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Nutrition-dependent control of insect development by insulin-like peptides

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Abstract

In metazoans, members of the insulin-like peptide (ILP) family play a role in multiple physiological functions in response to the nutritional status. ILPs have been identified and characterized in a wide variety of insect species. Insect ILPs that are mainly produced by several pairs of medial neurosecretory cells in the brain circulate in the hemolymph and act systemically on target tissues. Physiological and biochemical studies in Lepidoptera and genetic studies in the fruit fly have greatly expanded our knowledge of the physiological functions of ILPs. Here, we outline the recent progress of the structural classification of insect ILPs and overview recent studies that have elucidated the physiological functions of insect ILPs involved in nutrient-dependent growth during development.

Introduction

Nutrients are critical environmental signals influencing growth and development in animals. Although each cell in a multicellular organism responds directly to nutrition, the growth and development of the entire organism needs to be coordinated by adjusting growth between tissues and controlling the consumption of stored nutrients. The coordination of systemic organismal growth in response to the nutritional status is primarily mediated by the insulin-like peptide (ILP) family, which includes insulin and insulin-like growth factors (IGFs) in vertebrates, as well as multiple ILPs in invertebrates.

In vertebrates, insulin and IGFs regulate metabolism, growth and development in response to nutritional availability. Although insulin and IGFs have similar amino acid sequences, they have different physiological functions that are meditated by distinct receptor tyrosine kinases (RTKs), the insulin receptor and IGF-I receptor, respectively [1]. The major function of insulin is to control carbohydrate and lipid metabolism [2], whereas that of IGFs is to promote tissue and body growth during development [3]. Numerous studies have shown that the key regulator of the activities of insulin and IGFs is the nutritional status [4]. The production and secretion of insulin by pancreatic β-cells are tightly regulated by the nutrient

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status [5]. Nutritional availability also influences the production, serum concentration, and action of IGF-I in regulating appropriate tissue and body size [6]. Another class of ILP family peptides in vertebrates, relaxins and relaxin-like peptides, function through leucine-rich repeat-containing G protein-coupled receptors (GPCRs) and have multiple functions, especially associated with reproduction [7].

ILPs have been identified and characterized in a wide variety of invertebrate phyla and in arthropods, including insects [8]. In insects, ILPs are involved in multiple biological processes, including growth, metabolism, reproduction, immunity, behavior, stress resistance, diapause, and lifespan [8-15]. Recently, powerful genetic studies using the fruit fly *Drosophila melanogaster* have greatly enhanced our understanding of the conserved functions of ILPs, as well as their downstream signaling pathways called the insulin/IGF signaling (IIS) pathways [9-15]. In this review, we will first focus on the structural classification of ILPs in insects. We will then overview the recent progress in our understanding of the physiological functions of insect ILPs, especially as it relates to nutrient-dependent growth during development. Through this review, we aim to provide insights into the diverse yet conserved roles of insect ILPs in the coordination of systemic organismal growth, as well as tissue-specific growth, in response to the nutritional status during development.

Structural classification of insulin-like peptides in insects

ILP family members have been identified in multiple insect species, with their numbers varying significantly between only one in some orthopteran species and more than 40 in the silkworm *Bombyx mori* [8, 16]. The amino acid sequences of insect ILPs are highly divergent between insect orders, except for some critical residues (such as cysteines) that are necessary for tertiary structure formation. However, they can be classified into at least three groups based mainly on the sequence features of their precursors: insulin-like peptides, IGF-like peptides, and DILP7-like peptides (Figure 1) [17, 18]. The first group, insulin-like peptides, shares the most common structural feature of the ILP family, and most insect ILPs are classified into this group (Figure 1A). The common feature of insulin-like peptides is a conserved domain organization of their precursors, consisting of a signal peptide, with a B-chain, C-peptide, and A-chain, similar to the vertebrate ILP family. After cleavage of the signal peptide, the C-peptide is most likely removed to generate a mature heterodimeric peptide consisting of the A- and B-chains, such as in vertebrate insulin or relaxins. The second group is the putative IGF-like peptides. The recently identified *Bombyx* IGF-like peptide (BIGFLP) retains the C-peptide, resulting in a single-chain polypeptide, which is similar to vertebrate IGFs [19]. One of the characteristic features of IGF-like peptides is that they have a relatively shortened C-peptide compared with other insect ILPs. The third group, DILP7-like peptides, is characterized by an unusually conserved sequence shared by several insects and even some molluscan species. Precursor polypeptides with long conserved sequences are found in *Drosophila* (DILP7), the African malaria mosquito *Anopheles gambiae* (AgamILP5), the yellow fever mosquito *Aedes aegypti* (AaegILP5), the red flour beetle *Tribolium castaneum* (TcILP4), and molluscs such as the owl limpet *Lottia gigantea* (MIP4) and the California sea hare *Aplysia californica* (MIP1) [20, 8]. To date, the conserved biological function of these unique ILPs has not yet been clarified. In *Drosophila*,

