

# SIGNALING AND FUNCTION OF INSULIN-LIKE PEPTIDES IN INSECTS

---

Qi Wu<sup>1,2</sup> and Mark R. Brown<sup>2,3</sup>

<sup>1</sup>Department of Cellular Biology, <sup>2</sup>Biomedical and Health Sciences Institute,

<sup>3</sup>Department of Entomology, University of Georgia, Athens, Georgia;

email: qiwu@uga.edu; mbrown@bugs.ent.uga.edu

**Key Words** peptide hormone, growth, metabolism, aging, reproduction

■ **Abstract** Insulin-like peptides (ILPs) exist in insects and are encoded by multi-gene families that are expressed in the brain and other tissues. Upon secretion, these peptides likely serve as hormones, neurotransmitters, and growth factors, but to date, few direct functions have been demonstrated. In *Drosophila melanogaster*, molecular genetic studies have revealed elements of a conserved insulin signaling pathway, and as in other animal models, it appears to play a key role in metabolism, growth, reproduction, and aging. This review offers (a) an integrated summary of the efforts to characterize the distribution of ILPs in insects and to define this pathway and its functions in *Drosophila* and (b) a few considerations for future studies of ILP endocrinology in insects.

## INTRODUCTION

Insulin is the most extensively studied peptide hormone, largely because its failure to regulate carbohydrate metabolism in humans results in diabetes, and type II diabetes mellitus is the most common metabolic disorder worldwide. In 1925, the Nobel Prize was awarded to two Canadian scientists, Sir Frederick G. Banting and John J.R. MacLeod, for their work on the “anti-diabetic principle from the pancreas” (<http://www.discoveryofinsulin.com/>; <http://digital.library.utoronto.ca/insulin/>). In association with Charles H. Best and James B. Collip, their contribution was to develop a procedure whereby this “principle” could be extracted and purified to a uniform potency for the treatment of diabetes. That invertebrates, as well, may possess such a hormone was foreseen by Collip in his attempt to extract an insulin-like factor from a clam (114). Later, Nobel Prizes were awarded to Fredrick Sanger (1958) for defining the structure of insulin and to Rosalyn Yalow (1977) for developing the radioimmunoassay for insulin. As indicated by its first name, “isletin,” the earliest studies had shown that the islets of Langerhans in the pancreas of mammals and even fishes were the primary source of insulin.

Now, some 80 years later, insulin is but one member of a peptide family that includes relaxins/insulin-like peptides (ILPs) and insulin-like growth factors (IGFs)

in vertebrates; in fact, 10 such peptides are known for humans. Although insulin is a gut hormone, IGFs are found in diverse tissues and relaxins in the brain and reproductive tract. After synthesis as pre-propeptides and signal peptide cleavage, the propeptides with contiguous B-C-A peptides are packaged in secretory granules. The A and B peptides are linked covalently by two interchain disulfide bonds and one intrachain disulfide bond, and the C peptide, which aids this linkage, is clipped out by convertases at the basic residues at each end in all but the IGFs. The linked A and B peptides, each with 20 to 30 amino acids, are the bioactive form (5000–6000 Da) of insulin and related peptides. These peptides may be stored or released from the secretory granules at the cell surface in response to cellular or molecular stimuli. Once released, the peptides can diffuse to local target cells or be transported to distant ones where they act through different receptors and signaling pathways to regulate a great variety of processes.

Reports on the existence of ILPs or bioactivity of insulin in insects were relatively numerous from the mid-1960s to the late 1980s (60, 114, 128, 131). With the more recent application of molecular techniques and genomics, genes for ILPs have been identified in species from Orthoptera, Diptera, and Lepidoptera, and soon Hymenoptera, now that the honey bee genome database is available. Genes for enzymes needed to process peptide hormones before secretion have been cataloged for the fruit fly, *Drosophila melanogaster* (121), and the mosquito *Anopheles gambiae* (97). After secretion into the hemolymph, binding proteins may transport and facilitate ILP action, as known for IGF in mammals, and proteins with high-affinity binding of insulin or gene homologs have been identified in insects (25, 113) and in a mosquito baculovirus (2). Once ILPs are bound by receptors, this complex may be internalized in target cells and the insulin degraded by a specific enzyme, for which a homolog has been identified in *D. melanogaster* (65).

Over the past decade, genetic studies of *D. melanogaster* have shown that ILPs acting through a conserved insulin signaling pathway regulate development, longevity, metabolism, and female reproduction (21, 38, 40). For other insects, demonstrated direct functions of ILPs are surprisingly rare, and an extensive review in 1989 (131) reached the conclusion that ILPs in insects likely do not regulate circulating carbohydrate levels in the same way as insulin in vertebrates. Herein, we offer (a) an integrated summary of the efforts to characterize the distribution of ILPs in insects and to define this pathway and its functions in *D. melanogaster* and (b) several considerations for future studies of ILP endocrinology in insects.

## IDENTIFICATION OF INSULIN-LIKE PEPTIDES

### Other Invertebrate Phyla and Arthropods

Not surprisingly, ILPs and elements of insulin signaling pathways are conserved across the higher invertebrate phyla. More than 30 putative ILP genes were

identified in the genome of the nematode *Caenorhabditis elegans*, and with the exception of *ins-1* and *ins-18*, all lacked a putative C peptide (88). These two genes are expressed in neurons, and alteration of their expression affects ILP signaling in this animal (79). For mollusks, seven ILP genes in *Lymnea stagnalis* (114) and one in *Aplysia californica* (34) have been characterized. All molluscan ILPs have longer A and B peptides with additional cysteines, which form an extra interchain disulfide bond. In these animals, neurosecretory cells in the cerebral ganglia are the source of ILPs that, along with insulin, regulate carbohydrate metabolism and other functions (114). Only one structurally related ILP, androgenic gland hormone, has been characterized from a noninsect arthropod, the crustacean isopod *Armadillium vulgare* (84). The ILP has a variant amino acid sequence and a glycan moiety on the A peptide, and the recombinant peptide is bioactive once the C peptide is cleaved. Insulin immunoreactivity has been localized in the synganglion of ticks (28) and in the digestive tissues, e.g., hepatopancreas, of the lobster *Panulirus argus* (37) but not in its neuroendocrine tissues. A putative insulin receptor was partially characterized from the shrimp *Penaeus japonicus* (20) to explain the metabolic activity of insulin (64).

## Lepidoptera

The unintended discovery of the first insect ILP, named bombyxin (also small prothoracicotrophic hormone, PTTH, 4K-PTTH, and PTTH II), has been reviewed recently (52). A large PTTH (30 kDa) and the ILP were purified for structural characterization from mass extractions of heads from the silkworm, *Bombyx mori*, on the basis of their stimulation of adult development when injected into brainless pupae. The PTTH was active only in *B. mori*, and the ILP was active at subnanomolar concentrations only in a related species, *Samia cynthia*. Similarly, the *B. mori* ILP stimulated prothoracic glands isolated from *S. cynthia* larvae, but not those from *B. mori*, to produce ecdysteroid hormones that regulate molting and metamorphosis in insects. Synthetic *B. mori* and *S. cynthia* ILPs have been used for structure and function studies (77,78). Notably, a putative ILP from *Manduca sexta* did stimulate ecdysteroidogenesis in the homologous glands (47). These results, to date, are the best-documented direct effects of insect ILPs.

Subsequent molecular studies led to the most surprising discovery of 38 different ILP genes in *B. mori*. They are classified into seven subfamilies (A to G) according to their amino acid sequence similarities (58, 138, 139). This information aided the molecular identification of three ILPs in the convolvulus hawkmoth, *Agrius convolvuli* (55), and five ILPs in *S. cynthia* (78). With the exception of five pseudogenes in *B. mori*, all lepidopteran ILP genes encode propeptides with contiguous B, C, and A peptides. In *B. mori*, the ILP genes typically are arranged in pairs or triplets in the genome and have opposing transcription, thus indicating that their multiplication arose by unequal crossing over (58, 138, 139). These and other features are characteristic of so-called processed genes or retroposons thought to be generated by reverse transcription of processed mRNAs and subsequent

reinsertion into the genome (58); thus, an intron-containing ancestral gene may have been lost during the duplication of processed genes. Presumably, selection of *B. mori* for increased silk protein production over the past 3000 years has contributed to the proliferation of ILP genes.

Characterization of ILP gene expression in Lepidoptera has focused on the larval brain, where transcripts are localized in four or more pairs of medial neurosecretory cells (MNC) (54–56, 138, 139). In addition, a regulatory transcription element was identified in the *B. mori* F1 ILP gene that was essential for neurosecretory cell expression (103). An early study showed that bombyxin mRNA levels in brain were unchanged in fifth-instar *B. mori* from eclosion to pupation, as quantified from Northern blots (1). Later, ILP A and B family transcripts were detected in a variety of *B. mori* larval and adult tissues (ganglia, epidermis, testis, ovary, fat body, silk gland, Malpighian tubule, midgut, and hindgut) by RT-PCR and in situ hybridization (56).

For other lepidopteran species, insulin antisera were used to quantify or track the purification of putative and often bioactive ILPs in extracts of corpus cardiacum (CC) corpus allatum (CA) complexes (114, 128, 131), hemolymph, and midgut (36, 75). Later, antisera to a *B. mori* ILP were used to verify its localization in the MNC of *B. mori* larval brains, and axons from these cells extend to the CA, which serves as a release site (73). Whether these antisera recognize other ILPs in *B. mori* has not been reported; nevertheless, they have been used for immunocytochemistry with other insects. For the greater wax moth, *Galleria mellonella*, immunostaining was present at all times in brain MNC and CC cells of the last larval instar, pupae, and adults, whereas staining in the lateral neurosecretory cells of brains and cells in the thoracic ganglia of these stages was temporary (143). For *M. sexta*, immunostaining was evident in the brain MNC and CA of second and subsequent larval instars but only in the brain MNC of pupae and adults (7). In a similar study, antisera to *S. cynthia* ILP A and B consistently immunostained up to 16 brain MNC and the CA from the earliest larval instars to adults (136).

