ISJ 17: 186-195, 2020 ISSN 1824-307X

REVIEW

# Insulin-like peptides in model insects

**D Li, X Chen, F Zhu*, K Chen***

*School of Life Sciences, Jiangsu University, Zhenjiang 212013, China*

 *Accepted September 9, 2020*

## Abstract

Recent years, invertebrate animals with clear genetic background and completed genome sequence have become very popular in biological research. Insulin and insulin-like peptides (ILPs) are a class of peptides having important physiological functions, including promotion of cell proliferation and differentiation, and body growth and development. To date, many invertebrates have been identified with multiple ILP families, whose structures and functions have become increasingly clear to scientists. This review summarizes the ILPs identified in the model Lepidopteran insect *Bombyx mori*, in the aspects of structures and classification, functions and pathways. A brief discussion on the ILPs from several other model insects, including *Drosophila melanogaster*, *Aedes aegypti*, and *Apis mellifera,* was also included.

**Key Words**: insects; insulin-like peptides; *Bombyx mori*; bombyxin; signaling pathway

## Introduction

Insulin is a well-known hormonal peptide in the human body that regulates carbohydrate metabolism, and malfunction in the regulation can result in type II diabetes. There are also peptides that exhibit similar sequence and structural similarity to insulin, known as insulin-like peptides (ILPs), which regulate many different biological processes and have certain physiological activities similar to that of insulin, but their secretion sites are different and possess distinct functions (Okamoto *et al*., 2009). ILPs are hallmarked by the presence of a conserved cysteine knot motif, and their precursor proteins share similar structure domains, i.e., signal peptide followed by the B, C, and A chain. ILPs have in general about 50 to more than 200 amino acids in length, and are not only conversed in pretty much all vertebrates but also in most invertebrates and even unicellular organisms, and have thought to be emerged very early during the evolution of eukaryotes (Souza and López, 2004).

Insects compose the largest and most diverse class in the arthropods and many are important economically and scientifically. Starting 1960s, many insulin-like peptides (ILPs) have been reported in insects, and the ILPs from several model organism will be discussed in detail in this review. ILPs have been demonstrated as key regulators in the animal body for growth and development, survival and proliferation. Insulin-like peptides in other arthropods, such as crustaceans, have also been characterized and found exclusively produced by a male-specific androgenic gland (Cronin, 1947). Significant research has been carried out on these crustaceans, and proved that the ILPs of these species were crucial in sexual differentiation and spermatogenesis, which have been discussed in detail elsewhere (Abdu *et al*., 2002; Aflalo *et al*., 2006; Barki *et al*., 2006; Ventura and Sagi, 2012). A more distant invertebrate, the nematode *Caenorhabditis elegans* encodes a remarkable 40 ILPs in its genome (Pierce *et al*., 2001). These ILPs were found primarily expressed in the nervous system, and differs in disulfide bond arrangement (Matsunaga *et al*., 2017; Zheng *et al*., 2018). In this review, various aspects of insect ILPs will be discussed, including classifications, structures, functions and pathways in several model insects, including *Bombyx mori*, *Drosophila melanogaster*, *Aedes aegypti*, and *Apis mellifera*, with a more detailed discussion into *B. mori*.

*Corresponding authors*:
Keping Chen and Feifei Zhu
School of Life Sciences
Jiangsu University
Zhenjiang, Jiangsu, 212013, China
E-mail: kpchen@ujs.edu.cn and feifzhu@ujs.edu.cn

## Classification of insulin-like peptides in *B. mori*

Insulin-like peptide in *B. mori*, also known as bombyxin, was first identified in 1984 by Nagasawa, who found that a 19 amino acids long peptide at the N terminus of 4K-prothoracicotropic hormone (4K-PTTH) was highly homologous to insulin and insulin-like growth factors in vertebrates. This was the first time that insulin-like peptide was identified in invertebrates (Nagasawa *et al*., 1984). Up to now,

a total of 38 bombyxins belonging to 12 families have been identified in *B. mori* (Yoshida *et al*., 1998). Among them, 32 bombyxin genes have been successfully cloned, and based on their sequence similarities, have been divided into 7 families, namely families A-G (Ingvarsson *et al*., 1988; Ikuyo *et al*., 1997; Tsuzuki *et al*., 1997; Iwami *et al*., 2008; Iwami *et al*., 1990; Kawakami *et al*., 1989; Kondo *et al*., 1996). Familes A, B and C contain 10, 12, and 6 bombyxin genes, respectively, and families D, E, F and G have only one bombyxin gene in each family (Table 1) (Ingvarsson *et al*., 1988). Another 5 bombyxin families, namely families V, W, X, Y and Z, were predicted by Aslam *et al*. through analysis of the *B. mori* genome and then confirmed experimentally (Table 1). Family V contains two bombyxin genes, and families W to Z have one gene in each family (Aslam *et al*., 2011).

In 2009, Okamoto *et al*. purified an 8 kDa insulin-like peptide from the hemolymph of *B. mori* (Okamoto *et al*., 2009). Sequencing analysis showed that the peptide was more similar to insulin-like growth factors (IGFs) of vertebrates than to bombyxin (Okamoto *et al*., 2009). It was found that ecdysone could stimulate the secretion of the peptide and the secreted peptide could further promote the growth of specific tissues in the adult. Therefore, this ILP peptide was also named *B. mori* insulin-like growth factor peptide (BIGFLP). Both ILPs and IGFs have the conserved cysteine knot motif, but IGFs hold additional domains, e.g., a C domain between the A and B chains, and/or D, E domains at the C terminus (Lecroisey *et al*., 2015).

**Spatial distribution of insulin-like peptides in *B. mori***

Early studies by Northern blot hybridization could only detect bombyxins in the brain tissue of the silkworm, but later RT-PCR analysis showed that they could also be expressed in other tissues, such as ganglia, epidermis, testis, ovary, fat body, silk gland, malpighian tubule, midgut and hindgut of *B. mori*, although the expression levels were much lower than that in the brain (Iwami *et al*., 1996). Bombyxin families A-G, as well as family V and W, were mainly expressed in four pairs of central nerves in the brain of *B. mori*, while the expression in other tissues was very low (Table 1) (Iwami *et al*., 1996). Family X was mainly found in the fat body, family Y was mainly found in the brain and ovary at the larval stages, and family Z was highly expressed in follicular cells (Aslam *et al*., 2011). Our group has previously characterized the *BmILP* gene in *B. mori* and found it has identical sequence to bombyxin Z1 and had the highest expression level in the ovary tissue, especially in the mated females, suggesting a role of *BmILP* in the regulation of egg maturation (Chen *et al*., 2016).