DILP8 has recently been identified as a new ILP and has a unique effect on developmental timing and systemic body growth [21**, 22**, 23]. Compared with other *Drosophila* ILPs, DILP8 has atypical number of amino acid residues between two cysteine residues in the B chain, and between forth and fifth cysteine residues in the A chain. Although direct sequence comparisons show no clear DILP8 homologues in non-dipteran insects or nematoceran (mosquito) genomes [21**], it is possible that functional orthologs of DILP8 exist in other insects.

Insect ILPs act on target tissues by activating an RTK named insulin-like receptor (InR), which shows high similarity with mammalian insulin and IGF type-I receptors. Although multiple ILPs exist in each insect genome, it typically encodes only one or two InRs [8]. However, it remains possible that some ILPs act through alternative receptors such as GPCRs. Recently, Veenstra hypothesized that the receptors for DILP7 and DILP8 in *Drosophila* are candidate relaxin receptors, LGR4 (CG34411) and LGR3 (CG31096), respectively [24]. If this is indeed the case, DILP7 and DILP8 can be classified as relaxin-like peptides, although they have no relaxin-specific GPCR-binding motif, RxxxRxxI/V, in the B-chain [25].

Nutrient-dependent secretion and the transcription of insulin-like peptides by medial neurosecretory cells in the brain

The principal ILP-producing cells that are tightly associated with nutrient-dependent systemic growth regulation are the several pairs of medial neurosecretory cells (mNSCs), also known as insulin-producing cells (IPCs), in the brain. In this section, we will focus on the regulatory mechanisms of the secretion and transcription of insect ILPs in brain mNSCs.

In *Bombyx*, bombyxins produced by mNSCs are axonally transported to and released from the neurohemal organ called the corpora allata (CA) [16] (Figure 2). In *Drosophila*, mNSCs extend processes to the dorsal vessel (insect heart) [26], allowing the direct release of DILPs into the circulating hemolymph. DILPs produced by brain mNSCs are also axonally transported to the corpora cardiaca (CC), a pair of neurohemal organs in the ring gland. A recent study shows that the mNSC-derived DILP2 is also released within the brain and received by other specific sets of neurons, including several neurosecretory cells [27]. As in *Bombyx* and *Drosophila*, ILPs are produced in brain mNSCs in other insect species, including *Aedes* [28, 29], the desert locust *Schistocerca gregaria* [30], and the migratory brown planthopper *Nilaparvata lugens* [31**], suggesting critical and evolutionary conserved functions of mNSC-derived ILPs in insects.

In *Drosophila*, as in mammals, the secretion and transcription of each DILP are regulated directly or indirectly by multiple cues, including by several hormones and neurotransmitters [32, 15] (Figure 3). The secretion of DILPs from mNSCs highly depends on the nutritional condition. During *Drosophila* larval development, the major nutrient for systemic growth and development is amino acids. The availability of amino acids is mainly sensed by the fat body [33], a functional equivalent of the vertebrate liver and adipocytes, which in turn remotely regulates DILP secretion from brain mNSCs through unknown humoral signals called fat body-derived signals (FDSs) [34]. In addition to the amino acid-inducible FDSs, a

fat body-derived leptin-like protein called Unpaired 2 (Upd2) acts through GABAergic neurons to stimulate the secretion of DILPs in response to high-fat and high-sugar diets [35**]. Moreover, a fat body-derived small peptide called CCHamide-2 directly activates brain mNSCs to modulate DILP secretion primarily in response to glucose [36]. It has been suggested that the secretion of all DILPs is simultaneously induced by the depolarization of mNSCs [34]. However, a recent study demonstrated that the secretion of DILP3 is selectively stimulated by sugar during larval development [37*]. Although DILP2 and DILP5 secretion responds to amino acids [34], sugar stimulates the CC to release adipokinetic hormone (AKH), which acts directly on the mNSCs to promote the secretion of DILP3 [37*]. In addition to the hormones, dietary lipids derived from yeast can be a nutritional signal to regulate the release of DILP2 into hemolymph by modulating the activity of specific neurons in the brain [38].

