

## ADVANCED REVIEW

# Regulation of insulin and adipokinetic hormone/glucagon production in flies

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## Abstract

Metabolic homeostasis is under strict regulation of humoral factors across various taxa. In particular, insulin and glucagon, referred to in *Drosophila* as *Drosophila* insulin-like peptides (DILPs) and adipokinetic hormone (AKH), respectively, are key hormones that regulate metabolism in most metazoa. While much is known about the regulation of DILPs, the mechanisms regulating AKH/glucagon production is still poorly understood. In this review, we describe the various factors that regulate the production of DILPs and AKH and emphasize the need for future studies to decipher how energy homeostasis is governed in *Drosophila*.

This article is categorized under:

Invertebrate Organogenesis > Flies

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## KEYWORDS

AKH, *Drosophila*, hormone, insulin

## 1 | INTRODUCTION

Insulin and glucagon are key mammalian hormones produced from pancreatic islet cells that regulate the mobilization of fat and glucose in an antagonistic fashion (Freychet, 1990; Ganong, 1991). Under high blood sugar conditions, pancreatic  $\beta$ -cells release insulin, which in turn triggers glucose accumulation in the form of glycogen synthesis. Under low blood sugar conditions, insulin is repressed and pancreatic  $\alpha$ -cells release glucagon, which triggers the breakdown of glycogen to free sugars. Glucagon is also a lipolytic hormone that regulates lipid concentrations in plasma, including regulation of fatty acids, ketone bodies, and triglycerides (Schade, Woodside, & Eaton, 1979). Defects in insulin or glucagon pathways can result in various metabolic disorders, including, but not limited to type 2 diabetes, cardiovascular diseases, and cancer (Biddinger & Kahn, 2006). As such, numerous studies over the years have been performed to understand the molecular and cellular basis of insulin and glucagon regulation and signaling.

*Drosophila melanogaster* is a genetically tractable model for understanding the molecular basis of various physiological responses conserved in humans. The *Drosophila* genome encodes eight insulin-like peptides, DILP1–8, and a single glucagon-like hormone, adipokinetic hormone (AKH). Both DILPs and AKH display remarkable functional similarity to their mammalian counterparts (Das & Dobens, 2015; Owusu-Ansah & Perrimon, 2014; Wang, Tulina, Carlin, & Rulifson, 2007; Wu & Brown, 2006). The mammalian pancreas is known to co-opt neuronal transcription programs and resembles neurons in terms of their physiology and function (Arntfield & van der Kooy, 2011). Similarly, insulin-producing cells (IPCs) in the fly brain are neuronal cells that are functionally analogous to mammalian pancreatic  $\beta$ -cells. AKH-producing cells (APCs) in the fly corpora cardiaca (CC) are functional analogs of vertebrate pancreatic  $\alpha$ -cells. The vertebrate pancreas is derived from neuroectoderm. Similar to this, *Drosophila* IPCs are derived from a single pair of neuroblast cells from the neuroectoderm (Wang et al., 2007). APCs are of mesodermal origin (Park, Bustamante, Antonova, McLean, & Kim, 2011).

Because of the similarities between *Drosophila* and mammals with regards to insulin/DILPs and glucagon/AKH, *Drosophila* has become a relevant model system for studying the endocrine control of these hormones. After years of study by multiple labs, we now have a good understanding of *Drosophila* insulin-insulin like growth factor (IGF) signaling, including the regulation of insulin production and secretion (Padmanabha & Baker, 2014); however, our understanding of AKH has lagged behind. Here, we review factors known to regulate the production of DILPs and AKH from IPCs and APCs, respectively, and highlight the need for future work to decipher how energy homeostasis is governed in *Drosophila*.

## 2 | INSULIN AND GLUCAGON IN *DROSOPHILA*

The *Drosophila* genome encodes eight different DILPs, but not all are functional homologs of human insulin. Four of the DILP peptides, DILP1, 2, 3 and 5, are expressed in the IPCs of the fly brain (Brogiolo et al., 2001; Delanoue et al., 2016; Rulifson, Kim, & Nusse, 2002) and show characteristic conservation of cysteine residues and consensus cleavage sites found in human insulin (Figure 1; Blundell & Humbel, 1980; Brogiolo et al., 2001; Grönke, Clarke, Broughton, Andrews, & Partridge, 2010; Seidah & Chrétien, 1997). DILP4 is expressed in the embryonic mesoderm and the larval midgut; its biological function has not been characterized (Brogiolo et al., 2001). DILP6 is most similar to mammalian IGFs and is produced notably in the fat body, a functional homolog of human liver and adipose tissue, to relay growth signals during the non-feeding phase of larval development (Okamoto et al., 2009; Slaidina, Delanoue, Gronke, Partridge, & Léopold, 2009). DILP7 and DILP8 are relaxin-like peptides produced in the fly brain and imaginal discs, respectively (Colombani, Andersen, & Leopold, 2012; Garelli, Gontijo, Miguela, Caparros, & Dominguez, 2012; Yang, Belawat, Hafen, Jan, & Jan, 2008). Hence, unlike mammals, in which a single insulin peptide is expressed in pancreatic  $\beta$ -cells, flies express four DILPs that are functionally similar to human insulin (DILP1, 2, 3 and 5). With regards to glucagon, a subset of fly CC cells produce AKH (Buch, Melcher, Bauer, Katzenberger, & Pankratz, 2008; Rulifson et al., 2002), which is eight amino acids long. AKH has partial amino acid similarity to human glucagon and shares functional similarity (Figure 1; Noyes, Katz, & Schaffer, 1995; Schaffer, Noyes, Slaughter, Thorne, & Gaskell, 1990). In addition to the limited sequence and structural similarity of AKH, its receptor (AkhR) belongs to the GPCR family which is related to gonadotropin-releasing hormone (GnRH) receptor (Lindemans et al., 2009). Thus, AKH is considered to be a functional GnRH analog (Hauser & Grimmelikhuijzen, 2014).