The BILGFP peptide was mainly expressed in the fat body of *B. mori* from the pupal to adult stage (Okamoto *et al*., 2009). The fat body tissue of *B. mori* is functionally equivalent to the liver and adipocytes of vertebrates, and the liver was the main place where mammals secreted IGFs. BIGFLP was also found expressed in other tissues of *B. mori*, such as brain, ovary and testis. The median

**Table 1** Characteristics of bombyxin genes*

| Bombyxin gene family | Number of genes | Chromosome location | Primary expression cites | Notes |
|---|---|---|---|---|
| A | 10 | B1, B2, B4, A2-A4 clustered on an unknown chromosome; rest all on Chromosome 11 | Brain | A6 and/or A7 encodes bombyxin II |
| B | 12 | | Brain | |
| C | 6 | | Brain | |
| D | 1 | | Brain | |
| E | 1 | unknown | Brain | Encodes bombyxin IV |
| F | 1 | Chromosome 11 | Brain | |
| G | 1 | Chromosome 11 | - | |
| V | 2 | Chromosome 9 | Brain | |
| W | 1 | Chromosome 1 | Brain | |
| X | 1 | Chromosome 11 | Fat body | |
| Y | 1 | Chromosome 1 | Brain | |
| Z | 1 | Chromosome 1 | Follicular cells | |

* Data summarized based on reports from Mizoguchi and Okamoto, 2013, Aslam *et al*., 2011, and Iwami, 2000

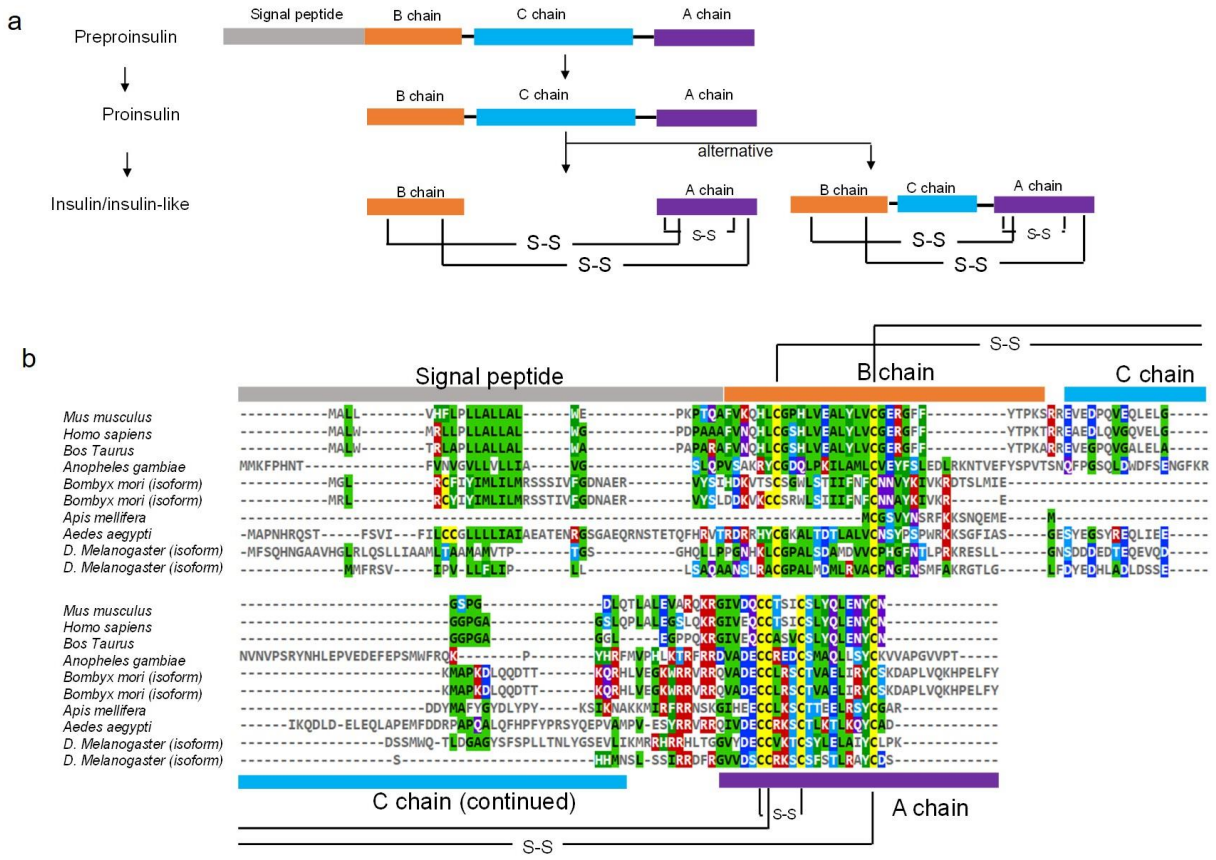


**Fig. 1** Structure of insulin and insulin-like peptides. (a) ILP maturation process and (a) alignment of representative insulin-like peptides from different species. Grey, orange, blue and purple bars above or below and amino acid sequence indicate the peptide domains for signal peptide, B chain, C chain, and A chain, respectively. S-S indicate disulfite bonds formed between the cysteine sites. Most species have more than one identified ILPs, and only one or two of the ILPs from one species was selected for alignment and comparison. *Mus musculus* (NP_032412.3), Homo sapiens (NP_001278826.1), *Bos Taurus* (NP_001172055.1), *Anopheles gambiae* (XP_314565.2), *Bombyx mori* (XP_021202224.1 and XP_021202225.1), *Apis mellifera* (NP_001171374.1), *Aedes aegypti* (XP_001657487.1), *Drosophila melanogaster* (NP_648359.1 and NP_996037.2). Conserved sites were colored according to amino acid hydrophobicity

neurosecretory cells (MNCs) in the brain have been identified to produce both BIGFLP and bombyxins, although the time of secretion varies between the two. *In vitro* experiments showed that the secretion of the BIGFLP gene in the ovary and testis was induced by ecdysone. The *B. mori* nephrocytes, which are functionally equivalent to glomerular podocytes of vertebrate kidneys, have been detected with high BIGFLP activity during the pupal stage, but the expression of BIGFLP gene was not detected. This indicated that the circulating BIGFLP was taken up and degraded by nephrocytes (Okamoto *et al*., 2011).

**Structures of ILP genes and proteins**

Compared to ILPs in higher animals, insect ILP genes are often intron-less (Mizoguchi and Okamoto, 2013). The human insulin gene contains two introns in two relatively conservative sites which are respectively located in 5' UTR and C-domain (Steiner *et al*., 1985), though sometimes the first intron can be inefficiently spiced (Wang *et al*., 1997). Whereas, there are no introns for bombyxin families A-G, W, X, and Y. Two genes V1 and V2 in the family V were found to contain one intron in the UTR of each gene, and the gene in family Z1 contained two introns that are similar to those ILPs found in vertebrates (Aslam *et al*., 2011). In *B. mori*, 25 of ILP genes were found clustered on chromosome 11, and 3 were located on chromosome 1, 2 were located on chromosome 9, and 1 on chromosome 11, while the locations for the rest ILPs have not been identified yet (Table 1) (Mizoguchi and Okamoto, 2013).

