



Physiological and Pathological Regulation of Peripheral Metabolism by Gut-Peptide Hormones in *Drosophila*

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The gastrointestinal (GI) tract in both vertebrates and invertebrates is now recognized as a major source of signals modulating, *via* gut-peptide hormones, the metabolic activities of peripheral organs, and carbo-lipid balance. Key advances in the understanding of metabolic functions of gut-peptide hormones and their mediated interorgan communication have been made using *Drosophila* as a model organism, given its powerful genetic tools and conserved metabolic regulation. Here, we summarize recent studies exploring peptide hormones that are involved in the communication between the midgut and other peripheral organs/tissues during feeding conditions. We also highlight the emerging impacts of fly gut-peptide hormones on stress sensing and carbo-lipid metabolism in various disease models, such as energy overload, pathogen infection, and tumor progression. Due to the functional similarity of intestine and its derived peptide hormones between *Drosophila* and mammals, it can be anticipated that findings obtained in the fly system will have important implications for the understanding of human physiology and pathology.

Keywords: gut hormone, *Drosophila*, metabolism and endocrinology, disease model, nutrient sensing, gut bacteria, tumor-induced wasting, stress sensing

INTRODUCTION

More than 100 bioactive gut-peptide hormones are produced by enteroendocrine cells (EEs) in the gastrointestinal (GI) tract, which is thus considered as the biggest endocrine organ in vertebrates (Sun et al., 2018). Emerging therapies, which are based on gut-peptide hormones and have been proven to be efficient in the treatment of metabolic disorders (Alexiadou et al., 2019), have drawn increasing attention to the gut-peptide-hormone modulation of systemic energy balance, including carbo-lipid metabolism in the liver, adipose tissue, muscle, heart, kidney, pancreas, bone, immune cells, as well as brain (Martin et al., 2019). The *Drosophila* intestine exhibits high similarities with the mammalian GI tract, not only in structure and physiology (Figure 1), but also in the production of gut-peptide hormones and its impact on metabolism homeostasis (Miguel-Aliaga et al., 2018). Research using the *Drosophila* system has addressed several fundamental issues regarding the function and regulation of gut-peptide hormones. In this review, we will summarize the types, metabolic impacts, stress sensing, and

participating signaling pathways of adult fly gut-peptide hormones under both physiological and pathological conditions.

TYPES AND FUNCTIONS OF *DROSOPHILA* GUT-PEPTIDE HORMONES

The luminal surface of the *Drosophila* adult intestine comprises four cell types (Figure 1B): intestinal stem cells (ISCs), enteroblasts (EBs), enterocytes (ECs), and enteroendocrine cells (EEs). The epithelium is covered and protected by a peritrophic membrane (PM), equivalent to human mucus, from intestinal microbes. The epithelial monolayer is aligned on its basal side on the basement membrane (BM). There are visceral muscles (VMs) that drive peristaltic movements, trachea that provide

oxygen, and innervated neurons, underneath the BM (Miguel-Aliaga et al., 2018). Similar to that of vertebrates, the majority of gut-peptide hormones are produced by the fly EEs to target distal organs (Reiher et al., 2011; Liu and Jin, 2017). Interestingly, growing evidence indicates that other cell types, like ECs and VMs, also produce bioactive peptides in response to extracellular stresses, many of which have shown systemic metabolic influences and are considered as novel gut-peptide hormones (Figure 1C).

EE-Derived-Peptide Hormones

A few established proproteins, like allatostatin A (AstA), AstB/Mip, AstC, neuropeptide F (NPF), short neuropeptide F (sNPF), tachykinin (TK), diuretic hormone 31 (DH31), and CCHamides 1 (CCHa1) and CCHa2, which can be processed into over 20 mature peptides, are produced by both larval and adult EEs as shown using antibody detection and proteomic analysis (Veenstra et al., 2008; Veenstra, 2009; Reiher et al., 2011). The protein maturation of the prohormones into multiple bioactive peptides is processed by a conserved prohormone convertase Amon and, probably, other putative enzyme homologs, like dCPD, Phm, and Pal1/2 (Reiher et al., 2011). Emerging single-cell RNAseq (scRNAseq) technologies have identified additional gut-peptide hormones in the EEs (Guo et al., 2019; Hung et al., 2020; Table 1). Even though several gut-peptide hormones such as DH31 and Tk were previously shown by *in vitro* assays to stimulate gut mobility and possible nutrient delivery decades ago (Siviter et al., 2000; LaJeunesse et al., 2010), the investigation of their physiological roles is largely hampered due to lack of genetic tools. As most Gal4 lines for genes that encode gut-peptide hormones in EEs also target those neurons expressing the same genes in the brain, it is very difficult to distinguish their roles in the gut and brain.

We have established a *Tk-g-Gal4*, that is predominantly expressed in all Tk^+ EEs, the most abundant one accounting for ~40% EEs in the midgut (Song et al., 2014), and a very small portion of Tk^+ neurons in the brain. Using this *Tk-g-Gal4*, we are able to ablate Tk^+ EEs and diminish Tk production in the gut with rarely affecting its expression in the brain, thus revealing *in vivo* metabolic roles of gut Tk in intestinal and systemic lipid metabolism. Mature gut-peptides Tk1–Tk5, which are processed from the pro-Tk, target the G-protein coupled receptor (GPCR) Tkr99D in the ECs and triggers cAMP/PKA signaling to suppress the activity of Sterol regulatory-element binding protein (SREBP) and lipogenic programs in the gut, leading to decreased lipid production in the ECs and reduced lipid storage in the whole body. In addition, gut Tk6, another pro-Tk-derived mature peptide, activates another GPCR Tkr86C in the VMs and increases ILP3 production to modulate both local and systemic insulin signaling and lipid homeostasis (Poels et al., 2009; Amcheslavsky et al., 2014; Kamareddine et al., 2018). Tk has been reported to activate Malpighian tubules (MTs; Soderberg et al., 2011), as well as ILP-producing cells (IPCs) in the brain (Birise et al., 2011), via Tkr99D to regulate ILPs secretion and nutrient-deprivation response as well. Thus, it is believed that gut-derived Tk targets multiple tissues/organs to collectively regulate systemic energy homeostasis.

