2 or dALS (Okamoto *et al.* 2013). SDR antagonizes IIS under adverse dietary conditions.

***dAKH, the Drosophila counterpart of glucagon:*** Glucagon and AKHs are peptide hormones important for the maintenance of physiological levels of circulating sugars. AKH peptides were initially identified from a variety of insect species, mainly by immunochemical assays of crude extracts of CC, and were shown to be essential for energy regulation during insect flight [reviewed by Gäde (1990)]. dAKH is synthesized as a preprohormone containing a signal peptide, a single AKH of eight amino acids, and a C-terminal AKH precursor-related peptide (Schaffer *et al.* 1990; Noyes *et al.* 1995; Galikova *et al.* 2015). dAKH is enzymatically processed by proprotein convertases (PCs) before the active hormone is released into circulation. A mutant of the *Drosophila* PC-encoding gene *amontillado* (*amon*) phenocopies the loss of dAKH signaling, and is necessary and sufficient for dAKH-mediated regulation of carbohydrate metabolism. In addition, direct peptide profiling from CC cells has shown that *amon* mutants lack a mature dAKH peptide, linking it to dAKH maturation (Rhea *et al.* 2010).

The *dAKH* gene is expressed exclusively in the CC neuroendocrine cells of larvae and adults (Isabel 2004; Kim and Rulifson 2004; Lee and Park 2004). In larvae, the AKHergic neurons make connections to the prothoracic gland, IPCs, and dorsal vessel (heart), where the peptide is released into the hemolymph (Kim and Rulifson 2004; Lee and Park 2004). In the adult, AKHergic neurons send axons to the brain (protocerebrum) and crop (Lee and Park 2004). Targeted ablation of the AKHergic neurons by overexpression of the proapoptotic gene *reaper* strongly reduces trehalose levels in the larval hemolymph (Isabel 2004; Kim and Rulifson 2004; Lee and Park 2004). This is consistent with the idea that dAKH functions like mammalian glucagon by releasing carbohydrates into circulation. CC-ablated adult flies are also more resistant to starvation and a show lack of starvation-induced hyperactivity (Isabel 2004; Lee and Park 2004). Analysis of dAKH mutants showed that AKH signaling is an essential mechanism in lipid catabolism and maintenance of normoglycemia in the adult fly, but is dispensable for larval energy metabolism. These findings suggest that larval CC ablation might have other, dAKH-independent consequences or that the role of AKH signaling is conditional and dependent on the larval nutrition uptake (Galikova *et al.* 2015).

dAKH release into the hemolymph is regulated through membrane depolarization by ATP-sensitive $K^+$ -channels. These channels serve as intracellular AMP/ATP sensors that control membrane potential and hormone secretion. A rapid decrease in trehalose concentration triggers calcium influx into CC cells, which induces the release of dAKH into the hemolymph (Kim and Rulifson 2004). The inward flux of calcium into the CC cells, and subsequent dAKH secretion, is dependent on AMPK (Braco *et al.* 2012). Surprisingly, dAKH secretion is also triggered by high hemolymph trehalose concentration (Kim and Neufeld 2015). In addition, a recent study by Song *et al.* (2017) showed that larvae fed a high-sugar diet had higher dAKH signaling output in the fat body. The inhibition of this signal by knocking down dAKH downstream signaling components AKH receptor (AKHR), Ire1, Creb2, or CBP by RNAi significantly alleviates the high-sugar diet-promoted hyperglycemia (Song *et al.* 2017). Together, these observations suggest a model of biphasic regulation of dAKH, where its secretion is promoted by low and high hemolymph trehalose concentrations. Such regulation can be understood by the necessity to maintain constant hemolymph trehalose concentrations during the rapid larval growth phase, when insulin signaling is high, as well as during the wandering and pupal stages when feeding has ceased. In comparison, high glucose concentrations stimulate glucagon secretion from mouse pancreatic islets and glucagon, further promoting hyperglycemia in diabetic humans (Jiang and Zhang 2003; Salehi *et al.* 2006). Hence, it is possible that the *Drosophila* CC responds to sugars in a similar manner as the mammalian pancreatic α-cells. Further studies in well-defined nutritional regimes are required to uncover the elaborate regulation of dAKH.