To now, five structural forms of bombyxin proteins have been isolated from the head of *B. mori*, namely, bombyxins I, II, III, IV and V (Nagasawa *et al*., 1986; Jhoti *et al*., 1987; Maruyama *et al*., 1988). The amino acid sequences of bombyxins II and IV have been fully identified, which are the products of the bombyxin A6/A7 and

the E1 genes, respectively (Table 1). The structures of bombyxins I, III and V have not been fully analyzed, and the bombyxin genes encoding them have not been revealed. The five protein structural forms all contain heterodimers of a A chain and a B chain, which have 50 % and 30 % amino acid similarity to those of human insulin, respectively. The A chain and B chain are linked by disulfide bonds within and between two chains in exactly the same way as insulin (Figure 1a) (Iwami, 2000). During translation, a bombyxin precursor, which is composed of a signal peptide, a B chain, a C chain, and a A chain successively from the N-terminal to the C-terminal end, will first be formed (Iwami, 2000). After that, the mature peptide is formed by proteolytic cleavage of the signal peptide and the C chain, and thereby leaving it with a heterodimer structure linked by disulfide bonds (Figure 1a). The newly discovered BIGFLP has a similar precursor to that of the bombyxin. However, when the BIGFLP matures, C chain is not completely excised, and part of the sequence is retained, while the signal peptide is completely excised, thus the BIGFLP peptide has a different structural form that contains the B, C, and A domains consecutively as a monomeric structure (Figure 1a) (Okamoto *et al*., 2009). From an evolutionary point of view, the amino acid sequence of the ILPs can be very divergent between distant species, but all possess conserved cysteine sites that form inter- and intra- chain disulfite bonds (Figure 1b), indicating a common phylogenetic origin (Mcrory and Sherwood, 1997; Jin Chan and Steiner, 2000).

The overall three-dimensional structure of bombyxin is spherical, similar to that of human insulin, which will form a T shaped crystal in solution (Jhoti *et al*., 1987; Nagata *et al*., 1995b). Although, the C terminal structure of the B chain in bombyxin II is significantly different from that of human insulin. B chain extends in helical form, similar to that of relaxin, a protein hormone that belongs to the insulin superfamily, and this form is unnecessary for the activity of bombyxin. On the other hand, the B chain of human insulin extends in the form of sharp turn and beta strands, and is indispensable for its activity (Nagata *et al*., 1995b; Nagata *et al*., 1995a). Therefore, bombyxin is more similar to relaxin in this regard, and human insulin may have evolved different receptor recognition sites at the C terminal of the B chain and thus distinguishes itself from bombyxin and relaxin. Human insulin is composed of dimers and hexamers, however, bombyxin II is unlikely to form dimers or hexamers by structural prediction. Instead, there is a hydrophobic region on the surface of bombyxin II (Mizoguchi and Okamoto, 2013), which may be important for the binding of bombyxin II to other proteins.

**Physiological functions: growth-promoting effect**

Although many insulin-like peptide genes have been identified in the genome of invertebrates, knowledge about the physiological functions of these gene products is still limited. It was found that bombyxin could reduce the concentration of the major sugar content trehalose in the hemolymph of *B. mori* and the effect was dose-dependent. Trehalose is a major carbohydrate in insects for energy storage. In the midgut and muscle of *B. mori*, bombyxin can enhance the activity of trehalase and help hydrolyze trehalose in the hemolymph into glucose, and facilitate the transport of the sugars to other tissues (Satake *et al*., 1997; Satake *et al*., 1999). Similar to insulin secretion in mammals, glucose in the silkworm could also stimulate the release of bombyxin into the hemolymph, and the titer of bombyxin in the hemolymph would decrease after starvation treatment of the silkworm (Masumura *et al*., 2000). Bombyxin can also reduce the glycogen content in the fat body and improve the activity of glycogen phosphorylase. However, these stated functions of bombyxin only exerted at the larval stage of the silkworm, and bombyxin does not affect the concentration of glucose in the adult hemolymph (Satake *et al*., 1997; Satake *et al*., 1999).

Bombyxin has been reported to promote cell proliferation in the hematopoietic organ of the silkworm. When the hematopoietic organs of the silkworm larvae were cultured *in vitro*, the addition of hemolymph to the culture medium could significantly improve the cell proliferation (Nakahara *et al*., 2003). Studies indicated that this promoting effect was mainly attributed to bombyxin in the hemolymph. When the hematopoietic organs from the first day of the fifth instar larvae were cultured with bombyxin-II for 48h, and the number of blood cells in the culture medium increased in a dose-dependent manner (Nakahara *et al*., 2006), indicating that bombyxin-II could promote mitosis. In addition, when bombyxin was added to silkworm ovary culture, increased meiosis of the ovary cells were observed. However, this might be an indirect effect, because bombyxin can stimulate the ecdysone production in the ovary cells, and ecdysone has been reported to induce the meiosis of cells at low concentration (Orikasa *et al*., 1993). In another study, when synthetic bombyxin-II was added to the silkworm ovary culture, a series of morphological changes of the cells were induced (Volkman and Goldsmith, 1982). Most of the cells became larger, round and aggregate into clusters. Some other cell lines became attached tightly to the bottom of the culture flask to be fibrous and spindle-like (Tanaka *et al*., 1995).

Bombyxin was supplemented exogenously to *Precis coenia* and found to promote the growth of the wing imaginal disc (Nijhout and Grunert, 2002). When the wing imaginal disc of *P. coenia* larva was removed and cultured in a standard nutrient-rich tissue culture medium, the wing imaginal disc ceased to grow. However, when 20-hydroxyecdysone and hemolymph from silkworm larva with an appropriate concentration were added to the tissue culture, the wing imaginal disc started its normal growth (Nijhout and Grunert, 2002). Therefore, the promoting effect may come from some biological factors in the hemolymph. Additionally, when 20-hydroxyecdysone and bombyxin-II were added to the tissue culture medium, the division rate of cell nucleuses was increased. However, when bombyxin-II was pre-incubated with bombyxin antibody, this growth-promoting effect was inhibited. Similar results were

observed in *Manduca sexta* (Nijhout *et al*., 2007). This indicated that bombyxin acted as a growth promoting factor to wing imaginal discs of other insects (Nijhout and Grunert, 2002). Bombyxin, together with 20-hydroxyecdysterone, played a synergistic role in promoting the growth of imaginal discs of insect species in a dose-dependent manner. This effect was different from promoting cell proliferation described above because here bombyxin relies on 20-hydroxyecdysone as a synergistic compound, while it can function independently to promote proliferation of silkworm hematopoietic and ovary cells.