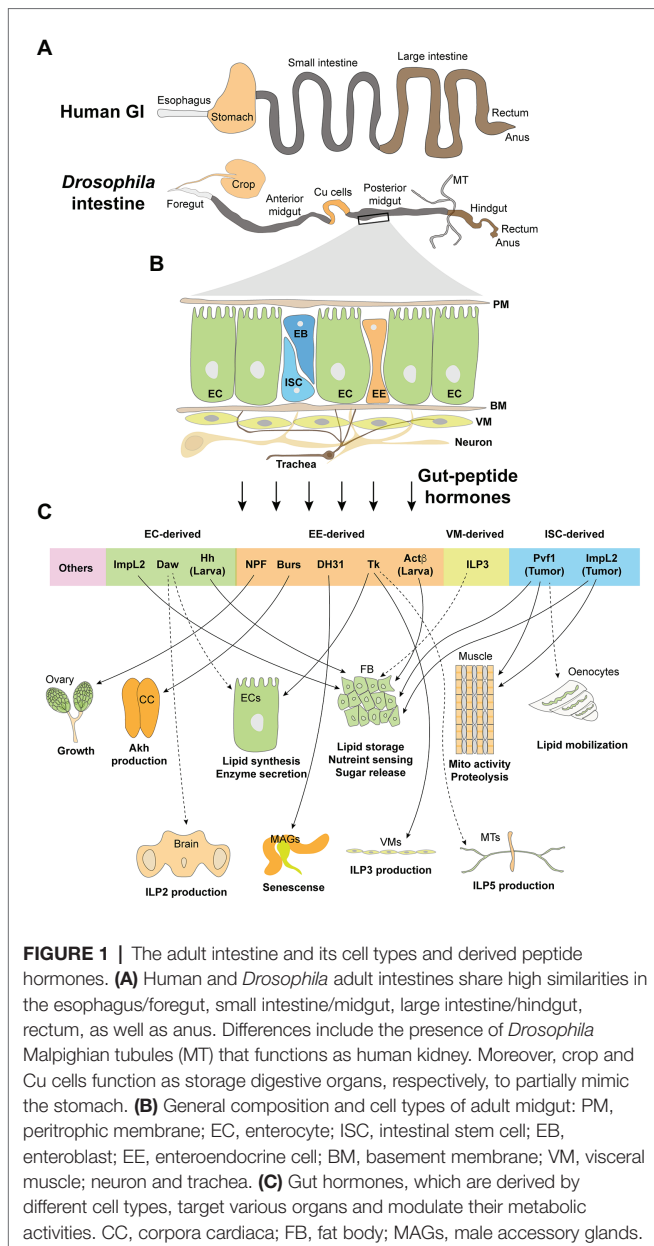


TABLE 1 | Summary of metabolic impacts of gut-peptide hormones in *Drosophila*.

Cell types	Gut hormones	Metabolic impacts	Targeted organs
EC	Daw	Decrease EC enzyme production and increase ILP2 production in IPCs (Chng et al., 2014; Ghosh and O'Connor, 2014)	ECs, IPCs
	ImpL2	Decrease lipid storage (Hang et al., 2014)	ECs, FB
	Hh (larva)	Increase lipid mobilization (Rodenfels et al., 2014; Zhang et al., 2020)	FB
	Tk	Increase lipid production in ECs and ILP3 release in VMs (Song et al., 2014; Kamareddine et al., 2018)	ECs, VMs
	NPF	Unknown (Ameku et al., 2018)	Ovary
EE	DH31	Unknown (Takeda et al., 2018)	MAGs
	Burs	Increase Akh release and lipid mobilization (Scopelliti et al., 2019)	Neuron
	Act β (larva)	Increase Akh response (Song et al., 2017)	FB
ISC/tumor	ImpL2	Increase lipid loss, glycemic level, and muscle wasting (Kwon et al., 2015)	Multiple
	Pvf1	Increase lipid loss and muscle wasting (Ghosh et al., 2019; Song et al., 2019)	FB, muscle, oenocytes
VM	ILP3	Increase systemic insulin signaling and lipid storage (Kamareddine et al., 2018)	Multiple

Single-cell RNAseq and biochemical analysis indicate that Tk⁺ EEs also produce DH31, Burs, NPF, Gpb5, and Nplp2, in different regions of the midgut (Chen et al., 2016a; Guo et al., 2019; Hung et al., 2020). Another study using the *Tk-g-Gal4* revealed that gut NPF non-autonomously controls mating-induced proliferation of germline stem cells *via* ovarian NPF receptor (NPF_R; Ameku et al., 2018). Like TkR99D, NPF_R is also expressed in MTs and is highly associated with nutrient-deprivation response (Chintapalli et al., 2012). It will be interesting to determine whether gut NPF regulates systemic metabolic homeostasis together with Tk.

Pros-Gal4, a driver that specifically targets all EEs in the midgut and a few neuroendocrine cells (Scopelliti et al., 2019), has recently been used for characterizing the functions of gut-peptide hormones in *Drosophila*. Even though the gut-peptide hormone Burs is produced by a small subset of Tk⁺ EEs (Chen et al., 2016a), a group has characterized its opposite role to that of Tk in lipid metabolic control by knocking down its expression in EEs with *Pros-Gal4* (Scopelliti et al., 2019). They demonstrated that Burs remotely activates the GPCR DLgr2 in a specialized type of neurons and subsequently inhibits the activity of neighbor neuroendocrine cells that produce adipokinetic hormone (Akh) in the corpora cardiaca (CC). Akh is the key hormone modulating lipid mobilization and carbohydrate release in the fat body *via* AkhR/cAMP/PKA cascade and promoting food seeking through a subset of neurons in the brain (Kim and Rulifson, 2004; Gronke et al., 2007; Huang et al., 2020). Gut-derived Burs, therefore, impairs Akh-associated triglyceride (TAG) breakdown and promotes systemic lipid accumulation.