***Regulation of carbohydrate metabolism in dAKH target tissues:*** The fly genome encodes one AKH-responsive GPCR, AKHR, which is expressed in the larval and adult fat body (Grönke *et al.* 2007; Bharucha *et al.* 2008). Consistent with the view of AKH being a lipolytic and glycolytic regulator, *AKHR* mutant animals display elevated triglyceride and glycogen levels compared to control animals, and are more resistant to starvation, probably due to changes in energy expenditure and reduced locomotor activity (Grönke *et al.* 2007; Bharucha *et al.* 2008). Overexpression of AKHR in the fat body leads to reduced triglyceride and glycogen stores (Grönke *et al.* 2007; Bharucha *et al.* 2008). The mechanism of GPCRs and the downstream intracellular events are well-documented in various model systems, as well as in humans (Pavlos and Friedman 2016). The downstream events following AKH binding to its receptor have also been studied in other insect species. For example, in adipocytes of the Lepidopteran *Manduca sexta*, AKHR activation leads to the cellular increase of classical second-messengers cAMP and $Ca^{2+}$ (Arrese *et al.* 1999). In *Drosophila*, the GPCR signal transducers G protein α q subunit (Gαq), G protein γ1 (Gγ1), and Phospholipase C at 21C (Plc21C) control cellular and organismal fat storage downstream of AKHR (Baumbach *et al.* 2014). Genetic modulation of the GPCR signaling components leads to an impairment of intracellular $Ca^{2+}$ through the inhibition of Store-Operated Calcium Entry (SOCE). As a consequence, lipid mobilization from the fat body is blocked through the regulation of Brummer lipase and diacylglycerol *O*-acyltransferase *midway* gene expression (Baumbach *et al.* 2014). Further details about AKHR signaling have been revealed in a recent study by Song *et al.* (2017), who showed that *Drosophila* AKHR employs an analogous signaling mechanism to mammalian glucagon, through the PKA-IRE-CREB2 pathway. Song *et al.* (2017) also elucidated a novel interaction between Activin and AKHR signaling in the fat body of chronically high-sugar-fed larvae. Activinβ derived from midgut enteroendocrine cells signals through the type I TGF-β

receptor Babo and downstream transcription factor dSmad2 in the fat body to regulate AKHR expression, resulting to hyperglycemia. Transcriptomic analysis of fat bodies overexpressing dAKH has revealed a breadth of downstream metabolic processes, including the PPP, glycolysis, and gluconeogenesis. Interestingly, dAKH and IIS were also shown to interact under conditions of high circulating trehalose through the regulation of dILP3 (Kim and Neufeld 2015). As a response to trehalose and dAKH signaling, elevated levels of circulating dILP3 were shown to both activate mTOR signaling in the larval fat body and prevent autophagy.

***Regulation of carbohydrate metabolism by transforming growth factor β/Activin signaling:*** The *Drosophila* TGF-β family signaling pathway has two separate branches that utilize different ligands, namely, the bone morphogenetic proteins (BMPs) and Activins (Upadhyay *et al.* 2017). The BMP and Activin ligands signal through a separate set of receptors and downstream effectors. Recent studies have revealed that the Activin branch, which signals through the Babo receptor, has an important role in carbohydrate metabolism. This branch includes three ligands: Daw, Activinβ, and Myoglianin (Upadhyay *et al.* 2017). Daw is highly expressed in the fat body and muscles (Bai *et al.* 2013; Mattila *et al.* 2015). Its expression is strongly induced by sugar feeding and, at least in larvae, the majority of sugar-induced gene expression of Daw is mediated by Mondo-Mlx, which binds to the *dawdle* promoter (Mattila *et al.* 2015). Moreover, Daw is a direct target of FOXO, which negatively regulates its expression (Bai *et al.* 2013).