### 2.1 | Larval versus adult endocrine system

In adult flies, sensing of dietary sugars is under the direct control of DILPs; however, in larvae, DILP3 secretion in response to sugars is AKH-dependent (J. Kim & Neufeld, 2015). This difference might be due to differences in the locations of APCs and IPCs in larvae versus adults. In both larvae and adults, IPCs are located in brain median neurosecretory cells. In larvae,

Human	-----MALWMRL-----L-PLLALLALWGPDPAAAFVNQHL <b>C</b> GS <del>H</del> LVEALYLV	42
DILP2	-----MSKPLSFI-SMVAVILLAS--STVKLAQ <b>G</b> TLCSEKLNEVLSMV	40
DILP3	-----MGIEMRCDRRILLPS-LLLLILMIGG----VQATMK <b>L</b> CGRKLPE TLSKL	45
DILP1	MFSQHNGAAVHGLRLQSLIIAAMLTAAMAMVTPTGS <b>G</b> HQLLPNGHKL <b>C</b> GPALSDAMDVV	60
DILP5	MF-----RSV-IPV-----LLFLIPLL--LSAQAA <b>N</b> SLRA <b>C</b> GPALMDMLRVA	39
	*	
Human	<b>C</b> GERGFFYTPKTRREA-----EDLQ-----	62
DILP2	<b>C</b> E EY-NPVI PHKRAMPGADSDLDALNPLQFVQEFEEEDNSISEPLRSA-----LFPGSY	93
DILP3	<b>C</b> VY <b>G</b> -FNAMTKR <b>T</b> LDPVNFN <b>Q</b> IDGFEDR-----SLLERLL	79
DILP1	<b>C</b> PH <b>G</b> -FNTLPRKRESLLGNSDDDEDTEQEV-----EDDSSM <b>W</b> QTLDGAGYSFSPLLTNLY	114
DILP5	<b>C</b> PNG-FNSMFAK <b>R</b> G <b>T</b> LGLFDYEDHLADLDS-----SE-S-----	71
	*	
Human	--GSLQPL--ALEGSLQKRGIVE <b>Q</b> CC <b>T</b> SI <b>C</b> SLYQLENYCN-----	98
DILP2	LGGVLSLA <b>E</b> VRRRTR <b>R</b> Q <b>G</b> IVER <b>C</b> CK <b>K</b> SCDMKALREY <b>C</b> SVVRN-	137
DILP3	SDSS-VQM---L <b>K</b> TRRLRD <b>G</b> V <b>F</b> DE <b>C</b> CL <b>K</b> SC <b>T</b> MD <b>E</b> VL <b>R</b> Y <b>C</b> AAK <b>P</b> RT	120
DILP1	GSEVL <b>I</b> K <b>M</b> --RRHRRHL <b>T</b> GGV <b>Y</b> DE <b>C</b> CV <b>K</b> T <b>C</b> S <b>Y</b> LELAI <b>Y</b> CL <b>P</b> K---	154
DILP5	--HHMNSL--SSIRRD <b>F</b> RGV <b>V</b> DS <b>C</b> CR <b>K</b> SC <b>S</b> FS <b>T</b> L <b>R</b> AY <b>C</b> DS----	107
	** * *	

\*Conserved cystine residues

Human	HSQ <b>C</b> T <b>F</b> T <b>S</b> D <b>Y</b> SKYLD <b>S</b> RR <b>A</b> Q <b>D</b> FV <b>Q</b> W <b>L</b> M <b>N</b> T	29
<i>Drosophila</i>	--EL <b>T</b> F <b>S</b> P <b>D</b> W-----	8
	** *	

\*Conserved residues

**FIGURE 1** (a) Amino acid sequence alignment of DILP1, 2, 3 and 5 with human insulin. Conserved characteristic of cystine residues have been highlighted. (b) Amino acid sequence alignment of fly AKH with human glucagon

the APCs are part of the ring gland, which is located between the two hemispheres of the larval brain and is made up of the CC, the corpora allata (CA), and the prothoracic gland (PG). During metamorphosis, the CC and CA separate from the PG and migrate to a location near the junction of the crop and the midgut (Gilbert, 1991). Thus, whereas in larvae, the CC is near the brain, in adults, the CC is near the esophagus (Toivonen & Partridge, 2009). This rearrangement of tissue organization may account for the different modes of insulin secretion regulation in larvae versus adults. To establish this, it will be important to elucidate the differences in nutrition-mediated responses of APCs in larvae versus adults.

### 3 | FACTORS REGULATING IPCs AND DILP PRODUCTION

Over the years, a number of studies have identified various factors that regulate the production of DILPs from IPCs (Table 1). Some of the factors have been summarized by previous reviews (Droujinine & Perrimon, 2016; Nässel, Kubrak, Liu, Luo, & Lushchak, 2013). Incorporating the latest literature, we grouped these factors into four broad categories: dietary nutrition-induced factors, neurotransmitters, neuropeptides, and hormonal factors.