Radioimmunoassays based on *B. mori* ILP antibodies have been used to profile the hemolymph ILP titer in *B. mori* during development (72, 102). A later study reported that ILP hemolymph titer in male *B. mori* went from  $\sim 40 \mu\text{g ml}^{-1}$  at eclosion to  $\sim 3 \text{ ng ml}^{-1}$  3 h post eclosion, and there was no change in females (106). In a review, ILP and ecdysteroid hemolymph titers were similar in life stages of *B. mori*, thus suggesting “that the two hormones function cooperatively” (71). Importantly, ILP hemolymph titer was profiled in parallel with ILP brain content, which ranged from less than 0.5 to 3 ng per brain (72), and ILP release has been stimulated from isolated *B. mori* brains (120).

## Orthoptera

The second insect ILP was isolated from the CC of the migratory locust, *Locusta migratoria*, as a 5-kDa peptide that had no sequence similarity to known peptides. Cloning and sequencing of the cDNA for this peptide revealed a locust

insulin-related peptide (LIRP) (48, 66). In contrast to the *B. mori* ILP genes, the LIRP gene contains introns, thus resembling the vertebrate insulin genes. The single copy of the LIRP gene is expressed as two transcripts that differ in the 5' region (63): One is expressed only in the brain, a putative neurohormone, and the other exists at low levels in essentially all tissues, a putative growth factor.

Later, LIRP was isolated for structural characterization (48) and localized to specific brain neurosecretory cells by immunocytochemistry (41). A significant quantity of LIRP is stored in the CC (30 ng per pair), presumably for release into hemolymph, but to date, no function is known in locusts, although it was reported that the native peptide failed to stimulate ecdysteroid production by locust prothoracic glands (62). Interestingly, the C peptide itself was found to depolarize and increase conductance of neurons isolated from the thoracic ganglia of *L. migratoria* (5), and functions are known for the insulin C peptide in mammals (118). Yet another peptide (pQSIDLFLLSPK) was isolated from the locust CC and found to be a proteolytic cleavage product from the extended B peptide (24). In bioassays it inhibits glycogen phosphorylase activity in locust fat body and was named glycogenolysis-inhibiting peptide (GIP).

Other studies of orthopteran species have used insulin and IGF antisera to localize ILPs in the nervous system of the cockroach *Periplaneta americana* and the stick insect *Carausius morosus* (92) and in midgut endocrine cells of *P. americana* (133). A strikingly complete immunocytochemical survey used a panel of 15 insulin antisera to define the distribution of immunostained cells and their axons in the brain, subesophageal ganglia, and CC-CA of the adult cockroach *Leucophaea maderae* (44). In addition, immunoreactive peptides were partially purified from brains, and in total such results point to the existence of multiple ILPs that may function as hormones and neurotransmitters.

## Diptera

A series of classic endocrine studies in the late 1970s were the first to demonstrate that brain MNCs in flies were the source of a hormone that decreased circulating glucose and trehalose levels (16, 31, 81). Such a hormone, when extracted from *D. melanogaster*, even had insulin bioactivity in mice (70), but it was the more comprehensive effort by Duve & Thorpe (32) that set high standards for subsequent work on insect ILPs. Their quest failed to isolate an ILP from the heads of the adult blow fly *Calliphora vomitoria* in sufficient quantity for structural characterization, but the highly purified ILP had activity in mammalian bioassays and lowered trehalose and glucose levels in flies made hyperglycemic by the extirpation of brain MNCs, which contain insulin-like immunoreactivity.

Given the pioneering application of genetic and molecular techniques to *D. melanogaster*, it is surprising that ILPs were identified long after an insulin receptor in this insect was characterized (see below). It was not until the *D. melanogaster* genome database was established that the seven ILP genes, *dilp1-7*, were identified (10, 130). Five of the *dilp* genes are clustered on chromosome 3, with *dilp6* and

*dilp7* on the X chromosome, and all have introns. At least one of the predicted peptides, DILP6, possesses notable variations: (a) The N terminus of the B peptide contains a decapeptide, a likely cleavage product, with no similarity to the locust GIP, and (b) the C peptide is truncated (KRRKR) similar to that of IGFs but still possesses proteolytic cleavage sites.

Tissue expression patterns for *dilp* genes in larvae and adults have been revealed by RNA in situ hybridization and immunocytochemistry. Transcripts for *dilp1*, 2, 3, and 5 exist in the same paired clusters of brain MNCs (10, 11). Transcripts also were detected in midgut (*dilp4*, 5, and 6), imaginal discs (*dilp2*), ventral nerve cord (*dilp7*), and salivary glands (*dilp2*) of larvae, but none were observed in fat body. In females, *dilp5* transcripts were localized in the follicle cells surrounding oocytes (51). Immunocytochemistry with antisera specific to DILP2 showed that brain MNCs with axons to the CC were the primary source of ILPs in larvae and females, and no significant immunostaining was seen in other tissues (13, 100). Two earlier studies using antisera to insulin or *B. mori* ILP reported immunostaining in the brain MNCs, other cells in the brain and fused ganglia, and peripheral neurons of larvae and adults (42, 142).

With the sequencing of the genome for the African malaria mosquito, *An. gambiae*, researchers identified seven ILP genes, *AgamILP1–7*, and the predicted ILP A and B peptides showed distinct similarities to the DILPs (61, 97). As in *D. melanogaster*, *AgamILP1–4* are arrayed proximally on chromosome 3, but a pair of genes, *AgamILP3/1*, are duplicated as *AgamILP6/7* approximately 23 kb away. Transcripts for *AgamILP2* and *AgamILP5* were detected in heads, thoraces, and abdomens of all life stages by RT-PCR, pointing to a growth factor function. *AgamILP1/7*, *3/6*, and *4* were detected only in the heads and thoraces of all life stages, and this distribution coincides with the ILP immunostaining of brain MNC and other cells (61) and a neurohormone function. In another mosquito, *Aedes aegypti*, the lateral neurosecretory cells of female brains were stained with a DILP antiserum, but no axons extended to the CC (13). The native structure and function of mosquito ILPs have yet to be defined.

## Other Insect Orders

There is limited evidence for ILPs in representative species of Hemiptera (111), Coleoptera, and Hymenoptera (82, 131). In another demonstration of the conservation of ILP function, the prothoracic glands from the last instar of a hemipteran, *Rhodnius prolixus*, were stimulated to secrete ecdysteroids in vitro by a *B. mori* ILP (117). Immunoassays with insulin antisera detected an ILP in larval head and midgut extracts from the beetle *Tenebrio molitor* (127) and immunostained cells in the brain, CA, and subesophageal ganglion of different life stages (110). An ILP purified from midguts of this beetle altered carbohydrate metabolism in the larval fat body in vitro (75). Royal jelly from the honey bee was the first insect-derived substance reported to possess insulin bioactivity (29), and later this activity was attributed to specific fatty acids (83).

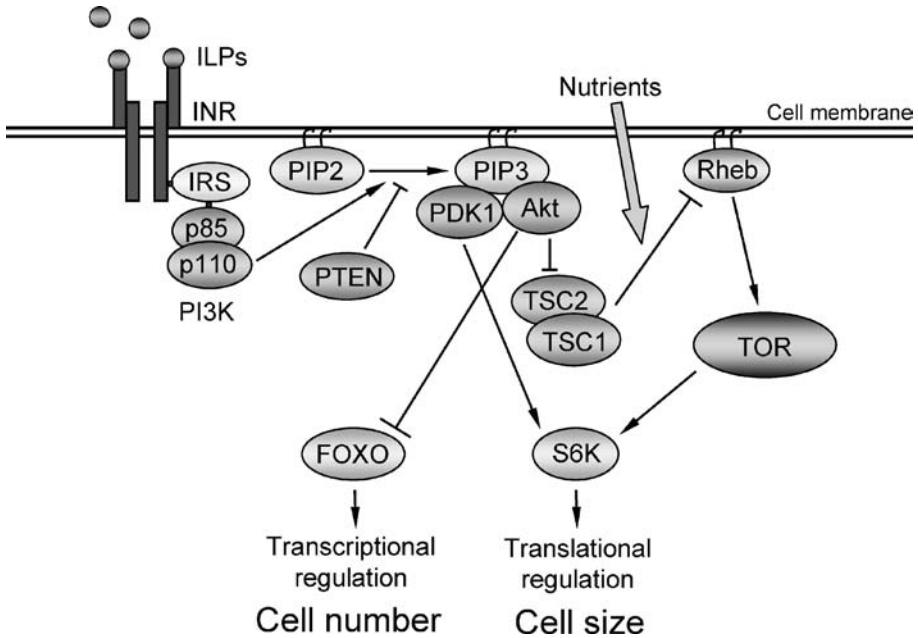
## SIGNALING PATHWAYS FOR INSULIN AND RELATED PEPTIDES

Two types of membrane-bound receptors are at the top of intracellular signaling pathways activated by insulin and related peptides in vertebrates. One type is a receptor tyrosine kinase (RTK), and although specific RTKs exist for insulin and IGF, the peptides can bind to both but with different affinities. These glycosylated receptors are homodimers of a proprotein with  $\alpha$ - and  $\beta$ -subunits that are cleaved and joined by disulfide bridges. The first insulin receptor to be characterized for invertebrates was purified from extracts of adult *D. melanogaster* on the basis of its binding of radiolabeled insulin and found to have the same physical and enzymatic properties as the human insulin receptor (87).

The other receptor type is a leucine-rich repeat-containing G-protein coupled receptor (LGR), and only recently have LGRs been shown to bind relaxins and activate the cAMP pathway in mammalian cell systems (18, 53). Given the conservation of ILP signaling in animals, it seems probable that an orphan LGR identified in *D. melanogaster* or *An. gambiae* will bind an ILP with basic amino acids in signature positions in the B peptide, as in the relaxins.