*dawdle*-null mutants display sugar intolerance similar to that of *mlx* mutants (Ghosh and O'Connor 2014; Mattila *et al.* 2015). On a carbohydrate-rich diet, most mutants die during larval stages and display delayed development, while the duration of larval development is normal and mutants pupariate in high numbers on a yeast diet. Moreover, *dawdle* mutants have high circulating trehalose and glucose as well as high glycogen and triacylglycerol levels (Ghosh and O'Connor 2014). Loss of *dawdle* also causes hemolymph acidification, possibly due to an accumulation of acidic TCA cycle intermediates. Daw seems to affect metabolic homeostasis through multiple mechanisms. It promotes secretion of dILPs, providing one of the many hormonal links between peripheral tissues and the IPCs (Ghosh and O'Connor 2014). Moreover, fat body-derived Daw influences signaling in the intestine, inhibiting expression of Amylases upon sugar feeding (Chng *et al.* 2014). Daw also contributes to the full activation of the sugar-responsive transcription factor Sugarbabe, possibly providing a feed-forward mechanism to the Mondo-Mlx-dependent activation of Sugarbabe (Mattila *et al.* 2015). Daw also maintains proteostasis in muscles, thereby extending life span (Bai *et al.* 2013). In addition to Daw, Activinβ was recently shown to contribute to carbohydrate metabolism. Chronic sugar feeding upregulates the expression of Activinβ from the enteroendocrine cells of the midgut (Song *et al.* 2017). It signals to the fat body, where it activates AKH signaling by upregulating AKHR expression, consequently causing hyperglycemia. In conclusion, Activin ligands are emerging as important carbohydrate-responsive signals that emanate from peripheral tissues.

***CCHamides, emerging sugar-responsive hormones:*** CCHamide is a short peptide hormone originally found in silkworms (*Bombyx mori*) (Roller *et al.* 2008). Subsequent work has led to the identification of two CCHamide genes, *CCHamide-1* and *-2*, in *Drosophila* (Hansen *et al.* 2011). CCHamide-1 and -2 signal through their respective GPCRs, which are homologs of Bombesin Receptor Subtype 3 (BRS-3) in mammals. Mice lacking BRS-3 develop mild obesity and display impaired glucose metabolism (Ohki-Hamazaki *et al.* 1997). CCHamide-2 is expressed mainly in the fat body and gut endocrine cells and its expression is nutrient-dependent (S. Li *et al.* 2013; Sano *et al.* 2015). It is downregulated by starvation and activated by refeeding with nutritious sugars (Sano *et al.* 2015). CCHamide-2 Receptor (CCHamide-2 R) is expressed mainly in the CNS, displaying high levels in the IPCs (Sano *et al.* 2015). CCHamide-2 promotes secretion of dILP2 and dILP5 as well as expression of dILP5 in the IPCs. Consequently, mutants of *CCHamide-2 R* are growth impaired. Moreover, mutants of *CCHamide-2* display strongly reduced feeding activity in both larvae and adults (Ren *et al.* 2015). In conclusion, CCHamide-2 is a carbohydrate-responsive hormone that mediates information from peripheral tissues to the CNS.

## Part III

### *Physiological processes linked to carbohydrate metabolism*

***Circadian clock and carbohydrate metabolism:*** Adult *Drosophila* feeding activity follows a circadian rhythm, with the highest feeding activity during the first few hours of daylight (Xu *et al.* 2008; Seay and Thummel 2011). This periodic feeding is reflected in the carbohydrate homeostasis of the animal, as circulating trehalose and glycogen levels increase a few hours after the highest feeding activity and are then gradually consumed during the remainder of the day (Seay and Thummel 2011). In contrast, triacylglycerol and protein levels do not display circadian oscillation (Seay and Thummel 2011). The circadian timekeeping system includes the central clock located in the brain and is composed of ∼150 clock-expressing neurons, as well as the peripheral clocks present in several peripheral tissues (Ito and Tomioka 2016). Cycling of the *Drosophila* feeding activity is controlled by the peripheral clock (Xu *et al.* 2008). In fact, a large number of metabolic genes, including *Zw*, display cyclic expression in the fat body, which depends on tissue autonomous clock activity (Xu *et al.* 2011). Moreover, flies lacking a functional clock in the fat body have significantly reduced glycogen storage along with starvation sensitivity, despite the fact that their total food consumption is higher than in control flies.