Using *Pros-Gal4* to eliminate the expression of DH31 in the midgut, another group revealed that gut-derived DH31

remotely regulates the senescent responses of male accessory glands (MAGs; Takeda et al., 2018), suggesting the hormonal effects of this gut peptide. Since DH31 has been reported to target MTs *via* the GPCR Dh31-R (Coast et al., 2001; Johnson et al., 2005), it could be anticipated that gut-derived DH31 also modulates MTs function, like water flux as well as systemic metabolic homeostasis.

Earlier studies indicated that AstC⁺ EEs, another large subpopulation of EEs in the midgut, also produce multiple gut peptides, such as AstA, AstB/Mip, CCHA1, and CCHA2 (Chen et al., 2016a; Guo et al., 2019). CCHA2 deficiency is associated with impaired ILPs production in larval brain (Ren et al., 2015; Sano et al., 2015). Even though, at least, fat-body-produced CCHA2 is shown to contribute to the regulation of ILP2 secretion (Sano et al., 2015), the origins of bioactive CCHA2 are still controversial. Using different drivers to examine whether brain- or gut-derived CCHA2 also targets adult IPCs in the brain and modulate systemic energy balance will help address this puzzle in the future (Ren et al., 2015; Sano et al., 2015).

Global *AstA* removal results in disruption of systemic lipid homeostasis and appetite control (Hergarden et al., 2012; Chen et al., 2016b). It is considered as a direct regulation of Akh and insulin releases by AstA, as the GPCR of AstA, AstA-R2, is highly expressed in both Akh- and insulin-producing cells (Hentze et al., 2015). However, these studies failed to distinguish the differential functions between gut- and brain-derived AstA. Another group used *Pros-Gal4* to knock down AstA expression in the midgut and revealed its systemic role as a peptide hormone in longevity modulation (Takeda et al., 2018). Therefore, it is possible that gut AstA modulates release of Akh or insulin to affect energy homeostasis, but further genetic validation is required.

Remarkably, flies lacking all EEs are relatively normal in terms of food intake and carbo-lipid metabolism but are shorter-lived (Amcheslavsky et al., 2014), indicating anticipated counter-regulatory impacts between distinct gut-peptide hormones on energy balance. We speculate that flies might execute differential metabolic impacts *via* certain gut-peptide hormones in the context of various stress responses.

In addition to adult EEs, larval EEs produce peptide hormones to regulate systemic metabolism as well. We have recently characterized Activin- β (Act β) as an important EE-derived peptide hormone in the larval midgut (Song et al., 2017). Using *Act β -Gal4*, *Tk-g-Gal4*, as well as *Pros-Gal4*, we uncovered that gut-derived Act β remotely activates Babo/Smox signaling in the fat body and enhances its Akh response, leading to carbohydrate breakdown and elevation of glycemic level.

EC-Derived-Peptide Hormones

Enterocyte is the biggest cell population in both larval and adult midguts and also produces multiple peptide hormones that regulate systemic energy homeostasis (Figure 1C and Table 1). ImpL2, an established hormone robustly blocking circulating ILP bioavailabilities and downregulating systemic insulin signaling (Honegger et al., 2008), is majorly expressed in the adult ECs (Hung et al., 2020) and is associated with the impairment of lipid metabolism caused by gut bacteria (Hang et al., 2014). Adult ECs also produce the TGF-beta/activin ligand, Dawdle (Daw), into the hemolymph to impair systemic carbo-lipid homeostasis. Possible mechanisms include that Babo/Smox signaling modulates the expression of carbohydrases and insulin secretion in the ECs and brain IPCs, respectively (Chng et al., 2014; Ghosh and O'Connor, 2014). In addition, larval ECs secrete Hedgehog (Hh), a conserved ligand that regulates metabolism and development across species (Teperino et al., 2014), *via* lipoprotein particles into the hemolymph to directly activate Ci/Bmm-axis and lipolysis in the fat body, resulting in systemic lipid loss (Rodenfels et al., 2014; Zhang et al., 2020).

There are a few cytokines, like Upd3 and Dpp (Jiang et al., 2009; Tian and Jiang, 2017), produced by ECs to maintain local

tissue homeostasis. ECs also secrete bioactive enzymes, including multiple trypsin for food digestion and peptidoglycan recognition proteins (PGRPs) that degrade gut-bacteria-derived peptidoglycans (PGNs) and blunt inflammatory responses (Guo et al., 2014; Charroux et al., 2018), to affect systemic carbo-lipid metabolism. It will be interesting to investigate whether they function as circulating hormones and directly affect metabolic activities of other organs in the future.

Peptide Hormones Derived by Other Intestinal Cell Types

Other gut cell types also produce peptide hormones that execute important metabolic roles (Figure 1C and Table 1). VM-secreted ILP3, which was previously characterized to maintain local insulin signaling and ISC activity in the gut (O'Brien et al., 2011), is currently found to contribute to insulin signaling in the whole fly (Kamareddine et al., 2018). ISCs that bear an active oncogene *yki* proliferate as malignant tumors and produce large amounts of bioactive peptide hormones, like ImpL2 and Pvf1, to impair lipid metabolism in the fat body (Kwon et al., 2015; Song et al., 2019). Despite the peptide hormones mentioned above, other cytokines/peptides, such as Delta (Dl) in ISCs (Ohlstein and Spradling, 2007), vein (vn) in VMs (Biteau and Jasper, 2011), PDF in neurons (Talsma et al., 2012), as well as Dpp in gut-associated trachea and hemocytes (Li et al., 2013; Ayyaz et al., 2015; Table 2), that influence ISC activity and local gut homeostasis have shown very limited impacts on metabolic activities of distal organs. Thus, we will not discuss them further in this review.