There are additional nutrient-dependent signals that affect mNSCs in adult *Drosophila*. In the adult fly, the CC also produce Limostatin (Lst), a peptide hormone that suppresses the secretion of DILPs from mNSCs [39]. Moreover, similar to the insulin release in mammals, a recent paper demonstrated that the secretion of DILPs is regulated by the direct sensing of glucose by GLUT1, the type-1 glucose transporter, which is expressed in mNSCs in the adult fly [40]. In *Bombyx*, glucose also stimulates the secretion of bombyxin into the hemolymph [41], suggesting the possibility that common ancestral mechanisms control the secretion of ILPs from insect mNSCs and mammalian, β-cells.

It has been suggested that the secretion and transcription of DILPs in mNSCs are regulated by different mechanisms [34, 40]. The transcription of each *dilp* gene in mNSCs is independently regulated [42], although a compensatory expression of *dilp* genes has also been demonstrated [43, 44*]. *dilp2* expression in mNSCs is already detectable in the late embryonic stage, whereas the expression levels of *dilp5* and *-3* are upregulated in the 2nd and 3rd instar, respectively [42]. Importantly, in both larval and adult stages, *dilp5* transcription is tightly regulated in a nutrient-dependent manner [42, 45, 34]. It has been shown that the transcription factors Eyeless (Ey) and Dachshund (Dac) synergistically and directly promote *dilp5* expression in mNSCs [46, 47]. However, how these two transcription factors are involved in the nutrient-dependent expression of *dilp5* remains unclear. Interestingly, Dach1/2 and Pax6, vertebrate homologs of the *Drosophila* Dac and Ey, have similar combinatorial effects on the activation of insulin expression in a mammalian β-cell-derived cell line [47].

Regulation of growth by the systemic and local action of insulin-like peptides during development

In insects, the larval feeding period is the specialized stage for systemic body growth. During this period, ILPs produced in mNSCs function as key signals that couple systemic and tissue-specific growth with nutritional availability. In *Drosophila*, for example, larval growth is severely retarded and the adult body size is significantly reduced, as if they are starved when mNSCs are genetically ablated [26], all mNSC-derived *dilps* are knocked out [44*], or *InR* is mutated (Figure 4). This phenotypic similarity clearly indicates that ILPs secreted from mNSCs are the major circulating hormones that activate InR to promote body

growth during larval feeding period. In addition to the systemic growth-promoting effect, systemically-circulating DILPs also affect specific cell/tissue growth, such as tracheal branching [48] and stem/progenitor cell maintenance and proliferation in the lymph gland [49] during larval development. The requirement of systemic ILPs from mNSCs for germline stem cell maintenance and proliferation as well as ovarian development has also been reported in adult flies and mosquitoes [50, 29, 8].

In holometabolous insects, many of the developing adult tissues, including the imaginal discs and primordia, undergo growth and differentiation during the wandering and pupal stages after larvae have stopped feeding [51]. During this period, the insect steroid hormone ecdysone plays critical roles in regulating adult tissue growth and differentiation. However, ILPs produced from the fat body also function, at least partially, as key systemic signals to regulate tissue and body growth. It has been shown in both *Bombyx* and *Drosophila* that the fat body predominantly produces ILPs called BIGFLP and DILP6, both of which are structurally classified as IGF-like peptides, during the post-feeding period in response to ecdysone [19, 52, 53] (Figure 3). *dilp6* mutants show an approximately 10% reduction in the final adult body size and weight [52, 53, 44*], indicating that some additional systemic growth occurs during the post-feeding period by utilizing stored nutrients in the fat body that were accumulated during the larval feeding period. Therefore, even after larvae stop feeding, fat body-derived ILPs play critical roles in regulating growth in a systemic manner.