The interaction between feeding and the circadian clock is bidirectional, as the circadian clock can be reset

by time-controlled feeding (Catterson *et al.* 2010; Xu *et al.* 2011). One mechanism mediating nutrient-dependent resetting of the circadian clock is through protein *O*-GlcNAcylation. *O*-GlcNAcylation is regulated in a circadian manner with inhibition of *Drosophila* Ogt in clock cells shortening the circadian period, while increased *O*-GlcNAcylation has the opposite effect (Kim *et al.* 2012). Central clock proteins, including Clock and Period, are modified by *O*-GlcNAc, which modulates their transcriptional activity (Kim *et al.* 2012; Kaasik *et al.* 2013). Protein *O*-GlcNAcylation is directly affected by the activity of the HBP, which is sensitive to glucose availability, providing a potential means for nutrient-dependent resetting of the circadian clock. Another point of interaction between nutrient sensing and circadian clock activity is through Mondo-Mlx-mediated intracellular sugar sensing. Mondo-Mlx directly regulates the expression of the Krüppel-like transcription factor Cabut (Havula *et al.* 2013; Bartok *et al.* 2015). The *cabut* promoter region is also bound by the circadian transcription factor CLK (Abruzzi *et al.* 2011), suggesting that sugar sensing and the circadian clock converge on Cabut regulation. Furthermore, Cabut overexpression leads to severe defects in circadian locomotor activity rhythms and deregulation of circadian cycling of metabolic targets, while having no effect on the core clock components (Bartok *et al.* 2015).

***Regulation of carbohydrate metabolism upon developmental transitions:*** During its life cycle, *Drosophila* undergoes different developmental stages with distinct metabolic needs. For example, during the larval stage, the body mass of *Drosophila* increases rapidly by ~200-fold, which requires metabolic reprogramming into an anabolic mode. Temporal analysis of gene expression during the embryonic stage has revealed widespread changes in metabolic gene expression before the transition from an embryo to a larva, which is termed the embryonic metabolic transition (EmbMT) (Tennessen *et al.* 2014a). The genes activated during the EmbMT encode glycolytic enzymes, lactate dehydrogenase, as well as TCA cycle components and other mitochondrial metabolic enzymes. Along with gene expression changes, metabolite profiles of embryos change during embryogenesis (An *et al.* 2014; Tennessen *et al.* 2014a). During embryonic development, the animal consumes its triacylglycerol and glycogen stores and concomitantly accumulates glycerol-3-phosphate. At the onset of the EmbMT, uric acid levels increase dramatically, possibly reflecting catabolism of nitrogen-containing metabolites (Tennessen *et al.* 2014a). Concomitantly, levels of some amino acids, such as glutamate and aspartate, decline (An *et al.* 2014).

The EmbMT metabolically prepares the animal for the transition into larval development, when the animal starts feeding and growing. Interestingly, the metabolic profile of larvae includes high glycolytic activity and the production of lactate (Tennessen *et al.* 2011). Although the involvement of tissue-specific hypoxia has not been ruled out, the metabolic profile of growing larvae resembles the Warburg-type metabolism of malignant and other highly proliferative cells, which rely on high rates of biosynthesis. Furthermore, *Drosophila* larvae produce high levels of L-2-hydroxyglutarate (L-2HG), a metabolite found at high levels in cancers (Li *et al.* 2017). L-2HG is a product of lactate dehydrogenase activity, which is highly upregulated upon the EmbMT. L-2HG accumulation may have functional relevance since it inhibits 2-oxoglutarate-dependent dioxygenases, which are important epigenetic regulators.