**Insulin-dependent signaling pathways**

In *B. mori*, bombyxin could regulate the molting process through the insulin signaling pathway. It was found that 24 h after the larvae were injected with bombyxin, silkworm molting were accelerated (Gu *et al*., 2015). An insulin-dependent signaling pathway in insects for development and growth was drawn to Figure 2. Bombyxin stimulates insulin receptor and serine/threonine protein kinase (Akt), which were then phosphorylated to activate downstream signal molecule target of rapamycin (TOR). The phosphorylation of serine/threonine protein kinase depended on phosphorylation of phosphatidylinositol-3 kinase (PI3K), indicating that PI3K was the upstream signal molecule of Akt and the activated TOR continues to phosphorylate the translation initiation factors 4E-binding protein (4E-BP) and p70 ribosomal protein S6 protein kinase (S6K) of its downstream signal molecules in eukaryotic cells and ultimately stimulate the molting of *B. mori*. Meanwhile, bombyxin could inhibit the phosphorylation of AMP-activated protein kinase (AMPK), but this inhibition was not dependent on PI3K, indicating that AMPK and PI3K were two different signal pathways of the downstream of insulin receptor. Chemical activators of AMPK could partially alleviate the inhibition of AMPK phosphorylation by bombyxin, indicating that AMPK is also involved in the TOR signal pathway and that PI3K/Akt and AMPK are two different downstream signal pathways of the insulin receptor and eventually converged on the TOR signal molecule to affect the molting of *B. mori*. In *B. mori*, serine/threonine protein kinase (Akt) have been identified, which consists of 493 amino acid residues, including a pleckstrin homology (PH) domain, a kinase domain and phosphorylation site that could be activated by bombyxin (Nagata *et al*., 2008).

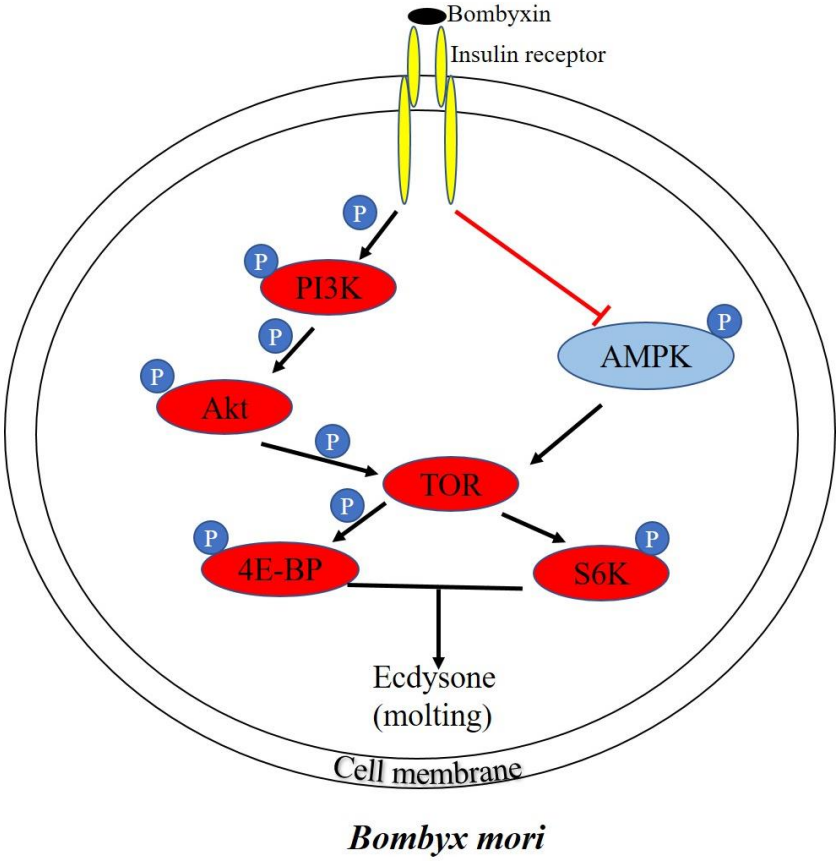


**Fig. 2** Insulin-dependent signaling pathway in *Bombyx mori*. "P" in the blue circle refers to phosphorylation, and red and blue ovals refer to proteins that are activated and inhibited, respectively. Black arrow and red bar indicate activation and inhibition. PI3K, phosphatidylinositol-3 kinase; AMPK, AMP-activated protein kinase; Akt, serine/threonine protein kinase; TOR, target of rapamycin; 4E-BP, 4E-binding protein; S6K, p70 ribosomal protein S6 protein kinase

There is species-specific regulation of ecdysone secretion. In *M. sexta*, when insulin and bombyxin II were used to activate the phosphorylation of insulin receptor and Akt, no stimulation of ecdysone was observed (Smith *et al*., 2014). Likewise, when PI3K inhibiting factor was used to inhibit the phosphorylation of Akt and 4E-BP, ecdysone secretion was not affected either. However, when extracellular signal regulated kinase (ERK) phosphorylation was inhibited, ecdysone secretion would be inhibited significantly (Smith *et al*., 2014). This indicated that ecdysis was mainly regulated through the ERK signal pathway. In *B. mori*, bombyxin could not activate the phosphorylation of ERK in prothoracic gland, but PTTH would (Gu *et al*., 2010; Gu *et al*., 2013). When the phosphorylation of ERK in *B. mori* was inhibited, it would not affect ecdysone secretion, but when the phosphorylation of Akt and 4E-BP was inhibited, ecdysone secretion would be affected. This indicated that silkworm molting was mainly regulated through the PI3K/Akt signal pathway (Gu *et al*., 2015).

**Insulin-like peptides in other insect species: the dipteran insect fruit fly**

The studies on the insulin-like peptides in *D. melanogaster* (DILP) were much more detailed than in *B. mori*. To date, 8 insulin-like peptides (DILP1-8) have been discovered in *D. melanogaster* (Brogiolo *et al*., 2001; Colombani *et al*., 2012; Garelli *et al*., 2012). Among them, DILP1, DILP2, DILP3 and DILP5 were mainly expressed in the median neurosecretory cells (MNCs) of the brain, similar to that of bombyxin (Brogiolo *et al*., 2001; Ikeya *et al*., 2002; Rulifson *et al*., 2002; Broughton *et al*., 2005; Nässel, 2012). The protein products encoded by these genes were structurally similar, represented by a heterodimer linked by disulfite bonds. At the adult stage, DILP2, DILP3 and DILP5 continued to express in MNC, however, DILP1 gene expression was not detected (Broughton *et al*., 2005). Although DILP2, DILP3 and DILP5 were all expressed in the MNCs, their temporal expression patterns were different. DILP 2 was expressed starting from the 1$^{st}$ instar larvae, while DILP3 was from the middle and later stage of the 3$^{rd}$ instar, and DILP 5 was from the 2$^{nd}$ instar (Ikeya *et al*., 2002).