The sensitivity to ILPs or IIS differs among developmental stages and tissues, and these differences can control the relative sizes of the adult tissues [54, 55*]. In the butterfly *Precis coenia* and the tobacco hornworm *Manduca sexta*, the wing discs change their sensitivity to nutrition or IIS with developmental stages [56, 57]. These differences can be explained by the changes in ecdysone titer during the final larval stage, since ILPs and ecdysone act through separate but synergistic pathways to modulate the growth of their wing discs *in vitro* [58, 59]. In addition, different organs show different sensitivities to IIS during development. In *Drosophila*, the genital disc and the wing disc differ in their sensitivity to IIS [55*]. Similarly, in the rhinoceros beetle *Trypoxylus dichotomus*, knockdown of *InR*, specifically during the post-feeding period, caused a 16% reduction in horn length in the adult, but caused only a 2% reduction in wing length and no reduction in the genitalia [60**]. In *Bombyx*, BIGFLP promotes the growth of adult imaginal discs or primordia, but not of larval tissues, which are degenerated or reconstructed during metamorphosis *in vitro* [19]. In this way, change in the relative size of adult tissues is caused by developmental stage- and tissue-specific modifications in their response to ILPs or IIS.

Recently, DILP8 was identified as a humoral factor that is released from growth-retarded or damaged imaginal discs to inhibit ecdysone production and systemic body growth [21**, 22**]. DILP8 mutation abolishes the developmental delay caused by imaginal disc growth perturbation, while DILP8 overexpression delays the pupariation timing. These unique functions of DILP8 clearly distinguish this ILP from other ILPs and indicate that this peptide may activate a receptor other than InR, as discussed above.

Insect ILPs not only act systemically but also in a local manner [61, 62]. Recent studies have demonstrated that DILPs act locally within the central nervous system in a nutrient-

dependent manner [63**, 64**, 65, 66*]. A subset of glial cells produce DILP6 during the larval feeding period, and its expression is inhibited by starvation. This nutrient-dependent expression and/or secretion of DILP6 activates the IIS in adjacent neural stem cells called neuroblasts, thereby leading to their exit from quiescence [63**, 64**, 66*]. Moreover, DILP6 has also been suggested to regulate the proliferation of perineural and cortex glia in the larval brain [65]. In the adult fly, midgut DILP3 functions as a local signal to regulate intestinal stem cell proliferation and growth in a nutrient-dependent manner [67]. In *Bombyx*, the ovariole sheath, which wraps around an array of follicles, produces BIGFLP during pupa-adult development [68] and may regulate early follicular growth in a paracrine manner.

Regulatory mechanisms of the activities of insulin-like peptides during development

In mammals, six classic IGF-binding proteins (IGFBPs) bind IGFs with high affinity and act as modulators of IGF activity. They act either to enhance or to dampen IIS by extending the half-life of IGFs, by changing their local and systemic availability, or by preventing them from binding to their receptor [69]. IGFBP3 can also interact with a third protein, called the acid-labile subunit (ALS), to form a trimeric complex in circulating blood. In addition, an IGFBP-related protein IGFBP7 (or IGFBP-rP), which shares approximately 30% similarity with IGFBP1 and IGFBP6 in its N-terminal domain, binds IGFs with a comparatively low affinity and also binds to the IGF-I receptor to act as a potent tumor suppressor in a wide variety of cancers [70, 71].

Insects also possess IGFBP-like proteins, including Neuroparsins [72] and Imp-L2 [73], which resemble mammalian IGFBP7. Although Neuroparsins and Imp-L2 show sequence homology to IGFBP7, this homology is restricted to different domains (Neuroparsins show similarity with the N-terminal domain, whereas Imp-L2 shows similarity with the C-terminal domain of IGFBP7). Therefore, Neuroparsins and Imp-L2 may have evolved from a common ancestral IGFBP7-like protein, although their functions are different. In *Schistocerca*, Neuroparsins directly bind to ILP (Scg-IRP) *in vitro* [30], suggesting that Neuroparsins act as potential modulators of ILP function. However, a recent study has shown that the mosquito neuroparsin-like factor called ovary ecdysteroidogenic hormone (OEH) promotes egg formation in parallel with ILP(s) by activating an RTK distinct from InR in *Aedes* [74*]. In *Drosophila*, Imp-L2 binds to circulating DILP2 and 5 and acts as a systemic inhibitor of IIS during development [75, 76] (Figure 3). Within the central nervous system, however, Imp-L2 functions as a positive regulator of DILP2-mediated IIS in some specific neurons [27]. Furthermore, two recent studies have demonstrated that Imp-L2 is secreted from tumors and creates insulin resistance in distant tissues, which drives a systemic wasting response in the adult fly [77, 78]. In parallel with mammals, the *Drosophila* homolog of ALS forms a trimeric complex with Imp-L2 and DILP2 in the circulating hemolymph, which inhibits IIS during larval development [79] (Figure 3).