In mammalian cell systems, the RTKs for insulin and IGF potentially can activate two signaling pathways that affect cell metabolism and growth. Once ligand binding occurs at the extracellular  $\alpha$ -subunits, the kinase regions of membrane spanning  $\beta$ -subunits undergo extensive tyrosine phosphorylation. In this state, the receptors interact at the intracellular membrane with adaptor proteins and insulin receptor substrates (IRS), which are phosphorylated by the receptor's kinase region. These proteins then bind and activate either a growth factor receptor-bound protein (GRB2) or a phosphatidylinositol-3-kinase (PI3K), each of which initiates a distinct pathway. The activated GRB2 activates the mitogen-activated protein kinase (MAPK) pathway, resulting in cell proliferation. In *D. melanogaster*, other ligands and RTKs activate the MAPK pathway during embryogenesis, and there are no reports that it plays a role in ILP signaling.

In the other pathway activated by PI3K, p110, the catalytic subunit, generates a membrane lipid messenger, phosphatidylinositol-3,4,5-triphosphate (PIP3), that activates kinases, including phosphoinositide-dependent protein kinase 1 (PDK1) and Akt/protein kinase B. These activated proteins in turn alter other proteins associated with insulin action, such as glucose uptake and lipid synthesis, or gene expression. An increasing number of proteins block or aid insulin action at specific steps in this pathway. This whole sequence of protein interactions forms the so-called conserved pathway (Figure 1) that has been extensively investigated in different animal models but only in one insect, *D. melanogaster*, for its effect on growth, longevity, and an increasing number of physiological processes (38). Putative homolog genes for many of these proteins have been identified in the mosquito *An. gambiae* (97). A summary based on concepts drawn from the phenotypic effects of these genetic manipulations follows, and the few relevant studies of other insects are included.



**Figure 1** Conserved insulin signaling pathway in insects. Homologs for the key components illustrated here have been characterized genetically and biochemically in *Drosophila melanogaster* and in a few other insects. Abbreviations: Akt, protein kinase B; FOXO, forkhead box-containing protein, O subfamily; ILPs, insulin-like peptides; INR, insulin receptor; IRS, insulin receptor substrate; p110, catalytic subunit of PI3K; p85, adaptor subunit of PI3K; PDK1, phosphatidylinositol-dependent kinase 1; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; PTEN, phosphatase and tensin homolog; Rheb, Ras-homolog expressed in brain; S6K, p70 ribosomal S6 kinase; TOR, target of rapamycin; TSC, tuberous sclerosis complex. See text for details.

## FUNCTIONAL SIGNIFICANCE OF THE CONSERVED PATHWAY

### Insulin Receptor

Ten years after the identification of an insulin receptor in *D. melanogaster*, its gene structure was reported (99) and its expression in embryos and cell lines was described, along with the effects of mutagenesis (33, 99). The *D. melanogaster* insulin receptor (DIR) is an RTK of ~400 kDa, and the degree of amino acid sequence conservation between DIR and the human insulin receptor is remarkable, as exemplified by the high affinity ( $K_d = 15$  nM) with which DIR binds human insulin (33, 137). However, to date, there is no report that the DIR binds or is

activated by a DILP or any other insect ILP. Another distinguishing characteristic is the 368-amino-acid extension of the  $\beta$ -subunit, which contains additional tyrosine phosphorylation sites (33), and its function has been examined (69). In some cells, this extension is cleaved from the receptor to give one isoform, which exists along with the extended isoform. In addition, partial nucleotide sequences for putative IGF receptors have been identified in *D. melanogaster* (89). The tissue distribution of DIR is poorly defined for all life stages of *D. melanogaster*, although DIR transcripts are reportedly ubiquitous up to mid-embryogenesis and then more localized in the nervous system and imaginal discs of larvae and in the nurse cells of ovaries and nervous system of females (17, 33, 39).

Structurally related insulin receptors in ovaries and other tissues from lepidopteran and mosquito species have been identified. In the last instar of *M. sexta*, a membrane protein (178 kDa) was detected with a human insulin receptor antibody in prothoracic glands, muscle, and fat body (115). Specific binding of a synthetic *B. mori* ILP to receptors on ovarian cells from three lepidopteran species and Sf9 cells has been reported, and the putative receptors possess the physical characteristics of an RTK (35). For the mosquito, *Ae. aegypti*, an insulin receptor was first identified as a PCR product from ovary cDNA, after it was found that bovine insulin stimulated the ovaries to produce ecdysteroid hormones (43, 95). Activity of the ovarian receptor was demonstrated, and receptor transcripts are present in adult brain. In contrast to the DIR, this receptor and one predicted for another mosquito, *An. gambiae* (97), do not have an extended  $\beta$ -subunit.

## Metabolism

Insulin in mammals induces cells to take up glucose and convert it to glycogen, to inhibit glycogen breakdown and gluconeogenesis, and generally to shift from catabolic to anabolic lipid and protein metabolism. As a test of the concept that insulin structure and function are conserved, most early studies focused on eliciting these regulatory effects in insects with vertebrate insulins or in mammalian bioassays with purified insect ILPs. Now with the identification of insect ILPs, this concept awaits validation by testing synthetic ILPs for direct effects in bioassays, and to date, only two such studies exist. When injected into neck-ligated *B. mori* larvae, a silkworm ILP (bombyxin II) lowered the concentration of trehalose, the major hemolymph sugar, and induced elevated trehalase activity (105). The injected ILP also lowered glycogen content and raised glycogen phosphorylase activity in the fat body. The apparent role of the injected ILP in *B. mori* larvae was to promote consumption of carbohydrate reserves and not the accumulation of reserves, as for insulin in mammals. When injected into adult *B. mori*, this ILP, however, had no effect on trehalose or lipid levels in hemolymph (106). Contrasting results for an ILP administered to different life stages of an insect suggest that ILP regulation of metabolism may be particularly complex.

Genetic manipulation of *D. melanogaster* offers another approach to define the regulation of carbohydrate and lipid metabolism through this pathway.

Targeted expression of a cell-death-promoting factor ablated brain MNCs in larvae that contain DILPs and elevated trehalose and glucose levels in hemolymph (11, 100). Although there is no significant difference in levels of proteins and glycogen, dwarf flies with mutations in *DIR* and the *IRS* gene exhibit up to a fivefold increase in lipids (6). Although homolog genes for glucose transporters and enzymes involved in glycogen synthesis have been identified in *D. melanogaster*, only one study has looked at glucose uptake and metabolism. Insulin had no effect on glucose uptake or lipid synthesis and reduced glycogen in *D. melanogaster* Kc cells, as above with silkworm larvae, but it did increase glucose oxidation and lactate production, thus providing biosynthetic precursors needed for growth (15). Relevant studies, described below, have focused on the regulation that this pathway exerts on cell size and growth in *D. melanogaster*, as mediated by nutrients.

## Cell Size and Growth

*DROSOPHILA MELANOGASTER* Altered expression of genes for ILPs and proteins in the conserved pathway produced phenotypic effects on the growth and organs of *D. melanogaster*. Overexpression of *dilp* genes ubiquitously results in bigger flies by increasing cell size and cell number of individual organs in a *DIR*-dependent manner (10). Genetic ablation of DILP MNCs caused developmental delay and growth retardation (100), and cells in the wings of these flies showed reductions in both size and number. These phenotypes are similar to those observed in flies homozygous for a partial loss-of-function mutation in *DIR* (10), but strong *DIR*-deficient mutants are recessive embryonic lethal, indicating a key developmental role for *DIR* (17, 33). Flies with a mutation in the *IRS* gene *chico* display reduced body size owing to an autonomous reduction in cell number and cell size (6). The growth deficiency phenotype of *chico* mutants resembles that of *DIR* mutants, suggesting that *DIR* requires *IRS* to affect growth. Targeted expression of the PI3K subunit gene *p110* as a dominant-negative in the developing wing imaginal discs causes a reduction in both cell size and cell number in the wing (134). Conversely, overexpression of an active, membrane-targeted variant of *p110* increased cell size and cell number. Furthermore, *p110* mutant larvae are incapable of growth beyond the size reached in the early third instar, indicating that the increase in larval cell size depends on PI3K (134).

A tumor suppressor protein in humans, *PTEN* (phosphatase and tensin homolog), is a negative regulator of this pathway because its phosphatase activity opposes that of PI3K. Overexpression of *PTEN* in *D. melanogaster* inhibits cell cycle progression at an early stage of mitosis and promotes cell apoptosis during eye development (49). The small-eye phenotype caused by *PTEN* overexpression can be rescued by overexpression of wild-type *p110*. Interestingly, overexpression of *DIR* also results in eye proliferation in the few survivors, but overexpression of both *DIR* and *PTEN* completely rescues this phenotype and blocks lethality, thus supporting the role of *PTEN* as a negative regulator.

PDK1 and Akt are mediators of the growth and proliferative responses regulated through this pathway. Biochemical studies demonstrate that Akt is activated by phosphorylation of a specific threonine by PDK1 and a particular serine by the target of rapamycin (TOR) kinase and its associated protein, rictor (8, 104). Flies deficient in *PDK1* exhibit lethality and apoptosis during embryogenesis, and overexpression of *PDK1* increases cell and organ size in a PI3K-dependent manner and enhances *Akt* gain-of-function phenotypes during eye morphogenesis (19, 98). Mutations in *Akt* reduce cell size, whereas its overexpression leads to increased cell size (108, 132). Overexpression of *Akt* rescues the phenotypes caused either by dominant-negative *p110* or overexpression of *PTEN*, which indicates Akt is downstream of both (108). Notably, unlike mutations in the upstream genes, *Akt* mutations do not affect cell proliferation, thus suggesting that it may be part of the downstream branch in this pathway (Figure 1).