#### 3.1 | Dietary nutrition-induced factors

Insulin secretion by IPCs is stimulated in a nutrition-dependent manner. Macronutrients including dietary sugars, fats, and amino acids are sensed by the target of rapamycin (TOR) signaling pathway in the fat body, which then releases factors into the circulation that stimulate the IPCs in an indirect manner (Colombani et al., 2003; Rajan & Perrimon, 2012). Dietary sugar and fat stimulate the larval fat body to release the Leptin-like peptide Unpaired 2 (Upd2), which binds to its receptor, Dome, on GABAergic neurons (Rajan & Perrimon, 2012). GABA inhibits the release of DILPs from IPCs (Enell, Kapan, Söderberg, Kahsai, & Nässel, 2010) and Upd2-Dome signaling releases this inhibition of IPCs by GABAergic neurons. Hence, nutrition drives stimulation of IPCs via the fat body. In adult flies, sugars directly stimulate  $Ca^{2+}$  in IPCs, suggesting that these cells sense glucose by a mechanism similar to mammalian pancreatic  $\beta$ -cells (Kr neisz, Chen, Fridell, & Mulkey, 2010; Park et al., 2014). Dietary amino acids also regulate larval IPCs, acting remotely via the fat body to regulate the release of DILPs (G minard et al., 2009). Sensing of dietary amino acids in particular results in stimulation of TOR signaling in the larval fat body, which then remotely stimulates DILP secretion by IPCs (Colombani et al., 2003; G minard et al., 2009). Two factors released from the fat body in response to dietary amino acids have been identified, Stunted (Sun) (Delanoue et al., 2016) and growth-blocking peptides (GBPs; Koyama & Mirth, 2016). Sun binds to Methuselah, a secretin-incretin receptor on IPCs, stimulating the release of DILPs and hence activating organ growth. In addition, GBP1 and CG11395 (GBP2) are secreted from the fat body when TOR activity is high to regulate IPCs (Koyama & Mirth, 2016). Interestingly, GBPs encode atypical ligands for the fly EGF receptor (EGFR; Meschi, L opold, & Delanoue, 2019). In a mechanism reminiscent of Upd2-Dome signaling, which releases the inhibition of IPCs by GABAergic neurons, activation of EGFR in a subpopulation of inhibitory neurons contacting the IPCs in response to fat body-derived GBPs allows for insulin secretion. An interesting question that remains to be addressed is whether, and how, specific amino acids stimulate the release of different factors from the fat body. Finally, dietary protein-specific regulation of insulin signaling has also been reported to stimulate protein-induced feeding inhibition in adult flies (Sun et al., 2017). Upon consumption of a protein-rich diet, a fat body-specific peptide called female-specific independent of transformer (FIT) is secreted. FIT acts peripherally on the IPCs to induce the release of DILP2, resulting in reduced feeding in adults.

#### 3.2 | Neurotransmitters

Some aminergic neurotransmitters, including serotonin, dopamine, and octopamine, regulate IPCs. Serotonergic neurons in the fly brain express NS3, a nucleostemin family GTPase that controls growth and body size by stimulating the release of DILPs from IPCs (Kaplan et al., 2008). Disruption of *ns3* leads to a build-up of DILP2 levels in the brain, consequently impairing larval growth and adult body size, suggesting that NS3 may act upstream of IPCs to regulate DILP2 levels. Although serotonergic neurons are closely juxtaposed to the IPCs, no direct connection between these two types of neurons has been reported. However, a direct role of serotonin in regulating IPCs has been established in other studies (Luo et al., 2012, 2014) and serotonin has been proposed to control DILPs via its 5-HT<sub>1A</sub> receptor, which is expressed in IPCs (Luo et al., 2012) to control DILP secretion. Knockdown of 5-HT<sub>1A</sub> results in reduced resistance to starvation, altered lipid metabolism, and an increase in the secretion of DILP2 and 5 (Luo et al., 2012, 2014). Surprisingly, however, under these conditions, no effect on growth has been observed. This can likely be explained by the finding that the 5-HT<sub>1A</sub> receptor is only expressed in adults and absent in larval IPCs when major nutrition-dependent growth occurs (Luo et al., 2012).