In addition to IGFBP-like proteins, a secreted decoy of InR (SDR) that is structurally similar to the extracellular domain of InR has been identified in *Drosophila* [80*]. Like Imp-L2 and ALS, the secreted protein SDR can directly interact with several circulating DILPs to

antagonize their activity during larval development (Figure 3). Phylogenetic analysis has shown that SDR is most closely related to InR among all *Drosophila* RTKs [80*], suggesting that SDR is duplicated from InR, but functions as a decoy receptor to negatively regulate IIS.

Although most insects including *Drosophila* only have a single InR gene, some insects have two InR genes. A recent study showed that two InR genes (*InR1* and *InR2*) in the planthopper *Nilaparvata* have opposite functions [31**]. In the developing *Nilaparvata* wing, InR1 activates canonical IIS and leads to the development of long-winged adults. However, InR2 physically binds to InR1 and inhibits the function of InR1. This inhibition shuts down the IIS in the developing wing and leads to the development of short-winged adults. It is possible that a similar regulatory mechanism is conserved in insects with two InR genes, such as *Tribolium* (*TcInR1* and *-2*) and the honey bee *Apis mellifera* (*AmInR1* and *-2*) [8].

Conclusion

Over the last decade, both genetic and biochemical analyses of the functions of insect ILPs have advanced our understanding of how animals coordinate their growth and metabolism, as well as how different cells/tissues communicate in response to nutrition. Although only a few model insects, such as *Drosophila*, have been used in such studies, evolutionarily and ecologically diversified insects can possess significant differences in the nutritional regulation of ILP functions. The recent application of powerful genetics such as RNAi, TALEN and CRISPR/Cas9 technologies in non-model insects should offer great opportunities for exploring new concepts and principles in the nutrition-dependent control of insect development in the future.

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be interesting to analyze the functional relationship and differences between SDR and the other ILP-binding protein complex Imp-L2/ALS [75, 76, 79]. [PubMed: 23307869]

Highlights

- Insulin-like peptides (ILPs) are encoded by multiple genes in insects.
- Insect ILPs are mainly produced by the brain medial neurosecretory cells (mNSCs).
- Transcription and secretion of ILPs are regulated by multiple nutritional signals.
- Insect ILPs regulate body and tissue growth by systemic as well as local actions.
- Activities of secreted ILPs are regulated by several binding proteins in hemolymph.