DIETARY REGULATION OF GUT-PEPTIDE-HORMONE PRODUCTION

Gut-peptide hormones act in concert to modulate the physiologies of both GI itself and other distal organs to ensure nutrient absorption, delivery, mobilization, as well as storage, after a meal. Note that, the production and release of gut-peptide hormones are directly controlled by the digested food in a

TABLE 2 | Summary of gut-produced cytokines/peptides in *Drosophila*.

Cell types	Gut peptides
ISC/EB	Dl, ILP6, egr, Hh, spi, wg, Upd1, and Upd2 (Ohlstein and Spradling, 2007; Jiang et al., 2009; Karpowicz et al., 2010; Osman et al., 2012; Tian et al., 2015, 2016; Doupe et al., 2018)
EC	Hh, Dpp, Gbb, Krn, PGRP-SA, PGRP-SB1, PGRP-SC1a, PGRP-SC1b, PGRP-SC2, PGRP-SD, PGRP-LB, Upd1, Upd2, and Upd3 (Werner et al., 2000; Bischoff et al., 2006; Jiang et al., 2009, 2011; Osman et al., 2012; Guo et al., 2014; Tian and Jiang, 2014, 2017; Lee et al., 2015; Iatsenko et al., 2016)
EE	AstA, AstC, AstB/Mip, CCHa1, CCHa2, sli, Orcokinin B, CCAP, Nplp2, Gbp5, and ITP (Veenstra et al., 2008; Biteau and Jasper, 2014; Veenstra and Ida, 2014; Ren et al., 2015; Chen et al., 2016a; Benguettat et al., 2018; Rommelaere et al., 2019; Hung et al., 2020)
Hemocyte	Dpp (Ayyaz et al., 2015) sNPF, DH44, PDF, and AstA
Neuron	(Talsma et al., 2012; Dus et al., 2015; Chen et al., 2016b; Shen et al., 2016)
Trachea	Dpp (Li et al., 2013)
VM	Vn, wg, and Dpp (Jiang and Edgar, 2009; Tian et al., 2016; Tian and Jiang, 2017)

feedback loop. For example, Tk production in the midgut is suppressed (Song et al., 2014), while Burs production is enhanced (Scopelliti et al., 2019), under the feeding condition. On the other hand, chronic high-caloric diets also perturb the production of gut-peptide hormones and systemic metabolic balance. Advanced imaging tools in *Drosophila* have demonstrated and visualized the *in vivo* nutrient sensing, which is associated with the production of gut peptides, in the midgut in response to individual component(s) in the food.

Amino Acids

A recent study using the cytoplasmic calcium reporter CaLexA has indicated that dietary amino acids like casein peptone and lysine directly activate intracellular Ca²⁺ cascade, an increase in which is associated with peptide release in multiple endocrine cells (Dus et al., 2015; Benguettat et al., 2018; Oh et al., 2019), in Tk⁺ and DH31⁺ EEs (Park et al., 2016). Consistently, the protein level and release of Tk peptides in EEs are increased in starved flies and other insects to control gut mobility and EC lipogenesis (Winther and Nassel, 2001; Song et al., 2014), while refeeding flies only yeast that contains plenty of amino acids, but not sucrose or coconut oil, blunts the increase in Tk peptide level (Song et al., 2014). Several transporters or receptors that sense distinct amino acids and trigger the downstream signaling pathways have been characterized in *Drosophila* (Maniere et al., 2020). However, their roles in EEs are not fully identified yet.

Carbohydrates

Nutrient deprivation reduces release of Burs from EEs into the hemolymph and causes intestinal Burs accumulation. Either sucrose refeeding or diminishing the expression of Glut1, a glucose transporter, in the EEs, alleviates Burs accumulation (Scopelliti et al., 2019), confirming the control of Burs release by dietary glucose or sucrose. In addition, starvation suppresses, while yeast refeeding restores, global larval CCHa2 mRNA levels (Sano et al., 2015). Interesting, the researchers found that glucose, but not amino acids, contained in yeast paste results in CCHa2 transcriptional suppression and further uncovered fat body TOR signaling as the sensor (Sano et al., 2015). It is possible that CCHa2 transcription in the EEs is similarly regulated by nutrients as well, but more biochemical and genetic evidence are required for further validation. In addition to hormone production and release, dietary carbohydrates also influence EE mass in the larval midgut. We have uncovered that chronic high-sucrose diet perturbs larval gut homeostasis and promotes EE differentiation with unknown mechanism(s), resulting in excessive Actβ production, enhanced Akh response in the fat body, and hyperglycemia (Song et al., 2017).

Lipids

The absorption of fatty acids in fly intestine has not been carefully studied, even though homologs of fatty acid transport proteins (FATPs), fatty acid translocase FAT (CD36), and fatty-acid-binding proteins (FABPs) that regulate fatty acid binding

and transport are all present in *Drosophila* (Adams et al., 2000). So far, there is no clear evidence suggesting a direct regulation of gut-peptide hormone release by dietary lipids in *Drosophila*. However, a group demonstrated that adult EE numbers are decreased by lipid-deleted food, while increased by high-cholesterol food (Obniski et al., 2018). They also revealed that dietary cholesterol, absorption of which is regulated by the Hr96/NPC2b axis in the intestine, influences endomembrane lipid composition and the subcellular localization, trafficking, as well as turnover, of the Delta/Notch complex in the ISCs. These changes further suppress Notch signaling in the EBs and promote EE differentiation and subsequent production of EE-derived peptide hormones (Obniski et al., 2018).