A key regulator of the EmbMT is the Estrogen-Related Receptor (ERR) (Tennessen *et al.* 2011). *ERR* expression is upregulated during late embryogenesis. *ERR* mutants die during the second larval instar and display severe metabolic problems, for example low levels of ATP and high levels of both trehalose and sorbitol. At the gene expression level, *ERR* mutants fail to activate glycolytic gene expression and consequently have low levels of L-2HG (Y. Li *et al.* 2013). It will be interesting to learn how the developmental metabolic switch interacts with environmental signals influencing carbohydrate metabolism. In fact, it is already known that ERR binds to HIF and is essential for activating HIF-dependent gene expression upon hypoxia (Y. Li *et al.* 2013). Furthermore, ERR is essential for HIF-independent gene regulation upon hypoxia, including upregulation of glycolytic transcripts, but how ERR mediates these HIF-independent responses remains to be elucidated.

Another metabolic transition during *Drosophila* development occurs during metamorphosis when mitochondrial respiration is very low, and it is strongly activated at the onset of the adult stage (Merkey *et al.* 2011). This corresponds to a dramatic change in the animal's mobility and feeding activity. Interestingly, the levels of HNF4 expression also strongly increase upon the transition to adulthood, which is followed by an upregulation of HNF4 downstream genes, including genes involved in glucose metabolism and mitochondrial OXPHOS (Barry and Thummel 2016). Notably, the effects of HNF4 on sugar tolerance are also observed during the late pupal stage or adult stage, in contrast to the larval phenotypes observed in *mlx* mutants (Havula *et al.* 2013). Thus, it is possible that carbohydrate metabolism is coordinated by distinct transcription factors during different developmental stages. As mentioned before, *Drosophila* IPCs undergo maturation during development. In the larval stage IPCs are nonresponsive to glucose, whereas in adults dILP secretion is glucose-responsive. Interestingly, IPC-specific knockdown of *HNF4* prevents the transition to glucose responsiveness. Similarly, mutations in human *HNF4a* cause Mature-Onset Diabetes of the Young I (MODY I), with impaired pancreatic β-cell function (Fajans and Bell 2011). In conclusion, HNF4 appears to mediate the developmental switch to rewire carbohydrate metabolism into the mode of high mitochondrial respiration and trigger the maturation of IPCs into glucose responsiveness upon reaching adulthood.

During developmental morphogenesis, the growth of individual cells is also closely regulated. Notch signaling promotes cell proliferation in specific developmental contexts, including the wing imaginal disc (Djiane *et al.* 2013). In this

setting, Notch signaling activates the expression of genes involved in glucose uptake and glycolysis through its downstream effector, Suppressor of Hairless (Slaninova *et al.* 2016). On the other hand, Notch signaling inhibits the expression of genes of the TCA cycle by activating the transcriptional repressor Hairy (Slaninova *et al.* 2016). Through these gene expression changes, Notch signaling reprograms cellular metabolism to match the needs of proliferative cells.

## Part IV

### *Drosophila as a model of carbohydrate metabolism-related pathophysiologies*

***Pathophysiologies of deregulated glycogen metabolism:*** In addition to understanding normal carbohydrate physiology, *Drosophila* has been increasingly used to model pathophysiologies related to ones observed in humans. Defects in glycogen autophagy lead to myopathies in humans. These myopathies can be hereditary or caused by drugs, such as chloroquine, which is used for the treatment of malaria. Interestingly, chloroquine feeding to *Drosophila* larvae leads to a dramatic accumulation of glycogen in autophagic vesicles upon starvation (Zirin *et al.* 2013). In addition, muscle sarcomere structure is impaired and locomotor function is reduced, suggesting that chloroquine-treated larvae can be used as a genetically tractable model to study myopathies caused by defective glycogen autophagy (Zirin *et al.* 2013). The accumulation of glycogen into autophagosomes can be prevented by inhibition of the autophagy machinery as well as activation of TOR signaling, which is known to regulate starvation-induced autophagy. Moreover, inhibition of *GlyS* in the muscle inhibits glycogen accumulation in chloroquine-treated larvae. Thus, *GlyS* function, either by driving glycogen synthesis or by acting as a scaffold between glycogen and the autophagy machinery, is essential for the myopathy phenotype (Zirin *et al.* 2013).