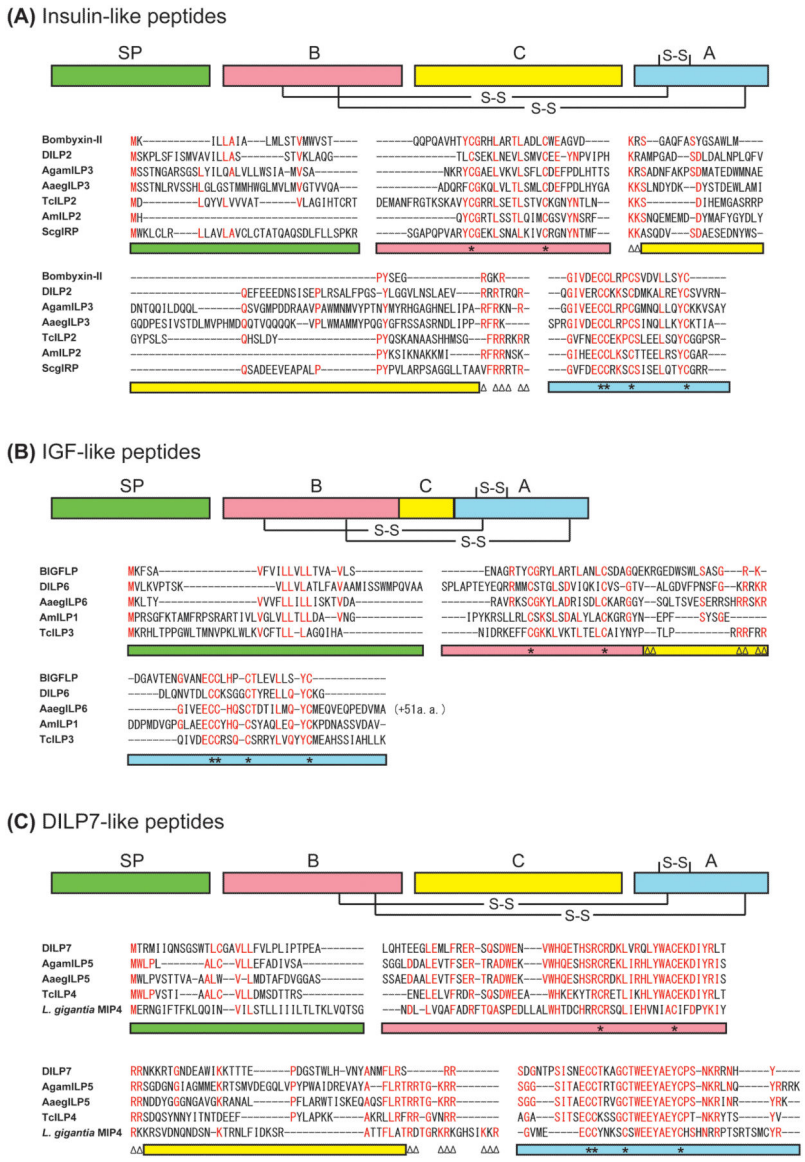

Figure 1.

Predicted insulin-like, IGF-like and DILP7-like peptides in insects. (A) Amino acid sequences of the representatives of predicted insulin-like peptides from *Bombyx* (bombyxin-II), *Drosophila* (DILP2), *Anopheles* (AgamILP3), *Aedes* (AaegILP3), *Apis* (AmILP2), *Tribolium* (TcILP2), and *Schistocerca* (ScgIRP) are aligned. (B) Amino acid sequences of the representatives of predicted IGF-like peptides from *B. mori* (BIGFLP), *Drosophila* (DILP6), *Aedes* (AaegILP6), *Apis* (AmILP1), and *Tribolium* (TcILP3) are aligned. (C) Amino acid sequences of the representatives of predicted highly conserved ILP group (DILP7-like peptides) from *Drosophila* (DILP7), *Anopheles* (AgamILP5), *Aedes* (AaegILP5), *Tribolium* (TcILP4), and *Lottia* (molluscan insulin-related peptide 4, MIP4) are aligned. Highly conserved amino acid residues are shown in red. Color bars indicate the predicted domains in the precursor peptides: green, signal peptide; red, B-chain; yellow, C-

peptide; blue, A-chain. Asterisks on the color bars below the alignment denote Cys residues, and paired triangles denote potential cleavage sites (dibasic amino acids).