Non-nutrient Components

An interesting study reported that 10 gustatory receptors are expressed in the EEs (Park and Kwon, 2011). These gustatory receptors can be activated by diverse dietary chemicals, such as caffeine, bitter compounds, and carbohydrates (Hanlon and Andrew, 2015; **Table 3**). Even though the ligand/receptor action in the gut is not characterized yet, it raises the hypothesis that diet might regulate gut-peptide hormone release and subsequent systemic metabolism *via* taste components in addition to nutrients. On the other hand, a recent study demonstrated that yeast particles trigger mechanical stress in the midgut and activate ISC proliferation (Li et al., 2018a). Several mechanical sensors, including TrpA1 and Piezo that activate Ca²⁺ cascade, are reported to be expressed in the adult EEs (Du et al., 2016; He et al., 2018), it will be likely that food containing indigestible particles or fibers would perturb the production and release of gut-peptide hormones independent of nutrients.

GUT MICROBIOTA AND PEPTIDE-HORMONE PRODUCTION

The gut microbiota emerges as a neglected metabolic organ based on a number of important discoveries of its products, including short-chain fatty acids (SCFAs), amino acids, and bacteriocin, that regulate host immunity and metabolism (Depetris-Chauvin et al., 2017; Li et al., 2018b; Qiao et al., 2019). The simpler microbiota and signaling systems of the *Drosophila* have provided researchers with a unique opportunity to study the impact of either commensal or pathogenic intestinal microbes on host feeding behavior and energy balance in a more controlled and targeted fashion (Capo et al., 2019). Several studies further

TABLE 3 | Gustatory receptor expression in adult EEs (Park and Kwon, 2011; Hanlon and Andrew, 2015).

Gustatory receptors	Producing cells	Putative ligands
Gr28a, Gr28b, Gr33a, Gr93a	Tk/NPF ⁺ EEs	Caffeine
Gr36c, Gr59a	Tk/NPF ⁺ EEs	Bitter compounds
Gr39a	Tk/NPF ⁺ EEs	Mating pheromone
Gr43a, Gr64a	Tk/NPF ⁺ EEs	Carbohydrates
Gr58c	NPF ⁻ EEs	Unknown

illustrated gut-peptide hormones as key regulators mediating host immune response and carbo-lipid metabolism in response to diverse gut microbiota.

Commensal Bacteria

Different groups have shown that axenic adult flies exhibit a delayed development, lipid and glycogen accumulation, and hyperglycemia (Shin et al., 2011; Wong et al., 2014). Impairment of systemic insulin signaling, which is associated with the dominant gut commensal microbiota (*Lactobacillus plantarum* and *Acetobacter pomorum*), is considered as a major regulator. *L. plantarum* produces branched-chain amino acids to directly activate host TOR and insulin signaling (Storelli et al., 2011), while SCFAs like acetate produced by *A. pomorum* modulate systemic insulin signaling via gut-peptide hormone production (Kamareddine et al., 2018). As we mentioned, both mRNA and peptide levels of gut Tk, which promotes ILP3 production in VMs via *Tkr99D* activation to modulate systemic insulin signaling and energy balance (Poels et al., 2009), are increased by intestinal acetate (Kamareddine et al., 2018). Even though the receptors sensing acetate are not yet identified in *Drosophila*, researchers indicated that intestinal microbial acetate activates immune responses via PGRP-LC/Rel signaling to increase Tk^+ EEs mass and Tk synthesis in the gut (Figure 2). Note that, acetate-activated Rel signaling is observed not only in Tk^+ EEs but also in $AstC^+$ EEs, ISCs, and ECs (Kamareddine et al., 2018). EC-derived *ImpL2* is previously reported to be suppressed by intestinal acetate content (Hang et al., 2014). Further, other hormones, like *AstA* and *CCHa2* produced in $AstC^+$ EEs, are probably associated with *ImpL2* production and systemic insulin response as well (Hentze et al., 2015; Ren et al., 2015). Therefore, it is quite likely that intestinal acetate modulates differential gut-peptide hormone production in multiple cell types via Rel activation and orchestrates their influences on insulin signaling and host metabolism (Figure 2).

Pathogenic Bacteria

A few studies have demonstrated that pathogenic bacteria also perturb host metabolism through gut-peptide hormones. Oral infection of *Vibrio cholerae*, a life-threatening bacterium for both human and *Drosophila*, does not influence the gut microbiota or the epithelial barrier but reprograms acetate metabolism in the gut through acetate metabolic genes, *CrbR* and *CrbS* (Hang et al., 2014). In response to intestinal microbial acetate switch, production of *ImpL2* and *Tk* is affected to impair systemic insulin signaling (Hang et al., 2014; Kamareddine et al., 2018). Dietary acetate supplementation further successfully alleviates *Vibrio cholerae*-disrupted host insulin signaling and metabolism. Note that, PGRP-LC/Rel signaling, which is shown to regulate *Tk* production, could be modulated by various PGNs derived by non-commensal bacteria as well (Royet and Charroux, 2013). Another group recently showed that septic, but not oral, infection of bacterial pathogen *Phototribadus luminescens*, *Phototribadus asymbiotica*, or non-pathogenic *Escherichia coli* modulate gut *Tk* production without affecting gut microbiota homeostasis, resulting in lipid accumulation