Recent studies show a tight but complex connection between this pathway and that mediated through TOR in response to nutrient availability. Null mutation of *TOR* in *D. melanogaster* results in severe growth defects, reductions in larval body size and imaginal discs, and lethality during the second instar (85, 140). Clonal analysis of imaginal discs shows that *TOR* mutant cells are smaller and have a slower proliferation, thus indicating that TOR autonomously regulates both cell growth and proliferation. Partial *TOR* mutation in larvae has the same effect as the withdrawal of amino acids, which is consistent with a role for TOR in growth control by sensing nutrient levels (140).

Genetic interaction studies indicate that TOR signaling is coupled to the conserved pathway by a complex of two tumor suppressor proteins, TSC1 and TSC2 (86). Mutation of *TSC1* and *TSC2* in *D. melanogaster* recapitulates alterations in the activity of *PTEN*-increased cell size and number, whereas coexpression of *TSC1* and *TSC2* has the opposite effects (90, 123). On the other hand, overexpression of *TSC1* and *TSC2* blocks the overgrowth phenotypes caused by *DIR* overexpression, while dominant-negative *TSC1/2* can rescue the lethality associated with loss of *DIR* function. Furthermore, the large cell phenotype of *TSC1* mutants cannot be suppressed by mutations in *DIR* or *Akt* or by overexpression of *PTEN* (90). These results and the fact that Akt destabilizes the TSC1/2 complex indicate that the negative regulator role of this complex is downstream of Akt.

The connection between TSC1/2 and TOR has been genetically mapped to a small GTP-binding protein, Rheb (Ras-homologue expressed in brain), in *D. melanogaster*. Mutation of *Rheb* results in growth defects similar to those caused by mutations in *chico* or other components in the pathway (107, 119, 141). Moreover, the phenotype of larval lethality caused by loss of TSC1 function is rescued by *Rheb* mutations. Genetic and biochemical evidence suggest that Rheb is a critical upstream activator of TOR (119), but its role is elusive.

Studies of animal models have shown that the ribosomal protein S6 kinase (S6K) is a downstream effector of this pathway. Loss of S6K function in *D. melanogaster* causes a severe reduction in body size by decreased cell size but does not alter cell

number (74). Moreover, S6K activity appears normal in *chico* mutants, suggesting that S6K regulates cell size by acquiring additional signals from other pathways (85, 93). Disruption of PI3K affects both cell size and cell number, thus implying that S6K is downstream of PDK1 and resides in a branch that controls cell growth and size but not cell number (59) (Figure 1). The large cell phenotype of *TSC1* mutation is suppressed by *S6K* mutation (90), thus indicating that TSC1 and TSC2 are upstream of S6K.

In addition, the transcription factor FOXO (forkhead box-containing protein, O subfamily) in *D. melanogaster* has been identified by genetic and biochemical approaches (91) as another critical target of Akt. Clonal analysis and genetic interaction assay show that FOXO regulates cell and organismal size by specifying cell number but not cell size (57, 91). Gain-of-function analysis reveals that under normal conditions excess FOXO is phosphorylated by Akt and kept inactive in the cytoplasm, but when deprived of nutrients or signaling through the DIR-IRS-PI3K pathway, unphosphorylated FOXO is transported to the nucleus, where it promotes factors that impede cell growth and proliferation (57).

Growth of insects is dependent on the metabolism of ingested nutrients or nutrients mobilized from reserves, and as alluded above, nutrient levels modulate signaling through this pathway. Results from a study of *D. melanogaster* larvae support this notion: (a) Mutation of *PI3K* suppresses cell growth in the same way as starvation, (b) ectopic induction of *DIR* or *PI3K* is sufficient to bypass the nutritional requirement for autonomous cell growth and DNA replication in starved larvae, (c) PI3K activity is downregulated in response to deprivation of dietary protein and amino acids, and (d) hyperactivation of *DIR* and *PI3K* led to reduced feeding and wandering (9). This study also showed that hyperactivation of this pathway increased nutrient storage in the fat body, whereas inhibition of PI3K activity depleted stored nutrients from the fat body, mimicking starvation (9). This and a more recent study point to the fat body in *D. melanogaster* as a key tissue that monitors nutrients through an amino acid transporter and the TOR pathway and that controls growth remotely through effects on this pathway in peripheral tissues (25). Autophagy of organelles and proteins in the fat body of *D. melanogaster* is induced by starvation or metamorphosis, and genetic analysis has shown that signaling through PI3K and TOR blocked starvation- or ecdysteroid-induced autophagy (101, 109). Together these findings show that nutrient intake leads to the activation of this pathway and in turn promotes anabolic metabolism, cell growth, and development, whereas nutrient deprivation has an opposing effect.

**OTHER INSECTS** For some time it has been known that insulin, IGF, and insect ILPs act as growth factors on insect cell lines and imaginal discs (15, 46, 60, 67, 114, 122, 128). A more comprehensive study showed that a putative ILP is a growth factor for wing imaginal discs in the butterfly *Precis coenia* (80). Such discs grow in vitro when supplemented with ecdysone and larval hemolymph, and *B. mori* ILP antibodies can remove the requisite hemolymph factor. A heat-stable

factor extracted from brains or synthetic *B. mori* ILP (bombyxin-II) can replace this activity in hemolymph, but ecdysone still is required.

## Reproduction

The presence of putative insulin receptors in the ovaries of lepidopteran species (35) hints at a role for ILPs in the reproductive physiology of insects, and studies of dipterans offer more evidence. Female *D. melanogaster* with genetically ablated DILPMNCs are smaller than wild ones and have greatly reduced fecundity (11, 51). Dwarf *D. melanogaster* females with mutant *DIR* expression have ovaries arrested at the previtellogenic stage, but treatment with a juvenile hormone (JH) analog, methoprene, restores vitellogenesis (126). In many insects, JH is the primary effector of reproduction, and this result may link these two endocrine pathways. In addition, *chico* mutation results in sterile females (6), and it impairs the proliferation of ovarian follicle cells and blocks egg chamber progression into vitellogenesis even when females have abundant food (30). Ovaries from wild-type females implanted into homozygous *chico* females were vitellogenic, but those from *chico* females implanted into wild-type females were not (93a). Thus, the *chico* mutation blocks the insulin signaling needed for yolk protein synthesis and uptake in the ovary but appears to have no effect on ovarian ecdysteroid production, hemolymph ecdysteroid levels, and even JH biosynthesis by the CA (in contrast to the reports below), because they were approximately the same in *chico* females and in wild-type females. Mutation of *Akt*, however, causes a cell-autonomous reduction in the size of ovarian follicle cells but does not affect cell proliferation (14).

Ovarian ecdysteroids are the primary effectors of vitellogenesis and egg maturation in many dipterans. As shown for the mosquito *Ae. aegypti*, bovine insulin stimulates ecdysteroid production by ovaries in vitro, and specific inhibitors of insulin receptor and PI3K activity blocked this stimulation (94). Ovaries from the blow fly *Phormia regina* were similarly stimulated in vitro with bovine insulin and a *B. mori* ILP (68), but isolated ovaries from long-lived *DIR*-mutant *D. melanogaster* produce little ecdysone compared with those from wild ones (129). Another neuropeptide, ovary ecdysteroidogenic hormone, originating from brain MNCs, stimulates the same process in ovaries of *Ae. aegypti* (12). This peptide is structurally related to neuroparsins in locusts that appear to be members of a superfamily that includes IGF-binding proteins in vertebrates (22). Although ecdysteroid hormones may be required for egg maturation in these dipterans, heterozygotic mutations in the ecdysteroid receptor increased the life span of *D. melanogaster* with no reduction in fertility (112), contradicting the often postulated tradeoff between reproduction and longevity.

Other components of the conserved pathway are directly involved in dipteran reproductive physiology. Insulin receptor expression in ovaries of *Ae. aegypti* females during a gonadotropic cycle was characterized (95), as described above, and an ovary-specific *Akt* was identified (96). Levels of insulin receptor transcript and protein increased in the ovaries during the first 24 hours after blood feeding and

disappeared thereafter (95). Interestingly, receptor transcripts and protein were not evident in earlier mosquito life stages. Initiation of vitellogenesis in the fat body of *Ae. aegypti* females is dependent on the ingestion of a blood meal that initiates an endocrine cascade and provides amino acids, which are monitored through TOR and S6K signaling, as shown by RNA interference of *TOR*, *TSC2*, and *S6K* expression (45). Finally, an oddity—insulin-fed *Anopheles* mosquitoes given infected blood meals had higher levels of malaria infection than those not fed insulin (4)—offers a hint that even host-parasite interactions may be regulated through this pathway.

## Longevity

The concept that ILPs acting through the conserved pathway stimulate growth and reproduction in organisms that, as a result, have a shorter life can be drawn from an early study of the butterfly *Pieris brassicae*. When injected into diapausing pupae of *P. brassicae*, vertebrate insulins induced diapause termination, synchronous adult development, and an apparent de novo synthesis of ecdysteroids (3). Now, many studies of *D. melanogaster* and other animal models offer more support for this concept (124, 125). Flies bearing a hypomorphic mutation in *DIR* or null mutations in *chico* display up to an 85% extension of life span (23, 126). Interestingly, the CA isolated from *DIR* mutant flies show decreased JH biosynthesis, and treatment of the long-lived *DIR* flies with a JH analog restored normal life expectancy. A subsequent study showed that the *DIR* and *chico* mutations in females altered JH synthesis by the CA for up to 10 days after eclosion (129a). In many adult insects, JH stimulates reproduction and inhibits diapause, but in its absence, adult life span may be extended. Because of this connection, it has been conjectured that DILPs acting through this pathway may directly regulate JH synthesis (124). More recently, insulin signaling is implicated in the deterioration of heart function in aging *D. melanogaster*. Flies with genetically ablated DILP MNCs (11) or mutations in *DIR* and *chico* had minimal heart deterioration over time and a longer life span (135). Downregulation of this pathway exclusively in the heart, by overexpressing *PTEN* or *FOXO*, also abolished age-dependent decline in heart function; but life span was normal. Together, these results show that this pathway has the potential to affect life span and organ senescence in *D. melanogaster*. These life-extending effects are comparable to those elicited by diet restriction but are not manifested in reduced metabolic rates (50).