When the larvae were starved, the transcriptional level of DILP3 and DILP5 would decrease, but the DILP2 transcription was not affected (Ikeya *et al*., 2002). Upon dietary restriction, the expression of DILP5 in adult drosophila was down-regulated, but DILP3 was not affected (Min *et al*., 2008). No expression of DILP4 was detected in the adult *D. melanogaster* (Grönke and Partridge, 2010). Genetic knockout of DILP1 and DILP2 resulted in a smaller body size of *D. melanogaster*. When DILP1-7 was overexpressed in *D. melanogaster*, the adult body size increased proportionally. Among them, DILP2 had the strongest promoting effect on the growth. The overexpression of DILP2 gene not only increased the size of MNC cell but also increased the number of cells (Brogiolo *et al*., 2001; Ikeya *et al*., 2002).

DILP2 was found down-regulated in a mutant *D. melanogaster* that had a prolonged life span, suggesting that DILP2 played a key role in regulating the life span of *D. melanogaster* (Hwangbo *et al*., 2004; Bauer *et al*., 2007; Lee *et al*., 2008). However, some scholars knocked down the DILP2 gene by RNA interference and found that the life span of *D. melanogaster* was not prolonged like expected, but trehalose storage in the adult *D. melanogaster* was increased, indicating that DILP2 was involved in sugar metabolism (Broughton *et al*., 2008). DILP6 was mainly expressed in the fat body tissue, and was positively regulated when *D. melanogaster* metamorphosed from larva to pupa (Okamoto *et al*., 2009). DILP7 gene was only expressed in specific neurons of the ventral nerve chain and a few neurons in the brain (Miguel-Aliaga *et al*., 2008; Yang *et al*., 2008).

DILP1-4 were clustered on chromosome 3 of *D. melanogaster*, while DILP5, DILP6 and DILP7 genes were located at two different sites on the X chromosome (Ikeya *et al*., 2002). The DILP1-7 precursor peptides contained 107-156 amino acids, and their primary structure included a signal peptide, B chain, C chain and A chain, and the cleavage sites lay between A chain and B chain and were similar to those of insulin and relaxin. Among the 8 DILPs, DILP6 was more similar to insulin growth factor. When DILP6 formed a tertiary structure, its C chain would not be completely cleaved by the protease, and thus some sequences were retained. Sequence similarity analysis showed that DILP2 had the highest amino acid sequence similarity with mature human insulin, reaching 35 % (Brogiolo *et al*., 2001; Ikeya *et al*., 2002).

Animals would constantly adjust their growth status, such as maturity or metamorphosis so as to adapt to the disorder (such as damage or tumor) occurred during the growth and development. Such processes require the connection between the tissues and organs of the animal so as to maintain the normality and symmetry of their bodies. It was found that the imaginal disc of *Drosphila melanogaster* could activate DILP8 spontaneously. When abnormality was detected during the growth and development of *D. melanogaster*, DILP8 would delay the metamorphosis of the body by inhibiting the synthesis of ecdysone and slowing down the growth of imaginal disc, and finally producing normal sized individuals (Garelli *et al*., 2012). When DILP8 was silenced or mutated, *D.* would develop asymmetrical bodies, irregular body size, and need more time to mature. Therefore, DILP8 appears to be a key regulatory factor to ensure the stability and robustness during the growth and development of *D. melanogaster*, however, this regulatory mechanism has not been clarified yet (Garelli *et al*., 2012).

**The dipteran insect mosquito**

When *A. aegypti* ingested the mammalian blood, it would transmit the parasites that caused diseases to mammals. Meanwhile, the mammalian blood was very important for the maturation of female *A. aegypti* eggs. Some studies have shown that after ingesting the mammalian blood, mosquitoes could stimulate the median neurosecretory cells of the brain to secrete a neuropeptide that could promote

the production of ecdysone in the ovary (Brown *et al*., 2008; Attardo *et al*., 2005). It was identified that the neuropeptide was insulin-like peptide which could activate multiple metabolic processes necessary for egg maturation. Up to now, 8 insulin-like peptides (AaegILP1-8) and 1 insulin receptor have been identified from the genome of *A. aegypti* (Riehle *et al*., 2006). Among them, AaegILP1, AaegILP3 and AaegILP8 were specifically expressed in the brain of female *A. aegypti* at the adult stage. Moreover, the precursors of these 8 insulin-like peptides were also composed of a signal peptide, B chain, C chain and A chain. In the formation of mature peptides, AaegILP6 was similar to insulin growth factor peptides (IGFs), whose C chain was not completely excised (Graf *et al*., 1997; Riehle *et al*., 2006).

It was found that AaegILP3 could stimulate the oocyte's uptake of yolk and the ovary's production of ecdysone in low concentration, indicating that AaegILP3 was a key regulatory factor for the production of eggs in *A. aegypti* (Brown *et al*., 2008). AaegILP3 also showed metabolic activity and could increase the storage of carbohydrate and lipid, and the function of AaegILP3 depended on the expression of mosquito insulin receptor (MIR), which have been previously detected in mosquito ovaries (Graf *et al*., 1997). The mature peptide MIR is a 400 kDa tetramer consisting of two 116 kDa α subunits and two 95 kDa β subunits. Immunohistochemistry analysis showed that MIR was located on the cytomembrane of follicle cells around egg cells and nutrient cells (Riehle and Brown, 2002). In the fat body, the insulin-like peptides of *A. aegypti* could also regulate the expression of yolk protein precursor gene. It was found in vitro that when 20-hydroxyecdysone and AaegILPs were added to the fat body culture separately, they could not stimulate the expression of yolk protein precursor gene. However, when 20-hydroxyecdysone and AaegILPs were incubated simultaneously in the fat body tissue culture, they played a strong synergistic role in regulating the expression of yolk protein precursor gene (Larsen *et al*., 2017; Roy *et al*., 2007).

In the genome of another mosquito species *Anopheles gambiae*, 7 insulin-like peptide genes (AgamILP1-7) have been identified so far (Riehle *et al*., 2002; Krieger *et al*., 2004). The arrangement of insulin-like peptide genes on the chromosomes of *A. gambiae* was similar to that of insulin. AgamILP1-4 genes clustered on chromosome III, about 23 kb away from AgamILP 6 and AgamILP 7, whereas AgamILP 5 was located on chromosome II. Among the AgamILPs, the two pairs Agam 3/Agam 6 and Agam 1/Agam 7 show high sequence homology, which were 98.7 % and 95.5 %, respectively (Riehle *et al*., 2002).