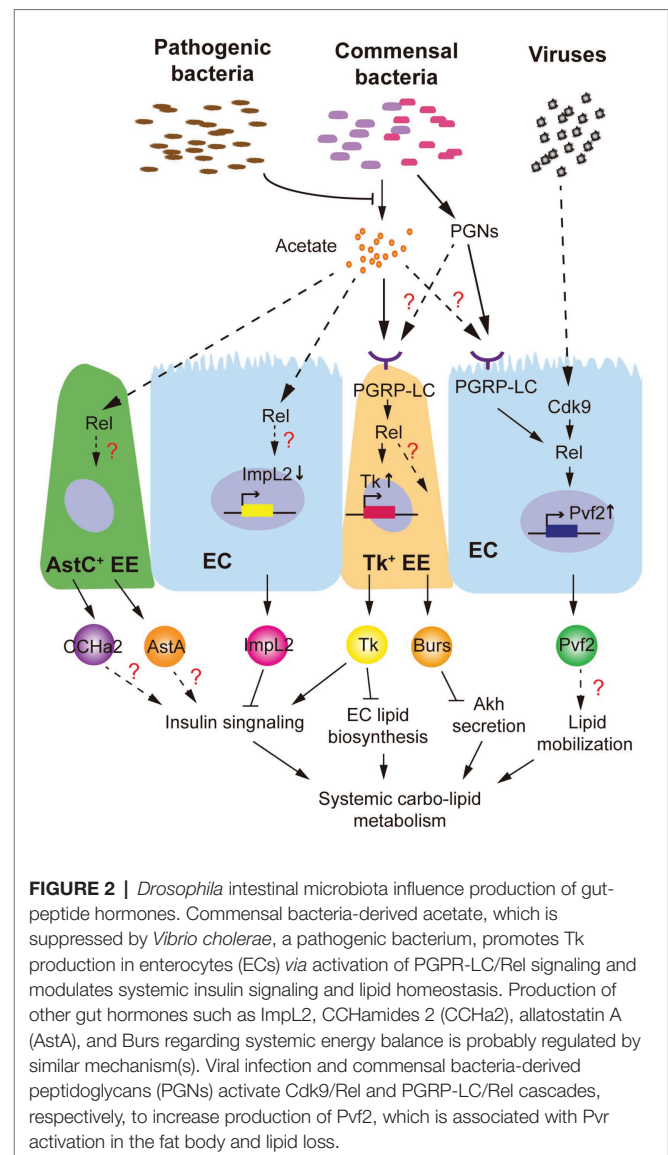


FIGURE 2 | *Drosophila* intestinal microbiota influence production of gut-peptide hormones. Commensal bacteria-derived acetate, which is suppressed by *Vibrio cholerae*, a pathogenic bacterium, promotes Tk production in enterocytes (ECs) via activation of PGRP-LC/Rel signaling and modulates systemic insulin signaling and lipid homeostasis. Production of other gut hormones such as *ImpL2*, *CCHa2* (CCHa2), allatostatin A (*AstA*), and *Burs* regarding systemic energy balance is probably regulated by similar mechanism(s). Viral infection and commensal bacteria-derived peptidoglycans (PGNs) activate *Cdk9/Rel* and PGRP-LC/Rel cascades, respectively, to increase production of *Pvf2*, which is associated with *Pvr* activation in the fat body and lipid loss.

in the gut and whole body (Harsh et al., 2019). This evidence indicates that gut-peptide hormone could be regulated by the circulating PGNs derived from non-commensal bacteria. Given the vast products such as virulence factors, secreted peptides, and metabolites produced by both commensal and non-commensal bacteria in the fly intestine, it will be necessary to dissect their impacts on gut-peptide hormones in terms of receptors sensing them and the signaling pathways they regulate.

Viruses

Drosophila C virus (DCV), a natural RNA virus for *Drosophila*, has recently been found to cause severe mortality as well as depleted stores of triglycerides and glycogen in adult flies (Arnold et al., 2013; Chtarbanova et al., 2014). The morphology and structure of midgut in DCV-infected flies are severely impaired. Importantly, the production of *Pvf2*, a peptide homolog of human platelet-derived growth factor (PDGF) and vascular endothelial

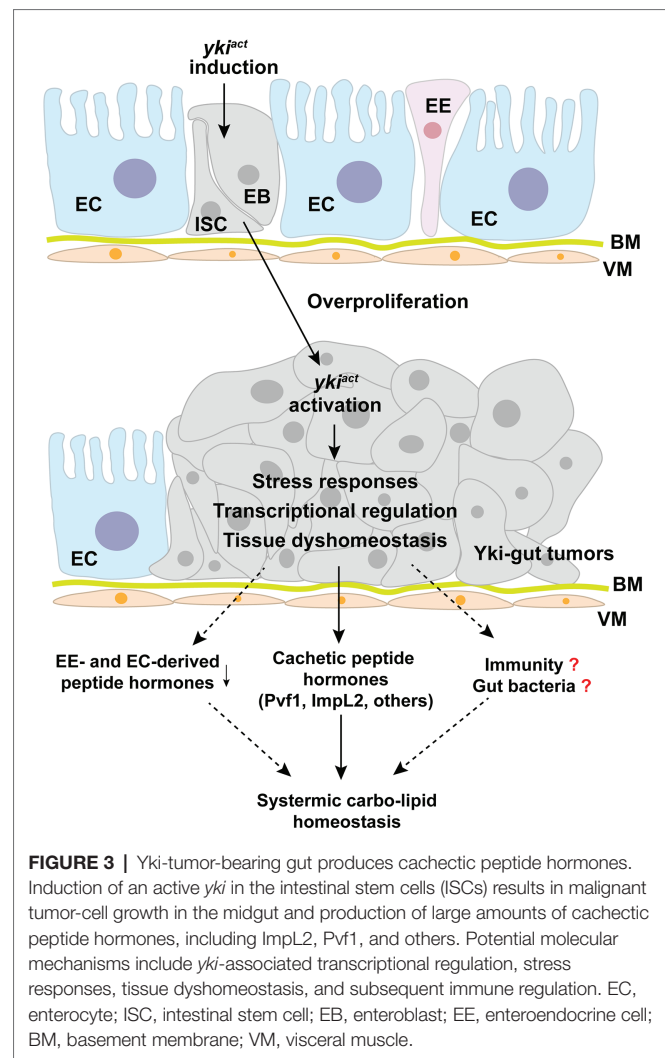
growth factor (VEGF), is dramatically upregulated in the ECs by the Cdk9/Rel pathway in response to viral infection (Sansone et al., 2015). Although in this study Pvf2 is shown to mediate intestinal antiviral immunity, its metabolic impact is not investigated yet. Interestingly, activation of Pvr, the receptor of Pvf2, in the fat body and oenocytes results in systemic lipid loss (Zheng et al., 2017; Ghosh et al., 2019; Song et al., 2019), phenocopying the DCV infection and suggesting a Pvf2/Pvr axis in the gut-to-fat-body communication. Infection of other RNA viruses like Flock House virus (FHV) also leads to the decline of systemic lipid accumulation (Chtarbanova et al., 2014), whether gut-peptide hormone is involved in FHV-associated metabolic disruption is an interesting question to address in the future.