Glycogen synthesis needs to be kept under strict tissue-specific control. Aberrant accumulation of glycogen in neurons coincides with aggressive neurodegeneration in humans, as observed in Lafora disease. In *Drosophila*, deregulated glycogen synthesis leads to neuronal loss, reduced locomotion, and short life span (Duran *et al.* 2012), demonstrating the causal relationship between neuronal glycogen and neurodegeneration. Also during the physiological aging process, glycogen clusters accumulate in neuronal processes. Inhibition of *Drosophila* GlyS expression in neurons improves neurological function with age and extends life span (Sinadinos *et al.* 2014). In conclusion, *Drosophila* has emerged as an important model to understand the regulation of glycogen metabolism and the underlying pathophysiologies.

***Modeling diabetes and its complications in Drosophila:*** While *Drosophila* displays a high degree of flexibility with respect to macronutrient content, chronic feeding of super-physiological concentrations (1 M) of sugar has been shown to cause a phenotype that resembles human obesity and diabetes. Larvae grown on a diet with high sucrose, but not on high fat or high protein diets, display high hemolymph glucose and high triacylglycerol levels (Musselman *et al.* 2011). This suggests that the physiological mechanism to cope with high-sugar intake and maintain glucose homeostasis has been saturated in this setting. Furthermore, high-sugar-fed larvae show signs of insulin resistance, including reduced phosphorylation of protein kinase AKT, a component of the IIS pathway. In adult animals, high-sugar feeding increases triglyceride storage while dietary sugar levels $> 10\%$ also shorten life span and reduce fecundity (Skorupa *et al.* 2008). Similar to larvae, signs of insulin resistance are observed in high-sugar-fed adults (Morris *et al.* 2012).

Stable isotope tracer experiments have unraveled differences in the metabolic profiles of larvae fed a high-sugar diet. High-sugar diet feeding leads to reduced fatty acid chain length and increased levels of desaturation, which are correlated with elevated expression of stearoyl-CoA desaturase 1 (Musselman *et al.* 2013), a target of Mondo-Mlx (Havula *et al.* 2013). Interestingly, loss-of-function of *king tubby*, a homolog of the mammalian obesity-associated gene *tubby* (Coleman and Eicher 1990; Shiri-Sverdlov *et al.* 2006), increase triglyceride storage and concomitantly lead to lower circulating glucose levels. Similar observations have been made following overexpression of Sugarbabe, a lipogenic Gli-similar transcription factor (Mattila *et al.* 2015). Thus, the lipogenic capacity of the fat body appears to be positively reflected in the ability to maintain the homeostasis of hemolymph glucose. High-sugar diet feeding also increases the levels of nonesterified fatty acids (NEFAs) (Musselman *et al.* 2013). Biosynthetic genes of CoA, a cosubstrate for lipogenesis, are activated following high-sugar diet feeding, and the inhibition of CoA biosynthesis leads to increased levels of NEFAs (Palanker Musselman *et al.* 2016). On the other hand, dietary supplementation of the CoA precursor pantothenate facilitates triglyceride biosynthesis, consequently lowering the levels of NEFAs and circulating glucose. Notably, however, this dietary intervention does not improve insulin sensitivity, suggesting that hemolymph glucose and lipid homeostasis can be uncoupled from insulin sensitivity (Palanker Musselman *et al.* 2016). One candidate for mediating the development of insulin resistance upon high-sugar feeding is the lipocalin NLaz. Lipocalins are secreted proteins, which bind small hydrophobic ligands and are known to promote insulin resistance in mammals (Yan *et al.* 2007). NLaz is under the control of the stress-activated JNK signaling pathway (Hull-Thompson *et al.* 2009), and it displays strongly elevated expression following high-sugar diet feeding (Pasco and Léopold 2012). Loss of NLaz expression suppresses insulin resistance in the fat body and improves the clearance of circulating glucose (Pasco and Léopold 2012).