## Other Functions

That this pathway is implicated in other functions is expected given the relative ease of genetically manipulating *D. melanogaster* and the continued interest in this pathway. *DIR* is expressed throughout the brain, particularly in optic lobe regions, and mutation of *DIR* disrupted connections between the optic lobes and brain in larvae and adults in the same way as mutation of a gene for an adaptor protein,

Dock (116). This protein binds to the extended form of the DIR  $\beta$ -subunit and is required for axon guidance in the nervous system, but mutations in *chico* had no effect, and thus DIR may interact with Dock and activate another pathway crucial for nervous system development. Behaviors are seated in the nervous system, and ethanol intoxication is regulated through this pathway in the nervous system of adult *D. melanogaster* (26). Targeted expression of a gene for an inhibitor of cAMP-dependent protein kinase A in the brain MNCs containing DILPs significantly increased adult ethanol sensitivity, as did global *DIR* and *chico* mutations and specific expression of *FOXO* in the nervous system.

## CONCLUSIONS

The founding concept that a peptide exists in insects with the same structure and regulatory role in glucose metabolism as insulin in mammals has expanded over the past four decades to include multiple ILPs and an intricate signaling pathway that coordinates the regulation of metabolism, growth, reproduction, longevity and other behavioral and physiological processes. Noticeably, this expanded concept is based on a preponderance of studies in which supporting conclusions are drawn from the genetic manipulations of one highly specialized fly species, *D. melanogaster*. In such studies, the effects of gene-specific mutations play out over the relatively long time needed to reach a particular life stage that is observed for an anomalous phenotype. The mutational effects on transcript or protein expression in target tissues or individuals should be quantified or assessed directly. This assessment may reveal a confounding effect, as described in one study in which the DILP MNCs were genetically ablated but other brain cells were seen to contain ILPs (11). The neuroendocrine action of ILPs in *D. melanogaster* is presumed, and future studies need to define the extent of ILP expression in the nervous system and other tissues and the hallmarks of peptide hormone actions that are only seconds to a few hours in duration. In particular, these issues must be resolved to further validate this expanded concept for insects in general:

1. The parameters of ILP synthesis, processing, storage, and release in cell sources must be determined, along with internal and external cues that govern these processes. High transcript or peptide levels in ILP-secreting cells may reflect increased storage or release. The action of other peptides originating from the propeptide ILPs, e.g., GIP and the C peptide, and that of the processed ILP must be assessed.
2. The hormonal effects of ILPs on target tissues usually are the result of rising or falling titers in hemolymph, and thus titer profiles must be correlated with a particular endocrine action of an ILP. ILPs acting as growth factors or neurotransmitters will have more local effects.
3. The binding of insect ILPs to an insect insulin receptor or LGR has yet to be demonstrated. Likely, this will necessitate the expression of such receptors

in heterologous cell systems for detailed binding studies of ILPs. In addition, receptor expression and turnover in tissues must be correlated with the associated ILP action, whether endocrine or local.

Nevertheless, this wonderfully integrative concept for ILP signaling in insects ties nutrient acquisition and reserves to the fundamentally important processes of development and reproduction and shows that it has the potential to exhibit an effect on the expression of genes required for these processes as profound as that exhibited by JH and ecdysteroids. Future investigations of ILP signaling must be spread to representatives of other insect orders that have proven so useful in defining the endocrinology and physiology of other peptide hormones.

## ACKNOWLEDGMENTS

Funding by the National Institutes of Health, USDA, and the state of Georgia over the past decade has provided QW, MRB, and past and present associates of the MRB laboratory with the support needed to explore the endocrinology of mosquitoes and other insects.

**The Annual Review of Entomology is online at <http://ento.annualreviews.org>**

## LITERATURE CITED

1. Adachi T, Takiya S, Suzuki Y, Iwami M, Kawakami A, et al. 1989. cDNA structure and expression of bombyxin, an insulin-like brain secretory peptide of the silkworm *Bombyx mori*. *J. Biol. Chem.* 264:7681–85
2. Afonso CL, Tulman ER, Lu Z, Balinsky CA, Moser BA, et al. 2001. Genome sequence of a baculovirus pathogenic for *Culex nigripalpus*. *J. Virol.* 75:11157–65
3. Arpagaus M. 1987. Vertebrate insulin induces diapause termination in *Pieris brassicae* pupae. *Roux's Arch. Dev. Biol.* 196:527–30
4. Beier MS, Pumpuni CB, Beier JC, Davis JR. 1994. Effects of para-aminobenzoic acid, insulin, and gentamicin on *Plasmodium falciparum* development in anopheline mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* 31:561–65
5. Bermudez I, Beadle DJ, Trifilieff E, Luu B, Hieter H. 1991. Electrophysiological activity of the C-peptide of the *Locusta* insulin-related peptide: effect on the membrane conductance of *Locusta* neurones in vitro. *FEBS Lett.* 293:137–41
6. Bohni R, Riesgo-Escovar J, Oldham S, Brogiolo W, Stocker H, et al. 1999. Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1–4. *Cell* 97:865–75
7. Nogueira BV, Muehleisen DP, Whisenton LR, Gray RS, Bollenbacher WE. 1997. Life cycle expression of a bombyxin-like neuropeptide in the tobacco hornworm, *Manduca sexta*. *J. Insect Physiol.* 43:47–53
8. Brazil DP, Hemmings BA. 2001. Ten years of protein kinase B signalling: a hard Akt to follow. *Trends Biochem. Sci.* 26:657–64
9. Britton JS, Lockwood WK, Li L, Cohen SM, Edgar BA. 2002. *Drosophila*'s insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Dev. Cell* 2:239–49

10. Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E. 2001. An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr. Biol.* 11:213–21
11. Broughton SJ, Piper MD, Ikeya T, Bass TM, Jacobson J, et al. 2005. Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc. Natl. Acad. Sci. USA* 102:3105–10
12. Brown MR, Graf R, Swiderek KM, Fendley D, Stracker TH, et al. 1998. Identification of a steroidogenic neurohormone in female mosquitoes. *J. Biol. Chem.* 273:3967–71
13. Cao C, Brown MR. 2001. Localization of an insulin-like peptide in brains of two flies. *Cell Tissue Res.* 304:317–21
14. Cavaliere V, Donati A, Hsouna A, Hsu T, Gargiulo G. 2005. dAkt kinase controls follicle cell size during *Drosophila* oogenesis. *Dev. Dyn.* 232:845–54
15. Ceddia RB, Bikopoulos GJ, Hilliker AJ, Sweeney G. 2003. Insulin stimulates glucose metabolism via the pentose phosphate pathway in *Drosophila* Kc cells. *FEBS Lett.* 555:307–10
16. Chen AC, Friedman S. 1977. Hormonal regulation of trehalose metabolism in the blowfly, *Phormia regina*: interaction between hypertrehalosemic and hypotrehalosemic hormones. *J. Insect Physiol.* 23:1223–32
17. Chen C, Jack J, Garofalo RS. 1996. The *Drosophila* insulin receptor is required for normal growth. *Endocrinology* 137:846–56
18. Chen J, Kuei C, Sutton SW, Bonaventure P, Nepomuceno D, et al. 2005. Pharmacological characterization of relaxin-3/INSL7 receptors GPCR135 and GPCR-142 from different mammalian species. *J. Pharmacol. Exp. Ther.* 312:83–95
19. Cho KS, Lee JH, Kim S, Kim D, Koh H, et al. 2001. *Drosophila* phosphoinositide-dependent kinase-1 regulates apoptosis and growth via the phosphoinositide 3-kinase-dependent signaling pathway. *Proc. Natl. Acad. Sci. USA* 98: 6144–49
20. Chuang NN, Wang PC. 1994. Characterization of insulin receptor from the muscle of the shrimp *Penaeus japonicus* (Crustacea: Decapoda). *Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol.* 108:289–97
21. Claeys I, Simonet G, Poels J, Van Loy T, Vercammen L, et al. 2002. Insulin-related peptides and their conserved signal transduction pathway. *Peptides* 23: 807–16
22. Claeys I, Simonet G, Van Loy T, De Loof A, Vanden Broeck J. 2003. cDNA cloning and transcript distribution of two novel members of the neuroparsin family in the desert locust, *Schistocerca gregaria*. *Insect Mol. Biol.* 12:473–81
23. Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, et al. 2001. Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* 292:104–6
24. Clynen E, Huybrechts J, Baggerman G, Van Doorn J, Van Der HD, et al. 2003. Identification of a glycogenolysis-inhibiting peptide from the corpora cardiaca of locusts. *Endocrinology* 144: 3441–48
25. Colombani J, Raisin S, Pantalacci S, Radimerski T, Montagne J, Leopold P. 2003. A nutrient sensor mechanism controls *Drosophila* growth. *Cell* 114:739–49
26. Corl AB, Rodan AR, Heberlein U. 2005. Insulin signaling in the nervous system regulates ethanol intoxication in *Drosophila melanogaster*. *Nat. Neurosci.* 8:18–19
27. Deleted in proof
28. Davis HH, Dotson EM, Oliver JH Jr. 1994. Localization of insulin-like immunoreactivity in the synganglion of nymphal and adult *Dermacentor*