GUT TUMORS AND PEPTIDE-HORMONE PRODUCTION

Tumor-bearing guts have recently been characterized as a differentiated endocrine organ that remotely impairs systemic metabolic homeostasis and causes a cachexia-like phenotype. Induction of an active oncogene *yki*, the homolog of human Yap1, in the ISCs leads to malignant tumorigenesis in the gut and subsequent wasting of host organs, including ovary degeneration, lipid loss, muscle dysfunction, hyperglycemia, as well as mortality (Kwon et al., 2015; Song et al., 2019). Because the affected flies eat normally, it is less likely that *yki*-gut tumors impair nutrient absorption in the gut. Integrating transcriptome analysis and RNAi screening, we have uncovered that *yki*-gut tumors produce various peptide hormones to disrupt the balance of systemic anabolism and catabolism (Figure 3).

First, *yki*-gut tumors release large amounts of ImpL2 to suppress IGF/insulin signaling and its associated anabolism in multiple host organs. As a consequence, ovary size and storages of lipid and glycogen in the fat body are decreased. The flies climb poorly, as the mitochondrial integrity and activity are both impaired in the skeletal muscles (Kwon et al., 2015). Second, *yki*-gut tumors produce excessive Pvf1, another hormone homologous to mammalian VEGF and PDGF, to extensively activate Pvr/MEK cascade and promote catabolism in the host organs, including lipid and carbohydrate breakdown in the fat body and muscular protein degradation. Small-molecule inhibitors against MEK/ERK strongly alleviate the wasting effects in *yki*-tumor-bearing flies, as well as C26-tumor cell models, providing pharmaceutical opportunities in prevention and treatment of cancer-associated cachexia (Song et al., 2019). Third, other potential tumor-derived peptide hormones have also been characterized using RNAi screening to regulate host wasting with unknown mechanisms (Song et al., 2019; Figure 3).

How *yki* activation in ISCs modulates production of cachectic peptide hormones is currently unknown. Possible mechanisms could include *yki*-induced direct transcriptional regulation of certain peptides and *yki*-associated ISC proliferation that enlarges the mass of peptide-producing cells. However, the transcriptional levels of these cachectic peptide hormones are increased far more than ISC marker genes and *yki*-target genes, like *diap1* and *Ex*, in the *yki*-gut tumors (Song et al., 2019). We, therefore, speculate



that *yki*-activation might also trigger unknown intracellular stress responses to increase peptide-hormone production in a cascade-amplification fashion (Figure 3).

Nevertheless, we also noticed that *yki*-gut tumors perturb midgut homeostasis by increasing the mass of ISCs but decreasing that of ECs and EEs. As expected, most of the endogenous immune-associated enzymes and peptide hormones that are produced by ECs and EEs are suppressed (Song et al., 2019; Figure 3). Whether these endogenous enzymes, which maintain intestinal bacteria balance and systemic immune response, and peptide hormones contribute to host wasting is another insightful question to be addressed.

OTHER PATHOLOGICAL CONDITIONS AND GUT-PEPTIDE HORMONES

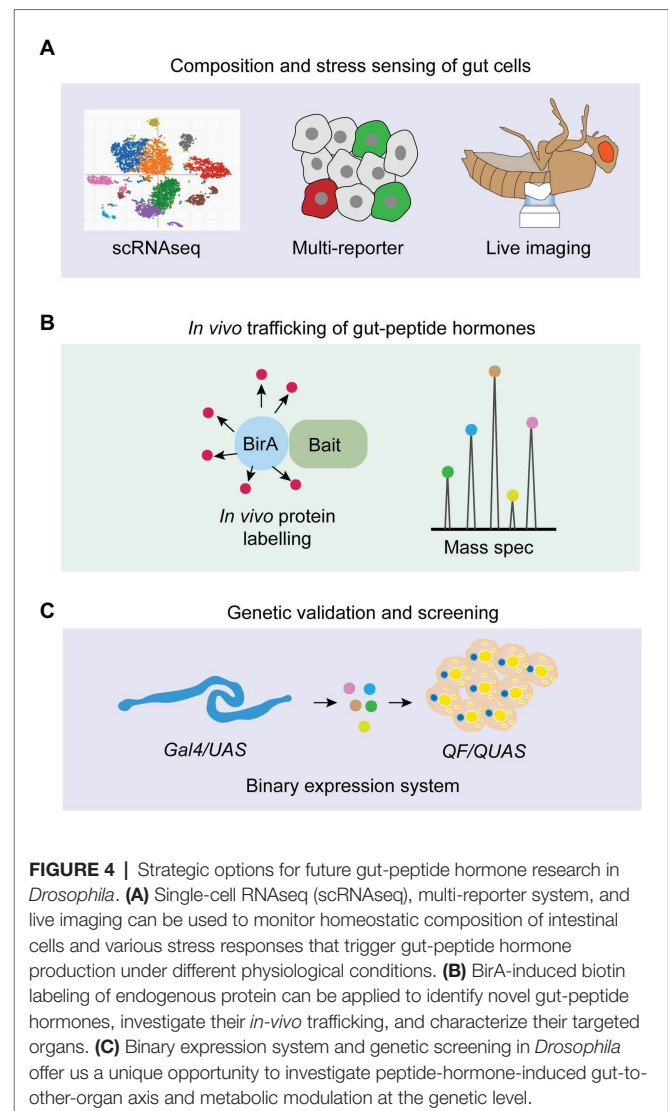
Drosophila model has also indicated high associations between systemic metabolism and other pathological conditions. For instance, aged flies exhibit less intestinal and systemic TAG storages (Karpac et al., 2013), while either diet restriction or