Other approaches to use *Drosophila* as a model for understanding diabetes and other metabolic pathophysiologies include genetic screens with type 2 diabetes-associated genes as well as screens to identify novel genes that affect circulating glucose levels. While genome-wide association (GWAS)

studies have been powerful in identifying genomic regions associated with metabolic disorders in human populations, the causative gene often remains uncertain. Complementing GWAS studies with functional experiments in *Drosophila* allows systematic discovery of candidate genes with an *in vivo* phenotype. Pendse and co-workers analyzed the sugar intolerance phenotypes of 83 *Drosophila* genes, which were homologs of human genes associated with type 2 diabetes or related metabolic traits (Pendse *et al.* 2013). This approach led to the identification of several new genes essential for sugar tolerance, including the homeobox transcription factor HHEX, mediating its function via activity in the intestine (Pendse *et al.* 2013). Another strategy utilizing *Drosophila* to identify human disease-associated candidate genes involves comparative analysis of gene expression data. Variants of the sugar sensing transcription factor ChREBP (MLXIPL, the mammalian ortholog of *Drosophila* Mondo) are strongly associated with circulating triglyceride levels in human (Kooner *et al.* 2008). Interestingly, human homologs of *Drosophila* Mondo-Mlx target genes are significantly overrepresented in the vicinity of triglyceride-associated SNPs, suggesting that novel candidate genes can be discovered through such comparative approaches (Mattila *et al.* 2015). RNAi screening for novel genes that affect circulating glucose levels have also uncovered $>$ 150 candidate genes involved in circulating glucose homeostasis (Ugrankar *et al.* 2015). These were further divided into muscle and fat body-specific genes, which displayed substantial overlap. Moreover, most genes that affect circulating glucose levels do not have a similar effect on trehalose, implying that these two forms of circulating sugars are independently regulated. This emphasizes the need to measure levels of both circulating metabolites when characterizing the metabolic phenotype of *Drosophila* mutants. Roughly half of the identified genes had been implicated in diabetes before, but several novel regulators of glucose homeostasis were observed, including the protein kinase-encoding gene *Ck1α* (Ugrankar *et al.* 2015). Ck1α displays a conserved role in maintaining glucose homeostasis, as adipose tissue-specific loss of the Ck1α homolog (CSNK1a1) leads to hyperglycemia in mice.

Untreated diabetes leads to several secondary problems, including kidney failure and diabetic nephropathy (Sharma *et al.* 2017). The *Drosophila* nephrocyte has anatomical, molecular, and functional similarities to the glomerular podocyte of vertebrates, a cell that forms the main size-selective barrier in the kidney (Weavers *et al.* 2009). A high-sucrose diet, which elevates circulating glucose, damages the nephrocytes, suggesting that *Drosophila* is a valid model for diabetic nephropathy (Na *et al.* 2015). A high-sucrose diet leads to loss of the Nephrin-like protein Sns, which is mediated by a glucosamine-dependent pathway (Na *et al.* 2015). This suggests that inhibition of the HBP might be a strategy to attenuate diabetic nephropathy. A sugar-rich diet and elevated circulating glucose levels also increase the risk of heart disease. In *Drosophila*, high dietary sugars increase cardiac arrhythmias and lead to structural deterioration of heart tissue (Na *et al.* 2013). Similar to the nephrocyte, this sugar-dependent toxicity can be alleviated by inhibition of the hexosamine biosynthesis pathway. These studies underline the possibility of using the fly as a model to test strategies for selective inhibition of the HBP and/or *O*-GlcNAc modification in treating diet-derived organ deterioration.