- variabilis* (Acari: Ixodidae). *Exp. Appl. Acarol.* 18:111–22
29. Dixit PK, Patel NG. 1964. Insulin-like activity in larval foods of the honeybee. *Nature* 202:189–90
  30. Drummond-Barbosa D, Spradling AC. 2001. Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Dev. Biol.* 231:265–78
  31. Duve H. 1978. The presence of a hypoglucemic and hypotrehalocemic hormone in the neurosecretory system of the blowfly *Calliphora erythrocephala*. *Gen. Comp. Endocrinol.* 36:102–10
  32. Duve H, Thorpe A, Lazarus NR. 1979. Isolation of material displaying insulin-like immunological and biological activity from the brain of the blowfly, *Calliphora vomitoria*. *Biochem. J.* 184:221–27
  33. Fernandez R, Tabarini D, Azpiazu N, Frasch M, Schlessinger J. 1995. The *Drosophila* insulin receptor homolog: a gene essential for embryonic development encodes two receptor isoforms with different signaling potential. *EMBO J.* 14:3373–84
  34. Floyd PD, Li L, Rubakhin SS, Sweedler JV, Horn CC, et al. 1999. Insulin prohormone processing, distribution, and relation to metabolism in *Aplysia californica*. *J. Neurosci.* 19:7732–41
  35. Fullbright G, Lacy ER, Bullesbach EE. 1997. The prothoracicotropic hormone bombyxin has specific receptors on insect ovarian cells. *Eur. J. Biochem.* 245:774–80
  36. Gadenne C, Trabelsi M, Lavenseau L. 1989. Existence and possible role of a substance immunologically related to insulin in the midgut of the European cornborer, *Ostrinia nubilalis* (Hbn). *Comp. Biochem. Physiol.* 93:375–81
  37. Gallardo N, Carrillo O, Molto E, Deas M, Gonzalez-Suarez R, et al. 2003. Isolation and biological characterization of a 6-kDa protein from hepatopancreas of lobster *Panulirus argus* with insulin-like effects. *Gen. Comp. Endocrinol.* 131:284–90
  38. Garofalo RS. 2002. Genetic analysis of insulin signaling in *Drosophila*. *Trends Endocrinol. Metab.* 13:156–62
  39. Garofalo RS, Rosen OM. 1988. Tissue localization of *Drosophila melanogaster* insulin receptor transcripts during development. *Mol. Cell Biol.* 8:1638–47
  40. Goberdhan DC, Wilson C. 2003. The functions of insulin signaling: size isn't everything, even in *Drosophila*. *Differentiation* 71:375–97
  41. Goltzene F, Holder F, Charlet M, Meister M, Oka T. 1992. Immunocytochemical localization of *Bombyx*-PTTH-like molecules in neurosecretory cells of the brain of the migratory locust, *Locusta migratoria*. A comparison with neuroparsin and insulin-related peptide. *Cell Tissue Res.* 269:133–40
  42. Gorczyca M, Augart C, Budnik V. 1993. Insulin-like receptor and insulin-like peptide are localized at neuromuscular junctions in *Drosophila*. *J. Neurosci.* 13:3692–704
  43. Graf R, Neuenschwander S, Brown MR, Ackermann U. 1997. 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.* 6:151–63
  44. Hansen GN, Hansen BL, Jorgensen PN, Scharrer B. 1990. Immunocytochemical localization and immunochemical characterization of an insulin-related peptide in the insect *Leucophaea maderae*. *Cell Tissue Res.* 259:265–73
  45. Hansen IA, Attardo GM, Roy SG, Raikhel AS. 2005. Target of rapamycin-dependent activation of S6 kinase is a central step in the transduction of nutritional signals during egg development in a mosquito. *J. Biol. Chem.* 280:20565–72
  46. Hatt PJ, Liebon C, Moriniere M,

- Oberlander H, Porcheron P. 1997. Activity of insulin growth factors and shrimp neurosecretory organ extracts on a lepidopteran cell line. *Arch. Insect Biochem. Physiol.* 34:313–28
47. Hayes GC, Muehleisen DP, Bollenbacher WE, Watson RD. 1995. Stimulation of ecdysteroidogenesis by small prothoracicotropic hormone: role of calcium. *Mol. Cell Endocrinol.* 115:105–12
48. Hetru C, Li KW, Bulet P, Lagueux M, Hoffmann JA. 1991. Isolation and structural characterization of an insulin-related molecule, a predominant neuropeptide from *Locusta migratoria*. *Eur. J. Biochem.* 201:495–99
49. Huang H, Potter CJ, Tao W, Li DM, Brogiolo W, et al. 1999. PTEN affects cell size, cell proliferation and apoptosis during *Drosophila* eye development. *Development* 126:5365–72
50. Hulbert AJ, Clancy DJ, Mair W, Braeckman BP, Gems D, Partridge L. 2004. Metabolic rate is not reduced by dietary restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in *Drosophila melanogaster*. *Exp. Gerontol.* 39:1137–43
51. Ikeya T, Galic M, Belawat P, Nairz K, Hafen E. 2002. Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*. *Curr. Biol.* 12:1293–300
52. Ishizaki H. 2004. Molecular characterization of the brain secretory peptides, prothoracicotropic hormone (PTTH) and bombyxin, of the silkworm *Bombyx mori*. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 80:195–203
53. Ivell R, Einspanier A. 2002. Relaxin peptides are new global players. *Trends Endocrinol. Metab.* 13:343–48
54. Iwami M. 1995. Multi-family genes encoding bombyxin and bombyxin-related peptides. See Ref. 120a, pp. 65–74
55. Iwami M, Furuya I, Kataoka H. 1996. Bombyxin-related peptides: cDNA structure and expression in the brain of the hornworm *Agrius convolvuli*. *Insect Biochem. Mol. Biol.* 26:25–32
56. Iwami M, Tanaka A, Hano N, Sakurai S. 1996. Bombyxin gene expression in tissues other than brain detected by reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridization. *Experientia* 52:882–87
57. Junger MA, Rintelen F, Stocker H, Wasserman JD, Vegh M, et al. 2003. The *Drosophila* forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. *J. Biol.* 2:20
58. Kondo H, Ino M, Suzuki A, Ishizaki H, Iwami M. 1996. Multiple gene copies for bombyxin, an insulin-related peptide of the silkworm *Bombyx mori*: structural signs for gene rearrangement and duplication responsible for generation of multiple molecular forms of bombyxin. *J. Mol. Biol.* 259:926–37
59. Kozma SC, Thomas G. 2002. Regulation of cell size in growth, development and human disease: PI3K, PKB and S6K. *Bioessays* 24:65–71
60. Kramer KJ. 1985. Vertebrate hormones in insects. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, ed. GA Kerkut, LI Gilbert, 7:511–36. New York: Pergamon
61. Krieger MJ, Jahan N, Riehle MA, Cao C, Brown MR. 2004. Molecular characterization of insulin-like peptide genes and their expression in the African malaria mosquito, *Anopheles gambiae*. *Insect Mol. Biol.* 13:305–15
62. Kromer E, Lagueux M. 1994. Studies on *Locusta* insulin related peptide. *Perspect. Comp. Endocrinol.* 220–25
63. Kromer-Metzger E, Lagueux M. 1994. Expression of the gene encoding an insulin-related peptide in *Locusta* (Insecta, Orthoptera). Evidence for alternative promoter usage. *Eur. J. Biochem.* 221:427–34

64. Kucharski LC, Schein V, Capp E, da Silva RS. 2002. In vitro insulin stimulatory effect on glucose uptake and glycogen synthesis in the gills of the estuarine crab *Chasmagnathus granulata*. *Gen. Comp. Endocrinol.* 125:256–63
65. Kuo WL, Gehm BD, Rosner MR. 1990. Cloning and expression of the cDNA for a *Drosophila* insulin-degrading enzyme. *Mol. Endocrinol.* 4:1580–91
66. Lagueux M, Lwoff L, Meister M, Goltzene F, Hoffmann JA. 1990. cDNAs from neurosecretory cells of brains of *Locusta migratoria* (Insecta, Orthoptera) encoding a novel member of the superfamily of insulins. *Eur. J. Biochem.* 187:249–54
67. Malaterre J, Strambi C, Aouane A, Strambi A, Rougon G, Cayre M. 2003. Effect of hormones and growth factors on the proliferation of adult cricket neural progenitor cells in vitro. *J. Neurobiol.* 56:387–97
68. Maniere G, Rondot I, Bullesbach EE, Gautron F, Vanhems E, Delbecq JP. 2004. Control of ovarian steroidogenesis by insulin-like peptides in the blowfly (*Phormia regina*). *J. Endocrinol.* 181:147–56
69. Marin-Hincapie M, Garofalo RS. 1999. The carboxyl terminal extension of the *Drosophila* insulin receptor homologue binds IRS-1 and influences cell survival. *J. Biol. Chem.* 274:24987–94
70. Meneses P, Los Angeles OM. 1975. A protein extract from *Drosophila melanogaster* with insulin-like activity. *Comp. Biochem. Physiol. A* 51:483–85
71. Mizoguchi A. 1994. Distribution and function of bombyxin. In *Perspectives in Comparative Endocrinology*, ed. KG Davey, RE Peter, SS Tobe, pp. 215–19. Ottawa: Natl. Res. Coun. Can.
72. Mizoguchi A. 1995. Measurement of the titers of *Bombyx* prothoracicotrophic hormone and bombyxin in the hemolymph of *Bombyx mori* by time-resolved fluoroimmunoassay. See Ref. 120a, pp. 129–38
73. Mizoguchi A, Ishizaki H, Nagasawa H, Kataoka H, Isogai A, et al. 1987. A monoclonal-antibody against a synthetic fragment of bombyxin (4K-prothoracicotrophic hormone) from the silkworm, *Bombyx mori*: characterization and immunohistochemistry. *Mol. Cell. Endocrinol.* 51:227–35
74. Montagne J, Stewart MJ, Stocker H, Hafen E, Kozma SC, Thomas G. 1999. *Drosophila* S6 kinase: a regulator of cell size. *Science* 285:2126–29
75. Mtioui A, Gourdoux L, Fournier B, Moreau R. 1993. Effects of intestinal insulin-like peptide on glucose catabolism in mealworm larval fat body in vitro: dependence on extracellular  $Ca^{2+}$  for its stimulatory action. *Arch. Insect Biochem. Physiol.* 24:113–28
76. Deleted in proof
77. Nagata K, Maruyama K, Nagasawa H, Urushibata I, Isogai A, et al. 1992. Bombyxin-II and its disulfide bond isomers: synthesis and activity. *Peptides* 13:653–62
78. Nagata K, Maruyama K, Kojima K, Yamamoto M, Tanaka M, et al. 1999. Prothoracicotrophic activity of SBRPs, the insulin-like peptides of the saturniid silkworm *Samia cynthia ricini*. *Biochem. Biophys. Res. Commun.* 266:575–78
79. Nelson DW, Padgett RW. 2003. Insulin worms its way into the spotlight. *Genes Dev.* 17:813–18
80. Nijhout HF, Grunert LW. 2002. Bombyxin is a growth factor for wing imaginal disks in Lepidoptera. *Proc. Natl. Acad. Sci. USA* 99:15446–50
81. Normann TC. 1975. Neurosecretory cells in insect brain and production of hypoglycaemic hormone. *Nature* 254:259–61
82. O'Connor K, Baxter D. 1985. The demonstration of insulin-like material in the honey bee, *Apis mellifera*. *Comp. Biochem. Physiol.* 81B:755–60