**TABLE 1** Factors regulating insulin-producing cells (IPCs) and AKH-producing cells (APCs) in fly larvae and adults

Factors regulating IPCs	Stage	References
Dietary sugars and fat	Larva and adult	Rajan and Perrimon (2012)
Dietary proteins	Larva and adult	Colombani et al. (2003), Delanoue et al. (2016), G�eminard, Rulifson, and L�eopold (2009), Koyama and Mirth (2016)
Neuropeptide F	Larva and adult	Kapan, Lushchak, Luo, and N�assel (2012), K.-S. Lee et al. (2008)
Serotonin	Larva and adult	Kaplan, Zimmermann, Suyama, Meyer, and Scott (2008), Luo, Becnel, Nichols, and N�assel (2012), Luo, Lushchak, Goergen, Williams, and N�assel (2014)
<i>Drosophila</i> tachykinin-related peptides	Larva and adult	Birse, Soderberg, Luo, Winther, and Nassel (2011)
Octopamine	Adult	Crocker, Shahidullah, Levitan, and Sehgal (2010), Luo et al. (2014)
Dopamine	Adult	Andreatta, Kyriacou, Flatt, and Costa (2018)
CCHamide-2	Larva and adult	Sano et al. (2015)
Leucokinin	Larva and adult	Yurgel et al. (2019), Zandawala et al. (2018)
Limostatin (Lst)	Adult	Alfa et al. (2015)
Adiponectin receptor (dAdipoR)	Larva and adult	Kwak et al. (2013)
Female-specific independent of transformer	Adult	Sun et al. (2017)
Allatostatin A (AstA)	Larva and adult	Hentze, Carlsson, Kondo, N�assel, and Rewitz (2015)
AKH	Larva	J. Kim and Neufeld (2015)
Dawdle (Daw)	Larva	Ghosh and O'Connor (2014)
Glass bottom boat (Gbb)	Larva	Ballard, Jarolimova, and Wharton (2010)
Stunted (Sun)	Larva	Delanoue et al. (2016)
Eiger	Larva and adult	Agrawal et al. (2016)
Growth-blocking peptide	Larva and adult	Koyama and Mirth (2016)
DILP6	Adult	Bai, Kang, and Tatar (2012)
DILP3	Adult	Gr�onke et al. (2010)
Factors regulating APCs	Stage	References
Dietary sugars	Larva	J. Kim and Neufeld (2015)
Insulin-like peptide 2 (DILP2)	Larva and adult	Post et al. (2018), Rulifson et al. (2002)
Pickpocket 28 (ppk28)	Adult	Waterson et al. (2014)
AKH negative feedback autoregulation	Adult	G�alikov�a et al. (2015)
TRPA1	Adult	Guntur et al. (2015)
Bursicon �	Adult	Scopelliti et al. (2019)
Allatostatin A (AstA)	Larva and adult	Hentze et al. (2015)
AMP-activated protein kinase	Larva and adult	Braco, Gillespie, Alberto, Brenman, and Johnson (2012)
Unpaired 2 (Upd2)	Adult	Zhao and Karpac (2017)

Insulin signaling is also known to interact with dopamine signaling (Gruntenko et al., 2016; Rauschenbach et al., 2015, 2017). Dopamine acts directly on IPCs via its receptor (Andreatta et al., 2018). Under normal conditions, DILP2 and DILP5 control ovarian growth and reproduction in females. Dopamine promotes ovarian dormancy via its receptor DopR1 expressed in IPCs in females. *DopR1* knockdown in IPCs reduces dormancy in flies by attenuating protein kinase cAMP-dependent signaling in IPCs. It will be interesting to learn if this response is sex-specific, that is, if *DopR1* is also expressed in male IPCs.

Octopamine regulates IPCs via a cAMP-dependent octopamine receptor, OAMB (Crocker et al., 2010; Luo et al., 2014). Octopamine-mediated stimulation of IPCs alters sleep by increasing cAMP and regulating K<sup>+</sup> currents in DILP2-expressing neurons. Moreover, octopamine increases cAMP in IPCs and knockdown of *OAMB* results in elevated *dilp3* transcript levels. Despite this understanding, whether regulation of IPCs by octopamine is direct or indirect is not clear.

### 3.3 | Neuropeptides

A number of neuropeptides have been reported to regulate IPCs. *Drosophila* short neuropeptide F (sNPF) modulates extracellular signal-related kinases in IPCs by acting through its receptor, sNPF1, resulting in expression of *dilps* (Kapan et al., 2012; K.-S. Lee et al., 2008). This sNPF-mediated increase in insulin secretion affects growth, metabolism, and lifespan through dFOXO signaling in the fat body. In addition, targeted manipulation of *sNPF1* in IPCs alters insulin signaling (K.-S. Lee et al., 2008). sNPF is produced in dorsal lateral peptidergic neurons (DLPs), which simultaneously produces the neuropeptide corazonin (CRZ) and have axons that impinge on the IPCs (Kapan et al., 2012). Whereas knockdown of both *sNPF* and *CRZ* results in improved survival of flies under starvation and alters carbohydrate and lipid metabolism, knockdown of *sNPF*, but not *CRZ*, decreases *dilp2* and *dilp5* transcript levels in the IPCs, suggesting that these co-released peptides might regulate DILPs via different mechanisms of action on IPCs. In addition, activation by *Drosophila* tachykinin-related peptides (DTKs) of their receptor, DTKR, also regulate IPCs (Birse et al., 2011). DTKR is expressed in IPCs. Activation of DTKR inhibits insulin signaling by reducing *dilp2* and *dilp3* transcript levels. Furthermore, knockdown of *DTKR* results in increased insulin signaling, which in turn results in increased circulating sugar levels in the fly blood. The CCHamide-2 (CCHa2) neuropeptide regulates IPCs and DILPs in a nutrition-dependent manner (Sano et al., 2015). CCHa2 is expressed in peripheral tissues, mainly the fat body and gut, whereas its receptor (CCHa2R) is expressed in IPCs in the larval brain (Sano et al., 2015). CCHa2 mutant larvae have reduced expression of *dilp5* and  $Ca^{2+}$  activity in IPCs. Furthermore, CCHa2 incubation in ex vivo culture triggers calcium waves in IPCs. When released, CCHa2 peptide acts on IPCs via its receptor, CCHa2R, mediating the release of DILPs. Another neuropeptide controlling IPCs stimulation is leucokinin, whose receptor, Lkr, is expressed on the surface of IPCs (Zandawala et al., 2018). Knockdown of *Lkr* in IPCs results in altered *dilp* transcription in a diet-specific manner, suggesting that leucokinin interacts with insulin signaling to control metabolism and stress response. In addition, starvation-induced sleep in flies is also under the control of leucokinin neurons-IPC connectivity (Yurgel et al., 2019). Finally, the neuropeptide Allatostatin A (AstA) positively regulates expression of *dilp3* in IPCs (Hentze et al., 2015). *Dar-2* (*AstA-R2*), the receptor for AstA, is expressed in IPCs. Excitation of AstA-producing neurons results in increased expression of both *dilp2* and *dilp3* in IPCs. In addition, knockdown of *Dar-2* in IPCs results in increased starvation resistance and affects key metabolic genes primarily mediated by insulin signaling in peripheral tissues, including *tobi* and *4EBP*.