***Mechanisms of dietary sugar-induced tumor growth:*** An unbalanced diet, obesity, and diabetes increase the risk of cancer (Khandekar *et al.* 2011). In *Drosophila*, specific combinations of oncogenes will produce tumor-like uncontrolled overgrowth, which allows the identification of genetic and dietary modifiers of tumor growth and metastasis (Gonzalez 2013). For example, a high-sugar diet strikingly increases the growth of Ras/Src-transformed tumors (Hirabayashi *et al.* 2013). Furthermore, sugar feeding converts local tumors into metastatic ones, thus significantly increasing their aggressiveness. High-sugar diet feeding activates salt-inducible kinases, which inhibit the growth-suppressing Hippo signaling pathway (Wehr *et al.* 2013; Hirabayashi and Cagan 2015). Consequent activation of the transcriptional cofactor Yorkie, which increases Wingless signaling in addition to driving the expression of growth-promoting and antiapoptotic genes, drives tumorigenesis (Hirabayashi and Cagan 2015). Wingless, in turn, promotes insulin receptor gene expression to promote insulin sensitivity of the tumor tissue, leading to a feed-forward cycle that enhances the malignant phenotypes of the tumors (Hirabayashi *et al.* 2013). While it remains uncertain which aspects of the sugar-induced growth of *Drosophila* tumors can be applied to human cancer, the fact that nutrient sensing and growth control pathways are highly conserved implies that this topic deserves a deeper look. However, the novel concepts emerging from *Drosophila* research should be actively tested in human tumor models.

## Concluding Remarks and Future Prospects

Recent years have brought to light a number of new mechanisms that control *Drosophila* carbohydrate metabolism. These include discoveries of regulatory networks, both intracellular and systemic, which control organismal carbohydrate homeostasis by directing specific metabolic responses in both an organ and cell type-specific manner. *Drosophila* developmental stages have unique metabolic needs, which has enabled research on metabolic reprogramming, for example the shift from the larval biosynthetic growth phase into energy-intensive high OXPHOS metabolism of the adult. Considering the importance of metabolic reprogramming in stem cells and cancer, an increased understanding of such mechanisms may have relevance much beyond *Drosophila* development. High-sugar intake and impaired carbohydrate metabolism are implicated in an increasing number of health conditions within human populations. Key advantages of *Drosophila* disease models are their amenability to a wide range of defined diets, which allows discovery of interactions between genes and nutrients, as well as the powerful genetic toolkit, which can be utilized in unbiased screens.

While research along the aforementioned topics will undoubtedly continue, new questions are likely to emerge. These include specific roles for different dietary sugars. In fact, some existing literature already indicates disparate physiological outcomes for different dietary sugars (Rovenko *et al.* 2015). As carbohydrate metabolism is connected to the whole carbon metabolism of the animal, future studies will likely address the complex interactions between carbohydrate metabolism and other types of nutrients, including macro- and micronutrients. For such questions, the use of fully defined "holidic" *Drosophila* diets will be an asset (Lee and Micchelli 2013; Piper *et al.* 2014). In addition, an important factor affecting animal metabolism is the intestinal microbiota. Interestingly, recent evidence shows that chronic high-sugar feeding influences *Drosophila* microbiota, promoting uracil-secreting bacteria (Whon *et al.* 2017). Although normally considered as pathogenic, these bacteria protect the host against the deleterious effects of a high-sugar diet. This underlines the need for a more careful look into the interactions between *Drosophila* dietary carbohydrates and microbiota. Finally, it should be recognized that the *Drosophila* genus includes a wealth of different species, each metabolically adapted to distinct nutritional conditions. In addition to insight into evolution, understanding the molecular mechanisms that underlie the natural variation of *Drosophila* species will provide a new understanding of the complex regulation of carbohydrate metabolism.

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