83. Okuda H, Kameda K, Morimoto C, Matsuura Y, Chikaki M, Jiang M. 1998. Studies on insulin-like substances and inhibitory substances toward angiotensin-converting enzyme in royal jelly. *Honeybee Sci.* 19:9–14
84. Okuno A, Hasegawa Y, Nishiyama M, Ohira T, Ko R, et al. 2002. Preparation of an active recombinant peptide of crustacean androgenic gland hormone. *Peptides* 23:567–72
85. Oldham S, Montagne J, Radimerski T, Thomas G, Hafen E. 2000. Genetic and biochemical characterization of dTOR, the *Drosophila* homolog of the target of rapamycin. *Genes Dev.* 14:2689–94
86. Pan D, Dong J, Zhang Y, Gao X. 2004. Tuberous sclerosis complex: from *Drosophila* to human disease. *Trends Cell Biol.* 14:78–85
87. Petruzzelli L, Herrera R, Garcia-Arenas R, Rosen OM. 1985. Acquisition of insulin-dependent protein tyrosine kinase activity during *Drosophila* embryogenesis. *J. Biol. Chem.* 260:16072–75
88. Pierce SB, Costa M, Wisotzkey R, Devadhar S, Homburger SA, et al. 2001. 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.* 15:672–86
89. Pietrokovski S, Shilo BZ. 2001. Identification of new signaling components in the *Drosophila* genome sequence. *Funct. Integr. Genomics* 1:250–55
90. Potter CJ, Huang H, Xu T. 2001. *Drosophila* Tsc1 functions with Tsc2 to antagonize insulin signaling in regulating cell growth, cell proliferation, and organ size. *Cell* 105:357–68
91. Puig O, Marr MT, Ruhf ML, Tjian R. 2003. Control of cell number by *Drosophila* FOXO: downstream and feedback regulation of the insulin receptor pathway. *Genes Dev.* 17:2006–20
92. Raabe M. 1986. Comparative immunocytochemical study of release sites of insulin, glucagon, and AKH-like products in *Locusta migratoria*, *Periplaneta americana*, and *Carausius morosus*. *Cell Tissue Res.* 267–71
93. Radimerski T, Montagne J, Rintelen F, Stocker H, van der Kaay J, et al. 2002. dS6K-regulated cell growth is dPKB/dPI(3)K-independent, but requires dPDK1. *Nat. Cell Biol.* 4:251–55
- 93a. Richard DS, Rybczynski R, Wilson TG, Wang Y, Wayne ML, et al. 2005. Insulin signaling is necessary for vitellogenesis in *Drosophila melanogaster* independent of the roles of juvenile hormone and ecdysteroids: Female sterility of the *chico* insulin signaling mutation is autonomous to the ovary. *J. Insect Physiol.* 51:455–64
94. Riehle MA, Brown MR. 1999. Insulin stimulates ecdysteroid production through a conserved signaling cascade in the mosquito *Aedes aegypti*. *Insect Biochem. Mol. Biol.* 29:855–60
95. Riehle MA, Brown MR. 2002. Insulin receptor expression during development and a reproductive cycle in the ovary of the mosquito *Aedes aegypti*. *Cell Tissue Res.* 308:409–20
96. Riehle MA, Brown MR. 2003. Molecular analysis of the serine/threonine kinase Akt and its expression in the mosquito *Aedes aegypti*. *Insect Mol. Biol.* 12:225–32
97. Riehle MA, Garczynski SF, Crim JW, Hill CA, Brown MR. 2002. Neuropeptides and peptide hormones in *Anopheles gambiae*. *Science* 298:172–75
98. Rintelen F, Stocker H, Thomas G, Hafen E. 2001. PDK1 regulates growth through Akt and S6K in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 98:15020–25
99. Ruan Y, Chen C, Cao Y, Garofalo RS. 1995. The *Drosophila* insulin receptor contains a novel carboxyl-terminal extension likely to play an important role in signal transduction. *J. Biol. Chem.* 270:4236–43
100. Rulifson EJ, Kim SK, Nusse R. 2002.

- Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* 296:1118–20
101. Rusten TE, Lindmo K, Juhasz G, Sass M, Seglen PO, et al. 2004. Programmed autophagy in the *Drosophila* fat body is induced by ecdysone through regulation of the PI3K pathway. *Dev. Cell* 7:179–92
  102. Saegusa H, Mizoguchi A, Kitahora H, Nagasawa H, Suzuki A, Ishizaki H. 1992. Changes in the titer of bombyxin-immunoreactive material in hemolymph during the postembryonic development of the silkworm *Bombyx mori*. *Dev. Growth Differ.* 34:595–605
  103. Salam SEA, Moto K, Sakurai S, Iwami M. 2001. Transcription element responsible for the brain cell-specific expression of the bombyxin gene that encodes an insect insulin-related peptide. *Zool. Sci.* 18:543–49
  104. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. 2005. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307:1098–101
  105. Satake S, Masumura M, Ishizaki H, Nagata K, Kataoka H, et al. 1997. Bombyxin, an insulin-related peptide of insects, reduces the major storage carbohydrates in the silkworm *Bombyx mori*. *Comp. Biochem. Physiol B Biochem. Mol. Biol.* 118:349–57
  106. Satake S, Nagata K, Kataoka H, Mizoguchi A. 1999. Bombyxin secretion in the adult silkworm *Bombyx mori*: sex-specificity and its correlation with metabolism. *J. Insect Physiol.* 45:939–45
  107. Saucedo LJ, Gao X, Chiarelli DA, Li L, Pan D, Edgar BA. 2003. Rheb promotes cell growth as a component of the insulin/TOR signalling network. *Nat. Cell Biol.* 5:566–71
  108. Scanga SE, Ruel L, Binari RC, Snow B, Stambolic V, et al. 2000. The conserved PI3'K/PTEN/Akt signaling pathway regulates both cell size and survival in *Drosophila*. *Oncogene* 19:3971–77
  109. Scott RC, Schuldiner O, Neufeld TP. 2004. Role and regulation of starvation-induced autophagy in the *Drosophila* fat body. *Dev. Cell* 7:167–78
  110. Sevala VM, Sevala VL, Loughton BG. 1993. Insulin-like molecules in the beetle *Tenebrio molitor*. *Cell Tissue Res.* 273:71–77
  111. Sevala VM, Sevala VL, Loughton BG, Davey KG. 1992. Insulin-like immunoreactivity and molting in *Rhodnius prolixus*. *Gen. Comp. Endocrinol.* 86:231–38
  112. Simon AF, Shih C, Mack A, Benzer S. 2003. Steroid control of longevity in *Drosophila melanogaster*. *Science* 299:1407–10
  113. Sloth AA, Hertz HP, Schaffer L, Kristensen C. 2000. A new secreted insect protein belonging to the immunoglobulin superfamily binds insulin and related peptides and inhibits their activities. *J. Biol. Chem.* 275:16948–53
  114. Smit AB, van Kesteren RE, Li KW, Van Minnen J, Spijker S, et al. 1998. Towards understanding the role of insulin in the brain: lessons from insulin-related signaling systems in the invertebrate brain. *Prog. Neurobiol.* 54:35–54
  115. Smith WA, Koundinya M, McAllister T, Brown A. 1997. Insulin receptor-like tyrosine kinase in the tobacco hornworm, *Manduca sexta*. *Arch. Insect Biochem. Physiol.* 99–110
  116. Song J, Wu L, Chen Z, Kohanski RA, Pick L. 2003. Axons guided by insulin receptor in *Drosophila* visual system. *Science* 300:502–5
  117. Steel CG, Vafofoulou X. 1997. Ecdysteroidogenic action of *Bombyx* prothoracicotropic hormone and bombyxin on the prothoracic glands of *Rhodnius prolixus* in vitro. *J. Insect Physiol.* 43:651–56
  118. Steiner DF. 2004. The proinsulin