**The hymenopteran insect honeybee**

Two insulin-like peptides (AmILP1 andAmILP2) and two insulin receptors (AmIR1 and AmIR2) have been identified within the genome of *A. mellifera* (Wheeler *et al*., 2006). At the critical stage of gender determination, the expressions of AmILP1, AmILP2 and AmIR2 were significantly different between the queen and worker bees (De Azevedo and Hartfelder, 2008; Wheeler *et al*., 2006). In queen larvae, the expression of AmILP1 gene was particularly high, and such high expression depended on the ingested royal jelly. In contrast, the expression of AmILP2 gene was higher in the worker bee larvae. Compared with the mature worker bees, the expression level of AmILP1, AmIR1 and AmIR2 in the heads of the mature queen bees was very low. In the worker bee, the expression level of AmILP1 decreased with aging, while in the queen bee, the expression level of AmILP1 increased with the aging process (Corona *et al*., 2007), indicating that the insulin signal pathway is related to the life span of *A. mellifera*. The expression difference between the queen and worker bee was relatively conservative during evolution, and the same phenomenon was also found in the bee *Polistes metricus* (Toth *et al*., 2007).

Based on phylogenetic analysis by Riehle *et al*., the ILPs of *A. aegypti*, *A. gambiae,* and *D. melanogaster* have higher homology to each other than to those of *B. mori,* indicating highly diverged sequences between insect orders, whereas, within the same insect order, the distances between the ILP families were greater than the orthologues within that order (Riehle *et al*., 2006).

**Conclusion**

From insects to mammals, insulin and insulin-like peptides play central roles in regulating the organ development and establishing adult body size. The signaling pathways of insulin and insulin-like peptides in *B. mori* as well as many other insects have been proposed, however, many details regarding the pathway, such as the interacting proteins of ILPs and the mechanisms of interaction, are still unclear. Comprehensive functional studies utilizing genetic editing, protein overexpression, and protein-protein interaction methods are anticipated to be increasingly demanding for the understanding the roles of many ILP genes and their protein products during animal growth and development.

**Acknowledgement**

This work was supported by the National Natural Science Foundation of China (31702186 and 31861143051).

**References**

Abdu U, Davis C, Khalaila I, Sagi A. The vitellogenin cDNA of cherax quadricarinatus encodes a lipoprotein with calcium binding ability, and its expression is induced following the removal of the androgenic gland in a sexually plastic system. Gen. Comp. Endocrinol. 127: 263-272, 2002.

Aflalo ED, Hoang TTT, Nguyen VH, Lam Q, Nguyen DM, Trinh QS*, et al.* A novel two-step procedure for mass production of all-male populations of the giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture. 256: 468-478, 2006.

Aslam AF, Kiya T, Mita K, Iwami M. Identification of novel bombyxin genes from the genome of the silkmoth *Bombyx mori* and analysis of their expression. Zool. Sci. 28: 609-16, 2011.

Attardo GM, Hansen IA, Raikhel AS. Nutritional regulation of vitellogenesis in mosquitoes: Implications for anautogeny. Insect Biochem. Mol. Biol. 35: 661-675, 2005.

Barki A, Karplus I, Manor R, Sagi A. Intersexuality and behavior in crayfish: The de-masculinization effects of androgenic gland ablation. Horm. Behav. 50: 322-331, 2006.

Bauer JH, Chang C, Morris SNS, Hozier S, Andersen S, Waitzman JS*, et al.* Expression of dominant-negative dmp53 in the adult fly brain inhibits insulin signaling. Proc. Natl. Acad. Sci. U. S. A. 104: 13355-13360, 2007.

Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E. An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. Curr. Biol. 11: 213-221, 2001.

Broughton S, Alic N, Slack C, Bass T, Ikeya T, Vinti G*, et al.* Reduction of DILP2 in drosophila triages a metabolic phenotype from lifespan revealing redundancy and compensation among DILPs. PLoS One. 3: 2008.

Broughton SJ, Piper MDW, Ikeya T, Bass TM, Jacobson J, Driege Y*, et al.* Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. Proc. Natl. Acad. Sci. U. S. A. 102: 3105-3110, 2005.

Brown MR, Clark KD, Gulia M, Zhao Z, Garczynski SF, Crim JW*, et al.* An insulin-like peptide regulates egg maturation and metabolism in the mosquito *Aedes aegypti*. Proc. Natl. Acad. Sci. U. S. A. 105: 5716-5721, 2008.

Chen X, Zhu F, Lv P, Nouara A, Yang Y*, et al.* Properties of the *Bombyx mori* insulin-like peptide (BmILP) gene. Invert. Surv. J. 76-88, 2016.

Colombani J, Andersen DS, Léopold P Secreted peptide DILP8 coordinates *Drosophila* tissue growth with developmental timing. Science. 336: 582-585, 2012.

Corona M, Velarde RA, Remolina S, Moran-Lauter A, Wang Y, Hughes KA*, et al.* Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. Proc. Natl. Acad. Sci. U. S. A. 104: 7128, 2007.

Cronin LE. Anatomy and histology of the male reproductive system of callinectes *Sapidus rathbun*. J. Morphol. 81: 209-239, 1947.

De Azevedo SV, Hartfelder K. The insulin signaling pathway in honey bee (*Apis mellifera*) caste development - differential expression of insulin-like peptides and insulin receptors in queen and worker larvae. J. Insect Physiol. 54: 1064-1071, 2008.

Garelli A, Gontijo AM, Miguela V, Caparros E, Dominguez M. Imaginal discs secrete insulin-like peptide 8 to mediate plasticity of growth and maturation. Science. 336: 579-582, 2012.

Grönke S, Partridge L. The functions of insulin-like peptides in insects. In: Clemmons D, Robinson I, Christen Y (eds) IGFs: Local repair and survival factors throughout life span. Research and Perspectives in Endocrine Interactions. Springer, Berlin, Heidelberg, 2010.

Graf R, Neuenschwander, S, Brown, MR and Ackermann, U. Insulin-mediated secretion of ecdysteroids from mosquito ovaries and molecular cloning of the insulin receptor homologue from ovaries of bloodfed *Aedes aegypti*. Insect Mol. Biol. 6: 151-163. 1997.

Gu S-H, Chen C-H, Hsieh Y-C, Lin P-L, Young S-C. Modulatory effects of bombyxin on ecdysteroidogenesis in *Bombyx mori* prothoracic glands. J. Insect Physiol. 72: 61-69, 2015.

Gu S-H, Hsieh Y-C, Young S-C, Lin P-L. Involvement of phosphorylation of adenosine 5′-monophosphate-activated protein kinase in ptth-stimulated ecdysteroidogenesis in prothoracic glands of the silkworm, *Bombyx mori*. PLoS One. 8: 2013.

Gu S-H, Lin J-L, Lin P-L. PTTH-stimulated erk phosphorylation in prothoracic glands of the silkworm, *Bombyx mori*: Role of Ca2+/calmodulin and receptor tyrosine kinase. J. Insect Physiol. 56: 93-101, 2010.