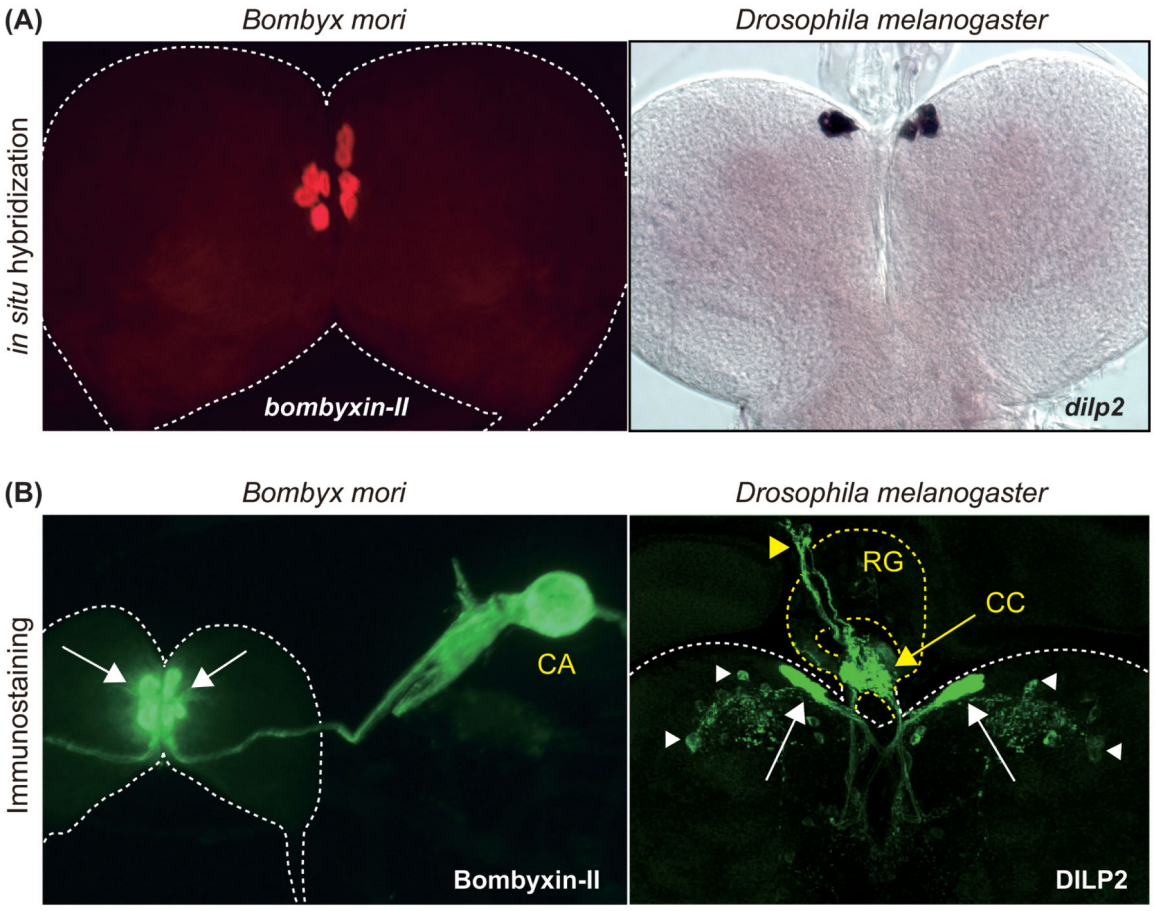

Figure 2.

Insulin-like peptides are mainly produced by brain mNSCs in insects. (A) Detection of *bombyxin-II* and *dilp2* mRNA in the larval brain by *in situ* hybridization. *bombyxin-II* and *dilp2* expression is observed in four and seven pairs of mNSCs in *Bombyx* and *Drosophila*, respectively. (B) Detection of Bombyxin-II and DILP2 localization in the larval brain by immunostaining. Bombyxin-II produced by mNSCs (white arrows) are axonally transported to the CA. DILP2 produced by mNSCs (white arrows) are axonally transported to the CC (yellow arrow) on the ring gland, and further transported to the dorsal vessel (yellow arrowhead). DILP2 signal can also be detected in specific sets of neurons within the brain (white arrowhead). CA, corpora allata; CC, corpora cardiaca; RG, ring gland.