### 3.4 | Humoral factors

Some hormones also govern DILPs production in IPCs. For example, the fat body derived hormone, Eiger, a *Drosophila* TNF- $\alpha$  ortholog, acts on the IPCs in a nutrition-dependent manner (Agrawal et al., 2016). Under nutrition-deprived conditions, Eiger is produced from the fat body and binds to its receptor, Grindelwald, on IPCs. This results in JNK-dependent inhibition of DILPs production. In addition, a human Decretin hormone ortholog, Limostatin (Lst), negatively regulates IPCs (Alfa et al., 2015). Lst is expressed in APCs in the CC under starvation conditions, binds to IPCs, and blocks insulin signaling through binding to its GPCR-family receptor, which is encoded by CG9918. Consistent with this, Lst loss-of-function mutant animals accumulate significantly higher than normal levels of DILP2 in the hemolymph. A larval fat-derived protein, Glass bottom boat (Gbb), is a TGF- $\beta$  family member that remotely controls the expression of *dilp2* mRNA (Ballard et al., 2010). Larvae mutant for *gbb* have elevated levels of *dilp2*. Furthermore, Gbb regulates amino acid uptake and lipid hydrolysis in larvae. However, it is unclear how Gbb controls IPCs from peripheral tissues. DILP6 is also known to negatively regulate *dilp2* transcription and secretion (Bai et al., 2012). *dilp6* transcription is under the control of the insulin-regulated transcription factor dFOXO, which positively modulates *dilp6* mRNA in the adult fat body. *dilp6* overexpression results in reduced *dilp2* transcripts and DILP2 peptide in IPC cell bodies and improves longevity. Thus, DILP6 appears to bridge dFOXO, peripheral fat tissue, and brain endocrine function via regulation of the IPCs. In addition, DILP3 also regulates the expression of *dilp2* in a feedback manner in the IPCs (Grönke et al., 2010). Finally, expression of *dilp2* and *dilp5* is downregulated in *dilp3* mutant flies, suggesting that DILP3 may act as a positive regulator of *dilp2* and *dilp5* in IPCs.

### 3.5 | Other factor(s)

Additional factors have been shown to regulate DILPs, but what they sense remains unclear. *Drosophila* adiponectin receptor (dAdipoR) is expressed in IPCs and regulates insulin secretion (Kwak et al., 2013). Inhibiting *dAdipoR* results in elevated sugar levels in the hemolymph and increased triglyceride levels. Furthermore, *dAdipoR* inhibition also results in accumulation of DILP2 in IPCs. Thus, dAdipoR appears to regulate the secretion of DILPs. However, the upstream regulator(s) of dAdipoR remains to be identified.

The *Drosophila* TGF- $\beta$ /Activin-like ligand Dawdle (Daw) affects insulin signaling by regulating the release of DILPs from larval IPCs (Ghosh & O'Connor, 2014). Loss of Daw does not affect *dilp* mRNA levels but does lead to accumulation of DILP2 in IPC cell bodies. It is not clear whether Daw controls DILP2 release by directly acting on IPCs or acts through a peripheral tissue. *Daw* is expressed in an array of tissues including muscle, fat body, gut, imaginal discs, and CNS (Parker, Ellis, Nguyen, & Arora, 2006; Serpe & O'Connor, 2006); however, what tissue(s) alter Daw production in response to dietary changes is not completely understood.

Although these factors control IPC-regulated *dilp* transcription, they are not necessarily involved regulating the release of DILPs from IPCs. Direct evidence for regulation of DILPs release has been limited. Recent studies have used genetic labeling of DILP2 with immunopeptides, which allows for ELISA-based quantification of circulating DILP2 levels, even at the picomolar concentrations normally found in the fly hemolymph (Park et al., 2014). Such approach will be important to characterize factors directly involved in the release of DILPs from IPCs.

### 3.6 | Factors regulating DILPs after release

A number of peptides have been identified that interact with secreted DILPs to regulate their post-release activity. Some of these facilitate insulin signaling by acting as transporter proteins. Others act as DILP antagonists. One such antagonistic factor is imaginal morphogenesis protein-late 2 (ImpL2). ImpL2 is primarily known as a downstream target of 20-hydroxyecdysone (Osterbur, Fristrom, Natzle, Tojo, & Fristrom, 1988; Zapf, Schoenle, & Froesch, 1985). However, recent reports have shown that it interacts with DILPs in an antagonist manner (Honegger et al., 2008). This ImpL2-DILP interaction is essential for starvation resistance in flies. ImpL2 has amino acid similarity to the carboxy-terminal immunoglobulin-like domains of the human tumor suppressor IGFBP-7. Overexpression of ImpL2 improves longevity in flies (Alic, Hoddinott, Vinti, & Partridge, 2011). Two recent studies have also identified the role of fly ImpL2 in organ wasting under nutritional stress. One study showed elevated levels of ImpL2 from over proliferating gut tissue (Kwon et al., 2015). This augmented ImpL2 results in reduced insulin signaling and organ wasting, including wasting of muscle, the fat body and the ovary. These phenotypes are rescued by the loss-of-function mutation of *Imp-L2*. In the other study, organ wasting was induced by transplantation of imaginal disc tumors. These tumors were found to secrete ImpL2, which triggers a wasting phenotype in adipose, muscular, and gonad tissues by interrupting insulin signaling (Figueroa-Clavevega & Bilder, 2015). The restricted role of ImpL2 is also known to enhance insulin signaling in a distinct subset of neurons in the larval brain (Bader et al., 2013). These IPC-innervated targeted neurons express ImpL2, which facilitates DILP2 uptake in these neurons. A fly homolog of vertebrate IGF-binding protein acid-labile subunit (known as IGFALS or ALS) has also been identified to interact with DILPs (Arquier et al., 2008). Similar to vertebrates, dALS partners with DILPs and ImpL2 to form a trimeric complex found in circulation. dALS is expressed in the larval IPCs and fat body, and is known to antagonize the role of DILPs in regulating metabolism and growth (Arquier et al., 2008).