Hwangbo DS, Gersham B, Tu M-P, Palmer M, Tatar, M. Drosophila dfoxo controls lifespan and regulates insulin signalling in brain and fat body. Nature. 429: 562-566, 2004.

Ikeya T, Galic M, Belawat P, Nairz K, Hafen E. Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the cns contributes to growth regulation in *Drosophila*. Curr. Biol. 12: 1293-1300, 2002.

Ikuyo Y, Seiji T, Salah Eldin Abdel S, Masaya I, Ahmed Mohamed K, Sho S*, et al.* Bombyxin F1 gene: Structure and expression of a new bombyxin family gene that forms a pair with bombyxin b10 gene. Zool. Sci. 14: 615-622, 1997.

Ingvarsson S, Asker C, Axelson H, Klein G, Sümegi J. Structure and expression of b-myc, a new member of the myc gene family. Mol. Cell. Biol. 8: 3168-3174, 1988.

Iwami M. Bombyxin: An insect brain peptide that belongs to the insulin family. Zoolog. Sci. 17: 1035-1044, 2000.

Iwami M, Adachi T, Kondo H, Kawakami A, Suzuki Y, Nagasawa H*, et al.* A novel family c of the genes that encode bombyxin, an insulin-related brain secretory peptide of the silkmoth *Bombyx mori*: Isolation and characterization of gene c-1. Insect Biochem. 20: 295-303, 1990.

Iwami M, Kawakami A, Ishizaki H, Takahashi S, Adachi-Yamada T, Suzuki Y*, et al.* Cloning of a gene encoding bombyxin, an insulin-like brain secretory peptide of the silkmoth *Bombyx mori* with prothoracicotropic activity. Dev. Growth Differ. 31: 31-37. 2008.

Iwami M, Tanaka A, Hano N, Sakurai S. Bombyxin gene expression in tissues other than brain detected by reverse transcriptionpolymerase chain reaction (RT-PCR) and *in situ* hybridization. Experientia. 52: 882-887, 1996.

Jhoti H, Mcleod AN, Blundell TL, Ishizaki H, Nagasawa H, Suzuki A. Prothoracicotropic hormone has an insulin-like tertiary structure. FEBS Lett. 219: 419-425. 1987.

Jin Chan S, Steiner, DF. Insulin through the ages: Phylogeny of a growth promoting and metabolic regulatory hormone1. Am. Zool. 40: 213-222, 2000.

Kawakami A, Iwami M, Nagasawa H, Suzuki A, Ishizaki H. Structure and organization of four clustered genes that encode bombyxin, an insulin-related brain secretory peptide of the silkmoth *Bombyx mori*. Proc. Natl. Acad. Sci. U. S. A. 86: 6843-6847, 1989.

Kondo H, Ino M, Suzuki A, Ishizaki H, Iwami M. Multiple gene copies for bombyxin, an insulin-related peptide of the silkmoth *Bombyx mori*: Structural signs for gene rearrangement and duplication responsible for generation of multiple molecular forms of bombyxin. J. Mol. Biol. 259: 926-937, 1996.

Krieger MJB, Jahan N, Riehle MA, Cao C, Brown MR. Molecular characterization of insulin-like peptide genes and their expression in the African malaria mosquito, *Anopheles gambiae*. Insect Mol. Biol. 13: 305-315, 2004.

Larsen ISB, Narimatsu Y, Joshi HJ, Siukstaite L, Harrison OJ, Brasch J*, et al.* Discovery of an O-mannosylation pathway selectively serving cadherins and protocadherins. Proc. Natl. Acad Sci. U S A. 114: 11163-11168, 2017.

Lecroisey C, Le Pétillon Y, Escriva H, Lammert E, Laudet V. Identification, evolution and expression of an insulin-like peptide in the cephalochordate branchiostoma lanceolatum. PLoS One. 10: e0119461-e0119461, 2015.

Lee K-S, Kwon OY, Lee JH, Kwon K, Min K-J, Jung S-A*, et al.* Drosophila short neuropeptide F signalling regulates growth by ERK-mediated insulin signalling. Nat. Cell Biol. 10: 468-475, 2008.

Maruyama K, Hietter H, Nagasawa H, Isogai A, Tamura S, Suzuki A*, et al.* Isolation and primary structure of bombyxin-iv, a novel molecular species of bombyxin from the silkworm, *Bombyx mori*. Agric. Biol. Chem. 52: 3035-3041, 1988.

Masumura M, Satake SI, Saegusa H, Mizoguchi, A. Glucose stimulates the release of bombyxin, an insulin-related peptide of the silkworm *Bombyx mori*. Gen. Comp. Endocrinol. 118: 393-399, 2000.

Matsunaga Y, Iwasaki T, Kawano, T. Diverse insulin-like peptides in *Caenorhabditis elegans*. Int. Biol. Rev. 1: 2017.

Mcrory JE, Sherwood, NM. Ancient divergence of insulin and insulin-like growth factor. DNA Cell Biol. 16: 939-949, 1997.

Miguel-Aliaga I, Thor S, Gould, AP. Postmitotic specification of *Drosophila* insulinergic neurons from pioneer neurons. PLoS Biol. 6: 538-551, 2008.

Min K-J, Yamamoto R, Buch S, Pankratz M, Tatar, M. Drosophila lifespan control by dietary restriction independent of insulin-like signaling. Aging cell. 7: 199-206, 2008.

Mizoguchi A, Okamoto N. Insulin-like and igf-like peptides in the silkmoth *Bombyx mori*: Discovery, structure, secretion, and function. Front. Physiol. 4: 217-217, 2013.

Nässel D. Insulin-producing cells and their regulation in physiology and behavior of *Drosophila*. Can. J. Zool. 90: 476-488, 2012.

Nagasawa H, Kataoka H, Isogai A, Tamura S, Suzuki A, Ishizaki H*, et al.* Amino-terminal amino acid sequence of the silkworm prothoracicotropic hormone: Homology with insulin. 226: 1344-1345, 1984.

Nagasawa H, Kataoka H, Isogai A, Tamura S, Suzuki A, Mizoguchi A*, et al.* Amino acid sequence of a prothoracicotropic hormone of the silkworm *Bombyx mori*. Proc. Natl. Acad. Sci. U. S. A. 83: 5840-5843, 1986.

Nagata K, Hatanaka H, Kohda D, Kataoka H, Nagasawa H, Isogai A*, et al.* Identification of the receptor-recognition surface of bombyxin-II, an insulin-like peptide of the silkmoth *Bombyx mori*: Critical importance of the B-chain central part. J. Mol. Biol. 253: 759-770, 1995a.

Nagata K, Hatanaka H, Kohda D, Kataoka H, Nagasawa H, Isogai A*, et al.* Three-dimensional solution structure of bombyxin-ii an insulin-like peptide of the silkmoth *Bombyx mori*: Structural comparison with insulin and relaxin. J. Mol. Biol. 253: 749-758, 1995b.