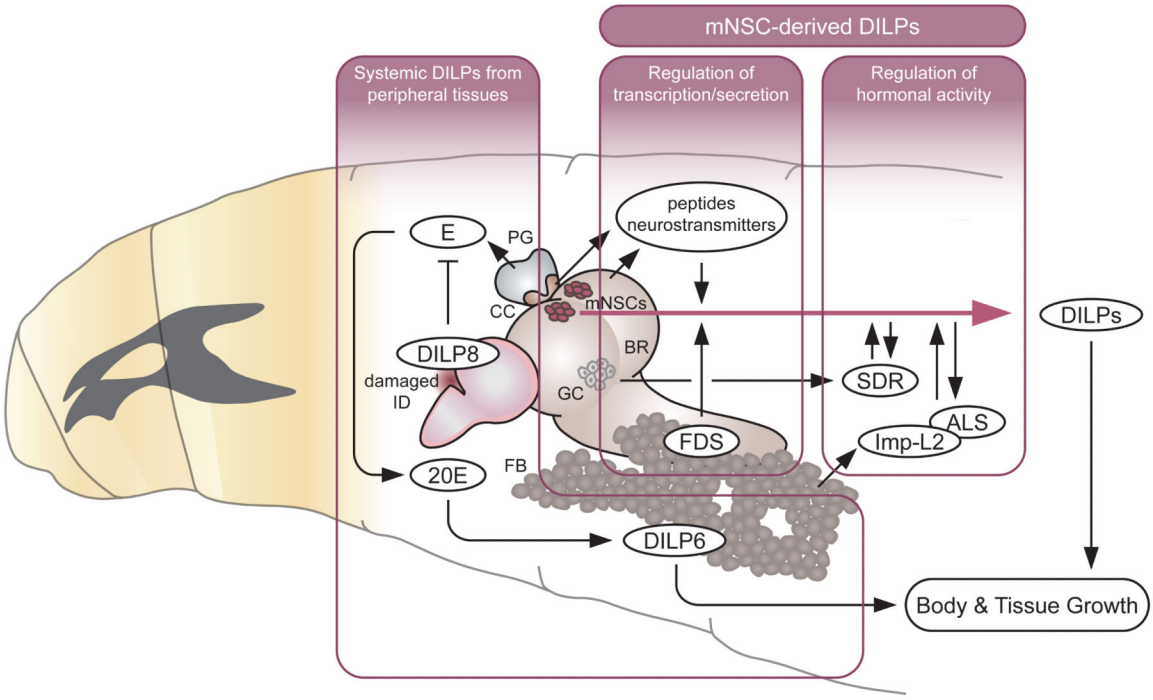

Figure 3.

Systemic function of DILPs during larval development and its regulation by multiple factors (see text for details).

DILP, *Drosophila* insulin-like peptide; E, ecdysone; 20E, 20-hydroxyecdysone (active form of ecdysone); FDS, fat body-derived signal; SDR, secreted decoy of insulin receptor; Imp-L2, ecdysone-inducible gene L2; ALS, acid-labile subunit; mNSCs, median neurosecretory cells; BR, brain; GC, glial cells; PG, prothoracic gland; CC, corpora cardiaca; ID, imaginal discs; FB, fat body.

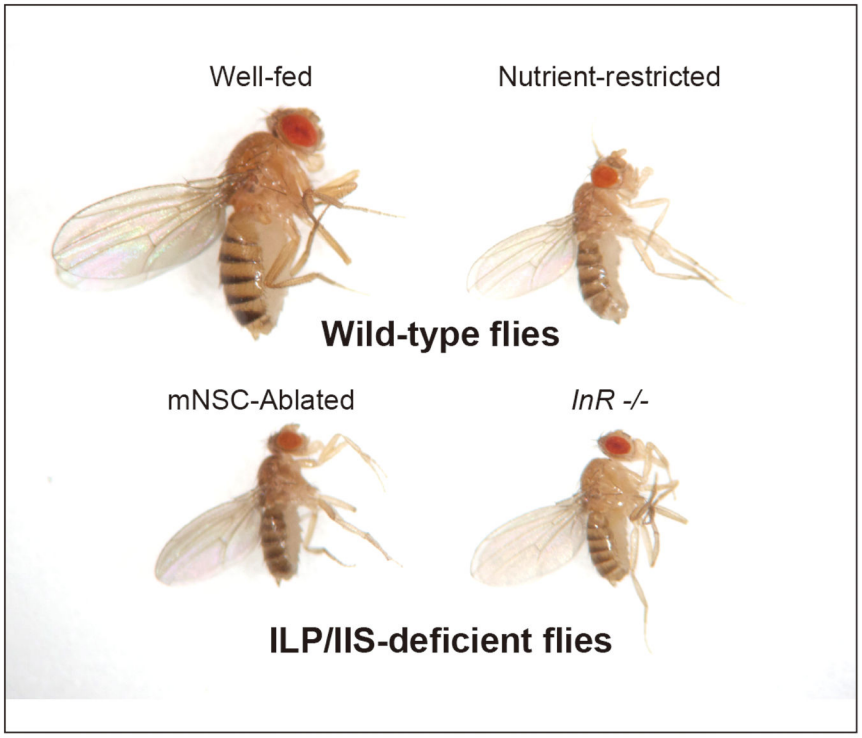

Figure 4.

Nutrient-restricted or ILP/IIS-deficient flies show severe growth defect. Wild-type flies, wild-type adult female flies raised either on a nutrient-rich diet (Well-fed) or low-protein diet (Nutrient-restricted). ILP/IIS-deficient flies, a brain mNSC-ablated female fly (DILP-producing mNSCs in the brain were genetically ablated using a *dilp2* promoter to express the pro-apoptotic gene, *reaper*) and an *InR* hypomorphic mutant female fly.