Certain peptides also interact with DILPs independently of ImpL2. One example is secreted decoy of insulin receptor (Sdr), which has structural similarity to the extracellular domain of the fly insulin receptor (InR), which directly interacts with DILPs (Okamoto et al., 2013). The Sdr-DILP interaction halts larval organ growth. Consistent with this, *Sdr* knockdown reverses growth inhibition. Based on the available evidence, Sdr is thought to function as a negative regulator of DILPs in flies (Okamoto et al., 2013). Unlike ImpL2, Sdr expression is not affected by the nutritional state of larvae.

Similar to humans, another group of antagonizing peptides, insulin-degrading enzymes (IDEs), are also known to interact with DILPs in flies (Tsuda, Kobayashi, Matsuo, & Aigaki, 2010). *Ide* knockout in IPCs results in reduced circulating trehalose levels in the hemolymph, improved fecundity and longevity, suggesting elevated levels of DILPs in circulation (Hyun & Hashimoto, 2011). Despite this reported evidence, the mechanism for IDE-mediated DILP degradation is still lacking.

## 4 | FACTORS REGULATING APCs

### 4.1 | Dietary nutrition-induced factors

Limited studies have been conducted to identify factors regulating AKH production or release by APCs. Sugars are known to positively stimulate APCs to release AKH in larvae (J. Kim & Neufeld, 2015); however, such observations have not been reported for adults.

Insulin signaling also regulates APCs. DILP2 released by IPCs accumulates in larval APCs (Rulifson et al., 2002). Although DILP2 is known to induce transcription of *Akh* mRNA in a DILP1-dependent manner in adults (Post et al., 2018), precisely how DILP2 regulates AKH production by APCs is unclear.

## 4.2 | Physiological factors

Physiological parameters such as gustation and odor perception are known to stimulate APCs. A critical water sensor that is encoded by the *pickpocket 28* (*ppk28*) gene and expressed in water sensing-gustatory neurons has been shown to regulate APCs in adult flies (Jourjine, Mullaney, Mann, & Scott, 2016; Waterson et al., 2014). Loss of *ppk28* results in an increase of AKH production, resulting in activation of AKH signaling and an increase in lipid reserves. The TrpA1 channel in flies detects UV radiation via sensing of light-induced production of H<sub>2</sub>O<sub>2</sub>. APCs express a H<sub>2</sub>O<sub>2</sub>-sensitive TrpA1 isoform and show a strong calcium response upon light and H<sub>2</sub>O<sub>2</sub> stimulation (Guntur et al., 2015). Both these responses are reduced in *dTRPA1* mutants, suggesting that UV light stimulates APCs via TrpA1-conferred H<sub>2</sub>O<sub>2</sub> production in both larvae and adults. AKH is known to accelerate heart rate and regulate sugar levels in hemolymph (Baumann & Gersch, 1982; Noyes et al., 1995; Scarborough et al., 1984), and UV is known to be an aversive stimulus for larvae (Sawin, Harris, Campos, & Sokolowski, 1994; Xiang et al., 2010), suggesting that UV sensitivity in APCs may act as a stress response in young larvae when exposed to strong radiation.

## 4.3 | Hormones

Enteroendocrine cells in the adult fly intestine respond to ingested nutrients by secreting Bursicon  $\alpha$ , a hormone that subsequently binds to its neuronal receptor, DLgr2 (also known as Rickets) (Scopelliti et al., 2019). Bursicon  $\alpha$ /DLgr2 binding leads to inhibition of AKH production in APCs, thereby repressing AKH signaling in the fat body. Impairing Bursicon  $\alpha$ /DLgr2 signaling in adult flies exacerbates oxidative metabolism and lipodystrophy.

## 4.4 | Neuropeptides

Similarly, what has been found for IPCs, the neuropeptide AstA also regulates APCs (Hentze et al., 2015). Its receptor, Dar-2 (also known as AstA-R2), is expressed in both IPCs and APCs. Although *Dar-2* knockdown does not influence the expression levels of *Akh*, it does lead to an increase in expression of *AkhR*, suggesting that AstA, which is differentially affected in response to dietary sugars and proteins, might regulate a balance between AKH and insulin signaling to mediate energy homeostasis. Stimulation of AstA increases the fly's preference for a protein-rich diet, whereas loss of AstA results in a preference for dietary sugars. Which tissue secretes AstA in response to ingested nutrition remains unknown. Although little is known about neuropeptide-mediated regulation of APCs in *Drosophila*, previous studies in locusts have identified peptides that control AKH release from the APCs. These peptides include tachykinin, crustacean cardioactive peptide, SchistoFLRF amide (myosuppressin), Proctolin and octopamine (Clark, Zhang, Tobe, & Lange, 2006; Nässel et al., 1995; Passier, Vullings, Diederer, & Van Horst, 1995; Veelaert et al., 1997; Vullings et al., 1998). These studies can guide future work to identify various neuropeptides that might regulate APCs in flies.