Nagata S, Hakuno F, Takahashi S-I, Nagasawa H. Identification of *Bombyx mori* AKT and its phosphorylation by bombyxin stimulation. Comp. Biochem. Physiol. B. 151: 355-360, 2008.

Nakahara Y, Kanamori Y, Kiuchi M, Kamimura, M. *In vitro* studies of hematopoiesis in the silkworm: Cell proliferation in and hemocyte discharge from the hematopoietic organ. J. Insect Physiol. 49: 907-916, 2003.

Nakahara Y, Matsumoto H, Kanamori Y, Kataoka H, Mizoguchi A, Kiuchi M*, et al.* Insulin signaling is involved in hematopoietic regulation in an insect hematopoietic organ. 52: 105-111, 2006.

Nijhout HF, Grunert LW. Bombyxin is a growth factor for wing imaginal disks in lepidoptera. Proc. Natl. Acad. Sci. U. S. A., 99: 2002.

Nijhout HF, Smith WA, Schachar I, Subramanian S, Tobler A, Grunert LW The control of growth and differentiation of the wing imaginal disks of manduca sexta. Dev. Biol. 302: 569-576, 2007.

Okamoto N, Yamanaka N, Endo Y, Kataoka H, Mizoguchi A. Spatiotemporal patterns of IGF-like peptide expression in the silkmoth *Bombyx mori* predict its pleiotropic actions. Gen. Comp. Endocrinol. 173: 171-182, 2011.

Okamoto N, Yamanaka N, Satake H, Saegusa H, Kataoka H, Mizoguchi A. An ecdysteroid-inducible insulin-like growth factor-like peptide regulates adult development of the silkmoth *Bombyx mori*. The FEBS J. 276: 1221-1232, 2009.

Orikasa C, Yamauchi H, Nagasawa H, Suzuki A, Nagata M. Induction of oocyte-nurse cell differentiation in the ovary by the brain during the initial stage of oogenesis in the silkworm, *Bombyx mori* (lepidoptera: *Bombycidae*). Appl. Entomol. Zool. 28: 1993.

Pierce SB, Costa M, Wisotzkey R, Devadhar S, Homburger SA, Buchman AR*, et al.* Regulation of daf-2 receptor signaling by human insulin and INS-1, a member of the unusually large and diverse *C. elegans* insulin gene family. Genes Dev. 15: 672-686, 2001.

Riehle M, Brown M. Insulin receptor expression during development and a reproductive cycle in the ovary of the mosquito *Aedes aegypti*. Cell Tissue Res. 308: 409-420, 2002.

Riehle M, Garczynski S, Crim J, Hill C, Brown M. Neuropeptides and peptide hormones in *Anopheles gambiae*. Science (New York, N.Y.). 298: 172-175, 2002.

Riehle MA, Fan Y, Cao C, Brown, MR. Molecular characterization of insulin-like peptides in the yellow fever mosquito, *Aedes aegypti*: Expression, cellular localization, and phylogeny. Peptides. 27: 2547-2560, 2006.

Roy SG, Hansen IA, Raikhel AS. Effect of insulin and 20-hydroxyecdysone in the fat body of the yellow fever mosquito, *Aedes aegypti*. Insect Biochem. Mol. Biol. 37: 1317-1326, 2007.

Rulifson EJ, Kim SK, Nusse, R. Ablation of insulin-producing neurons in flies: Growth and diabetic phenotypes. Science. 296: 1118-1120, 2002.

Satake S, Nagata K, Kataoka H, Mizoguchi A. Bombyxin secretion in the adult silkmoth *Bombyx mori*: Sex-specificity and its correlation with metabolism. J Insect. Physiol. 45: 939-945, 1999.

Satake SI, Masumura M, Ishizaki H, Nagata K, Mizoguchi A. Bombyxin, an insulin-related peptide of insects, reduces the major storage carbohydrates in the silkworm *Bombyx mori.* J. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 118: 349-357, 1997.

Smith WA, Lamattina A, Collins, M. Insulin signaling pathways in lepidopteran ecdysone secretion. Front. Physiol. 5: 19-19, 2014.

Souza AMFD, López JA. Insulin or insulin-like studies on unicellular organisms: A review. Braz. Arch. Biol. Technol. 47: 973-981, 2004.

Steiner DF, Chan SJ, Welsh JM, Kwok SCM. Structure and evolution of the insulin gene. Annu. Rev. Genet. 19: 463-484, 1985.

Tanaka M, Kataoka H, Nagata K, Nagasawa H, Suzuki, A. Morphological changes of bm-n4 cells induced by bombyxin, an insulin-related peptide of *Bombyx mori*. Regul. Pept. 57: 311-318, 1995.

Toth A, Varala K, Newman T, Miguez F, Willoughby D, Simons J*, et al.* Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. Science (New York, N.Y.). 318: 441-444, 2007.

Tsuzuki S, Masuta T, Furuno M, Sakurai S, Iwami, M. Structure and expression of bombyxin E1 gene: A novel family gene that encodes bombyxin-IV, an insect insulin-related neurosecretory peptide. Comp. Biochem. Physiol. B, Biochem. Mol. Biol. 117: 409-416, 1997.

Ventura T, Sagi, A. The insulin-like androgenic gland hormone in crustaceans: From a single gene silencing to a wide array of sexual manipulation-based biotechnologies. Biotechnol. Adv. 30: 1543-1550, 2012.

Volkman LE, Goldsmith PA. Generalized immunoassay for *Autographa californica* nuclear polyhedrosis virus infectivity in vitro. Appl. Environ. Microbiol. 44: 227-233, 1982.

Wang J, Shen L, Najafi H, Kolberg J, Matschinsky FM, Urdea M*, et al.* Regulation of insulin prerna splicing by glucose. Proc. Natl. Acad. Sci. U. S. A. 94: 4360-4365, 1997.

Wheeler DE, Buck N, Evans JD. Expression of insulin pathway genes during the period of caste determination in the honey bee, *Apis mellifera*. Insect Mol. Biol. 15: 597-602, 2006.

Yang C-H, Belawat P, Hafen E, Jan LY, Jan Y-N. *Drosophila* egg-laying site selection as a system to study simple decision-making processes. Science. 319: 1679-1683, 2008.

Yoshida I, Moto K, Sakurai S, Iwami MA. Novel member of the bombyxin gene family: Structure and expression of bombyxin g1 gene, an insulin-related peptide gene of the silkmoth *Bombyx mori*. Dev. Genes Evol. 208: 407-410, 1998.

Zheng S, Chiu H, Boudreau J, Papanicolaou T, Bendena W, Chin-Sang IA. Functional study of all 40 *Caenorhabditis elegans* insulin-like peptides. J. Biol. Chem. 293: 16912-16922, 2018.