impaired insulin signaling significantly extends lifespan and increases systemic TAG accumulation (Song et al., 2010; Luis et al., 2016). Recent studies have uncovered important clues regarding participation of gut-peptide hormones, including increased EE mass and bacterial load that are associated with gut-peptide production in aged adult midgut (Ayyaz and Jasper, 2013; He et al., 2018). Moreover, removal of gut-derived AstA and DH31 modulate fly longevity in an opposite manner (Takeda et al., 2018). It will be essential to examine whether gut-peptide hormones are involved in the regulation of aging and carbo-lipid metabolic homeostasis.

Another example is sleep deprivation, a well-established condition that disrupts systemic metabolic balance (Stahl et al., 2017). Researchers have revealed that an EC-derived amino acid, D-serine, is essential for sleep control (Dai et al., 2019), indicating a participating role of fly gut. Strikingly, a recent study further demonstrated that sleep deprivation results in accumulation of reactive oxygen species (ROS) and triggers consequent oxidative stress specifically in the gut, whereas diminishment of ROS accumulation in the gut improves survival without sleep in flies (Vaccaro et al., 2020). Given the fact that ROS regulates diverse metabolic signaling pathways, as well as tissue homeostasis and commensal bacterial control, in the gut (Ha et al., 2005; Ayyaz and Jasper, 2013; Xu et al., 2017), we speculate that ROS-associated production and release of gut-peptide hormone might function as a nexus between sleep and metabolic homeostasis and beyond.

CONCLUSION AND OUTLOOK

In this review, we have summarized the gut-hormone regulation of systemic metabolism and its essential impacts on physiological and pathogenic outputs, focusing on their genetic characterization, stress-sensing, as well as the mechanisms of act, in *Drosophila*. Despite the gained knowledge and ongoing functional validation of gut-peptide hormones, several fundamental questions in this field still remain unaddressed (Figure 4). In particular, the stress-sensing of intestinal cells regarding peptide production in response to multiple internal and external stimuli is largely unknown. Growing findings of novel stress responses, such as mechanical stress induced by different components in the food (He et al., 2018; Li et al., 2018a), local hypoxic response caused by bacterial infection (Valzania et al., 2018; Krejcová et al., 2019), immune response triggered by intestinal microbial metabolites (Kamareddine et al., 2018), as well as the newly-identified oxidative stress associated with sleep loss (Vaccaro et al., 2020), keep shaping our current understanding of intestinal phenomena. Therefore, integrating multi-reporter system, long-term live imaging (Martin et al., 2018), and scRNAseq to monitor diverse stress responses and study whether and how gut-peptide-hormone production is affected by them will add new dimensions for exploiting gut physiology and metabolic homeostasis (Figure 4A). This strategy will also help illustrate the compositional change



of different gut cells that produce distinct hormones and the orchestrating impacts on systemic metabolism under chronic conditions, like tumor progression and high-caloric diet (Figure 4A). *In vivo* trafficking of hormones is difficult to achieve due to limited genetic tools. Recent studies, which engineered a promiscuous biotin ligase, BirA, to specifically label secreted proteins including peptide prohormones (Stevens et al., 2019; Droujinine et al., 2020), are very promising to address the limitation (Figure 4B). They used a fused BirA to biotinylate all proteins in the muscle ER and detected biotin-labeled proteins in the blood to identify potential myokines. Moreover, they further detected biotin-labeled proteins in the other organs to characterize *in vivo* trafficking of these myokines from skeletal muscle to the fat body (Droujinine et al., 2020). Genetic validation is required to confirm the physiological outputs of gut-peptide hormone-induced interorgan communication. The binary expression systems such as LexA/LexAop (Kockel et al., 2016) and QF/QUAS (Riabinina and Potter, 2016) together with Gal4/UAS

offer us a unique opportunity. For instance, we could increase the release of a gut-peptide hormone using the *LexA/LexAop* system and simultaneously block its receptor or downstream signaling pathways in the receiving organ using the *Gal4/UAS* system to evaluate the physiological regulation of the particular gut-peptide hormone under study. Conversely, we can also use the *QF/QUAS* system to set up reliable readouts (e.g., Ca^{2+} signaling in Akh-producing cells induced by Burs) and screen for the potential stress pathways and trafficking regulators of the matched hormones (e.g., Burs) with the *Gal4/UAS* system (Figure 4C).

Taken together, the *Drosophila* organism with the accessibility of genetic tools, the simplicity of its genome, and the feasibility for disease modeling, will serve as a powerful system for the future research of gut-peptide hormones. Some of the metabolic regulations that are found in *Drosophila* have recently been shown to be similar in mammals. For example, similar to fly Tk regulation of ILP3/5 secretion, mammalian Substance P and neurokinin A also promote insulin release (Schmidt et al., 2000). Mammalian galanin inhibits insulin secretion as fly *AstA* does (Tang et al., 2012). Like fly *Pvfl*, VEGFs are also produced in malignant colon tumors and found to promote lipid mobilization (Sun et al., 2012; Bendardaf et al., 2017). These evidence might, therefore, become relevant in the context of

human physiology and pathologies such as diet-induced obesity and diabetes, infectious diseases, and cancer cachexia.

AUTHOR CONTRIBUTIONS

XZ wrote the part of bacteria-induced gut hormone production. GD wrote the section of gut hormone types. JL wrote the section of tumor-induced gut hormone production. XX wrote the part of dietary regulation. ER wrote the section of other conditions. WS discussed and organized the whole manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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