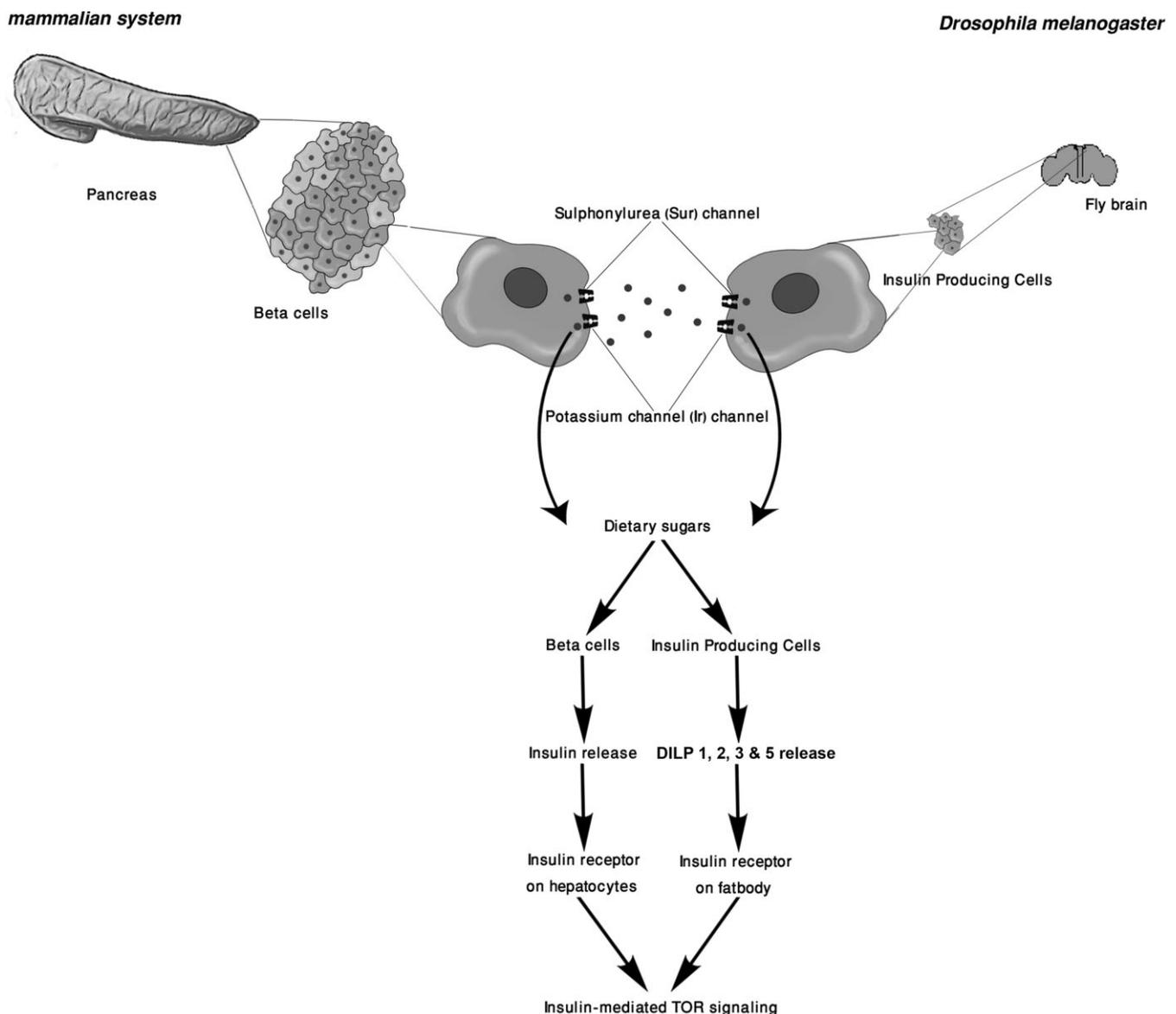
## 4.5 | Other factors

As observed for glucagon, *Akh* transcription is negatively regulated by AKH signaling (Gáliková et al., 2015; Gelling et al., 2003). When GFP is expressed under the indirect control of the *Akh* promoter using UAS/Gal4, loss of AKH signaling is associated with an increase in GFP signal, indicating higher *Akh* promoter activity in the absence of AKH signaling (Gáliková et al., 2015). In addition, AMP-activated protein kinase (AMPK) in APCs is critical for promoting normal AKH secretion (Braco et al., 2012). In line with this, a reduction of AMPK in APCs phenocopies APCs ablation, *Akh* knockdown, and deletion of the *AkhR*, in the context of reduced AKH secretion. Finally, a skeletal muscle-derived cytokine, Upd2 stimulates AKH secretion from APCs in adult flies (Zhao & Karpac, 2017). This Upd2/AKH inter-tissue communication controls systemic lipid homeostasis by stimulating lipogenesis in the fat body.

## 4.6 | Noncanonical role of AKH

It is largely believed that AKH is the functional homolog of vertebrate glucagon, as AKH is vital for regulating fat mobilization and disaccharide trehalose production under energy-demanding conditions (S. K. Kim & Rulifson, 2004; G. Lee & Park, 2004). However, some reports have challenged this view, as AKH is dispensable for lipid homeostasis in the third instar larval stage (Gáliková et al., 2015; Yamada, Habara, Kubo, & Nishimura, 2018) and as AKH can stimulate IPCs in a nutrition-sensitive manner (J. Kim & Neufeld, 2015). Furthermore, dietary sugar levels positively regulate APCs in the larval CC and

AKH stimulates IPCs to release DILP3 (J. Kim & Neufeld, 2015). RNAi-mediated knockdown of *Akh*, or a null mutation of *AkhR*, results in TOR inhibition in adipose tissues. Conversely, overexpression of AKH elevates TOR signaling in fat. Altogether, these results suggest that AKH secretion is an essential part of sugar-triggered signaling. One plausible explanation comes from the observation in mammals that glucose-sensitive cells in pancreatic  $\beta$ -cells express the ATP-sensitive potassium channels Sur and Ir (Figure 2; Aguilar-Bryan et al., 1995; S. K. Kim & Rulifson, 2004). Unlike adult flies, where the fly orthologs of these channels are expressed in IPCs (Fridell et al., 2009), in larvae, these channels are expressed in the APCs in the CC cells (S. K. Kim & Rulifson, 2004). However, there are contradicting reports with regards to the activation of APCs under different trehalose concentrations (J. Kim & Neufeld, 2015; S. K. Kim & Rulifson, 2004). One report describes the stimulation of  $Ca^{2+}$  in APCs in response to low trehalose (S. K. Kim & Rulifson, 2004), whereas another study reports the release of AKH peptides under high trehalose (J. Kim & Neufeld, 2015). Future work is required to systematically measure AKH release under different trehalose concentrations. In light of the current literature, it appears that the fly adult DILP–AKH system is more similar to the mammalian insulin–glucagon system than the larval system is. Indeed, APC–IPC interactions in *Drosophila* might be more complex than previously thought and warrant more investigation. Finally, apart from regulating metabolism in flies, AKH signaling has been also reported to control locomotor activity in *Drosophila* (G. Lee & Park, 2004).



**FIGURE 2** Conserved mechanism of glucose sensing in *Drosophila* and mammals. Mammals express ATP-sensitive potassium channels on pancreatic  $\beta$ -cells, enabling them to sense dietary sugars and produce insulin. Similarly, *Drosophila* expresses these orthologous channels on insulin-producing cells, which subsequently produce DILPs. Metabolic signaling converges on insulin-mediated TOR signaling

Flies exhibit prolonged hyperactivity under starvation conditions, which is believed to be associated with increased foraging. In contrast, flies devoid of AKH do not exhibit this type of hyperactivity.

## 5 | CONCLUSION

Misregulation of glucagon can result in various diseases, including hyperglucagonemia, hypoglycemia, idiopathic diabetes mellitus, and renal and hepatic disease (Trimble, 1976). This underscores the need for better understanding of the various regulatory mechanisms controlling glucagon. Here, we reviewed DILP and AKH regulation in *Drosophila* and highlight the fact that, in contrast to DILP production, which is known to be governed by many pathways, only a few mechanisms have been discovered to regulate AKH production. Identifying additional pathways that regulate AKH is likely to reveal important new insights into the mechanisms of metabolic homeostasis. The proglucagon (*GCG*) gene encodes precursors of glucagon peptides in humans. Three major hormones are encoded by *GCG*: glucagon, glucagon-like peptide 1 (GLP1) and glucagon-like peptide 2 (Kieffer & Francis Habener, 1999). GLP1 is an incretin hormone that regulates insulin release in  $\beta$ -cells in a nutrition-dependent manner (Baggio & Drucker, 2007; Holst, 2007; Meier & Nauck, 2005). Similarly, AKH also controls DILP release in flies in response to dietary sugars (J. Kim & Neufeld, 2015). However, empirical evidence of structural and functional homology of AKH and GLP1 is still lacking. In addition, similar to *GCG*, AKH prohormone is also processed into two different mature hormones, AKH and APRP. However, most studies have solely focused on AKH and the function of APRP remains uncharacterized (Gáliková et al., 2015). It will be interesting in future studies to examine the role and function of APRP in energy homeostasis.

It remains to be determined whether and how dietary proteins stimulate hormone production and secretion by IPCs and APCs and their coordinate regulation. Although recent studies have started to provide insights on protein-mediated control of the AKH-insulin endocrine system (Buch et al., 2008), much remains to be understood. For example, as one report suggests, dietary proteins have no effect on AKH levels under starvation conditions (Mochanová, Tomčala, Svobodová, & Kodrčík, 2018). Moreover, in addition to dietary sugars and proteins, fats have also been reported to regulate insulin signaling via hormonal control by peripheral tissues (Rajan & Perrimon, 2012). However, any potential role of dietary fats in the regulation of APCs remains elusive. Finally, the role in metabolic homeostasis of fly oenocytes, the equivalent of human hepatocytes that mobilize lipids from fat body and breakdown free fatty acids into ketone bodies (Billeter, Atallah, Krupp, Millar, & Levine, 2009; Gutierrez, Wiggins, Fielding, & Gould, 2007), is largely uncharacterized. These specialized cells might also release factors that regulate IPCs and/or APCs. In addition, it is likely that many factors released from peripheral organs, that is, gut and muscle tissues, that regulate the activity of IPC or APCs remain to be identified.

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## CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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