- C-peptide: a multirole model. *Exp. Diabesity Res.* 5:7–14
119. Stocker H, Radimerski T, Schindelholz B, Wittwer F, Belawat P, et al. 2003. Rheb is an essential regulator of S6K in controlling cell growth in *Drosophila*. *Nat. Cell Biol.* 5:559–65
  120. Suenobu A, Mizoguchi A, Ichikawa T. 2004. Relationship between firing activity of bombyxin-producing neurosecretory cells and hemolymph bombyxin titer in the silkworm *Bombyx mori*. *Gen. Comp. Endocrinol.* 137:219–26
  - 120a. Suzuki A, Kataoka H, Matsumoto S. 1995. *Molecular Mechanisms of Insect Metamorphosis and Diapause*. Tokyo: Ind. Publ. Consult.
  121. Taghert PH, Veenstra JA. 2003. *Drosophila* neuropeptide signaling. *Adv. Genet.* 49:1–65
  122. Tanaka M, Kataoka H, Nagata K, Nagasawa H, Suzuki A. 1995. Morphological changes of BM-N4 cells induced by bombyxin, an insulin-related peptide of *Bombyx mori*. *Regul. Pept.* 57:311–18
  123. Tapon N, Ito N, Dickson BJ, Treisman JE, Hariharan IK. 2001. The *Drosophila* tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation. *Cell* 105:345–55
  124. Tatar M. 2004. The neuroendocrine regulation of *Drosophila* aging. *Exp. Gerontol.* 39:1745–50
  125. Tatar M, Bartke A, Antebi A. 2003. The endocrine regulation of aging by insulin-like signals. *Science* 299:1346–51
  126. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. 2001. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292:107–10
  127. Teller JK, Rosinski G, Pilc L, Kasprzyk A, Lesicki A. 1983. The presence of insulin-like hormone in heads and mid-guts of *Tenebrio molitor* L. (Coleoptera) larvae. *Comp. Biochem. Physiol.* 74: 463–65
  128. Thorpe A, Duve H. 1984. Insulin-like and glucagon-like peptides in insects and mollusks. *Mol. Phys.* 5:235–60
  129. Tu MP, Yin CM, Tatar M. 2002. Impaired ovarian ecdysone synthesis of *Drosophila melanogaster* insulin receptor mutants. *Aging Cell* 1:158–60
  - 129a. Tu M-P, Yin C-M, Tatar M. 2005. Mutations in insulin signaling pathway alter juvenile hormone synthesis in *Drosophila melanogaster*. *Gen. Comp. Endocrinol.* 142:347–56
  130. Vanden Broeck J. 2001. Neuropeptides and their precursors in the fruit-fly, *Drosophila melanogaster*. *Peptides* 22:241–54
  131. Veenstra JA. 1989. Do insects really have a homeostatic hypotrehalosaemic hormone? *Biol. Rev. Camb. Philos. Soc.* 64: 305–16
  132. Verdu J, Buratovich MA, Wilder EL, Birnbaum MJ. 1999. Cell-autonomous regulation of cell and organ growth in *Drosophila* by Akt/PKB. *Nat. Cell Biol.* 1:500–6
  133. Verhaert PD, Downer RG, Huybrechts R, De Loof A. 1989. A substance resembling somatomedin C in the American cockroach. *Regul. Pept.* 25:99–110
  134. Weinkove D, Neufeld TP, Twardzik T, Waterfield MD, Leivers SJ. 1999. Regulation of imaginal disc cell size, cell number and organ size by *Drosophila* class I(A) phosphoinositide 3-kinase and its adaptor. *Curr. Biol.* 9:1019–29
  135. Wessells RJ, Fitzgerald E, Cypser JR, Tatar M, Bodmer R. 2004. Insulin regulation of heart function in aging fruit flies. *Nat. Genet.* 36:1275–81
  136. Yagi Y, Ishibashi J, Nagata K, Kataoka H, Suzuki A, et al. 1995. The brain neurosecretory cells of the moth *Samia Cynthia ricini*: immunohistochemical localization and developmental-changes of the samia homologs of the *Bombyx* prothoracicotropic hormone and bombyxin. *Dev. Growth Differ.* 37:505–15
  137. Yamaguchi T, Fernandez R, Roth RA.

1995. Comparison of the signaling abilities of the *Drosophila* and human insulin receptors in mammalian cells. *Biochemistry* 34:4962–68
138. Yoshida I, Moto K, Sakurai S, Iwami M. 1998. A novel member of the bombyxin gene family: structure and expression of bombyxin G1 gene, an insulin-related peptide gene of the silkworm *Bombyx mori*. *Dev. Genes Evol.* 208:407–10
139. Yoshida I, Tsuzuki S, Abdel Salam SE, Ino M, Korayem AM, et al. 1997. Bombyxin F1 gene: structure and expression of a new bombyxin family gene that forms a pair with bombyxin B10 gene. *Zool. Sci.* 14:615–22
140. Zhang H, Stallock JP, Ng JC, Reinhard C, Neufeld TP. 2000. Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. *Genes Dev.* 14:2712–24
141. Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D. 2003. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat. Cell Biol.* 5:578–81
142. Zitnan D, Sehnal F, Bryant PJ. 1993. Neurons producing specific neuropeptides in the central nervous system of normal and pupariation-delayed *Drosophila*. *Dev. Biol.* 156:117–35
143. Zitnan D, Sehnal F, Mizoguchi A, Ishizaki H, Nagasawa H, Suzuki A. 1990. Developmental changes in the bombyxin-like and insulin-like immunoreactive neurosecretory system in the wax moth, *Galleria mellonella*. *Dev. Growth Differ.* 32:637–45

## CONTENTS

---

SIGNALING AND FUNCTION OF INSULIN-LIKE PEPTIDES IN INSECTS, <i>Qi Wu and Mark R. Brown</i>	1
PROSTAGLANDINS AND OTHER EICOSANOIDS IN INSECTS: BIOLOGICAL SIGNIFICANCE, <i>David Stanley</i>	25
BOTANICAL INSECTICIDES, DETERRENTS, AND REPELLENTS IN MODERN AGRICULTURE AND AN INCREASINGLY REGULATED WORLD, <i>Murray B. Isman</i>	45
INVASION BIOLOGY OF THRIPS, <i>Joseph G. Morse and Mark S. Hoddle</i>	67
INSECT VECTORS OF PHYTOPLASMAS, <i>Phyllis G. Weintraub and LeAnn Beanland</i>	91
INSECT ODOR AND TASTE RECEPTORS, <i>Elissa A. Hallem, Anupama Dahanukar, and John R. Carlson</i>	113
INSECT BIODIVERSITY OF BOREAL PEAT BOGS, <i>Karel Spitzer and Hugh V. Danks</i>	137
PLANT CHEMISTRY AND NATURAL ENEMY FITNESS: EFFECTS ON HERBIVORE AND NATURAL ENEMY INTERACTIONS, <i>Paul J. Ode</i>	163
APPARENT COMPETITION, QUANTITATIVE FOOD WEBS, AND THE STRUCTURE OF PHYTOPHAGOUS INSECT COMMUNITIES, <i>F.J. Frank van Veen, Rebecca J. Morris, and H. Charles J. Godfray</i>	187
STRUCTURE OF THE MUSHROOM BODIES OF THE INSECT BRAIN, <i>Susan E. Fahrbach</i>	209
EVOLUTION OF DEVELOPMENTAL STRATEGIES IN PARASITIC HYMENOPTERA, <i>Francesco Pennacchio and Michael R. Strand</i>	233
DOPA DECARBOXYLASE: A MODEL GENE-ENZYME SYSTEM FOR STUDYING DEVELOPMENT, BEHAVIOR, AND SYSTEMATICS, <i>Ross B. Hodgetts and Sandra L. O'Keefe</i>	259
CONCEPTS AND APPLICATIONS OF TRAP CROPPING IN PEST MANAGEMENT, <i>A.M. Shelton and F.R. Badenes-Perez</i>	285
HOST PLANT SELECTION BY APHIDS: BEHAVIORAL, EVOLUTIONARY, AND APPLIED PERSPECTIVES, <i>Glen Powell, Colin R. Tosh, and Jim Hardie</i>	309

BIZARRE INTERACTIONS AND ENDGAMES: ENTOMOPATHOGENIC FUNGI AND THEIR ARTHROPOD HOSTS, <i>H.E. Roy, D.C. Steinkraus, J. Eilenberg, A.E. Hajek, and J.K. Pell</i>	331
CURRENT TRENDS IN QUARANTINE ENTOMOLOGY, <i>Peter A. Follett and Lisa G. Neven</i>	359
THE ECOLOGICAL SIGNIFICANCE OF TALLGRASS PRAIRIE ARTHROPODS, <i>Matt R. Whiles and Ralph E. Charlton</i>	387
MATING SYSTEMS OF BLOOD-FEEDING FLIES, <i>Boaz Yuval</i>	413
CANNIBALISM, FOOD LIMITATION, INTRASPECIFIC COMPETITION, AND THE REGULATION OF SPIDER POPULATIONS, <i>David H. Wise</i>	441
BIOGEOGRAPHIC AREAS AND TRANSITION ZONES OF LATIN AMERICA AND THE CARIBBEAN ISLANDS BASED ON PANBIOGEOGRAPHIC AND CLADISTIC ANALYSES OF THE ENTOMOFAUNA, <i>Juan J. Morrone</i>	467
DEVELOPMENTS IN AQUATIC INSECT BIOMONITORING: A COMPARATIVE ANALYSIS OF RECENT APPROACHES, <i>Núria Bonada, Narcís Prat, Vincent H. Resh, and Bernhard Statzner</i>	495
TACHINIDAE: EVOLUTION, BEHAVIOR, AND ECOLOGY, <i>John O. Stireman, III, James E. O'Hara, and D. Monty Wood</i>	525
TICK PHEROMONES AND THEIR USE IN TICK CONTROL, <i>Daniel E. Sonenshine</i>	557
CONFLICT RESOLUTION IN INSECT SOCIETIES, <i>Francis L.W. Ratnieks, Kevin R. Foster, and Tom Wenseleers</i>	581
ASSESSING RISKS OF RELEASING EXOTIC BIOLOGICAL CONTROL AGENTS OF ARTHROPOD PESTS, <i>J.C. van Lenteren, J. Bale, F. Bigler, H.M.T. Hokkanen, and A.J.M. Loomans</i>	609
DEFECATION BEHAVIOR AND ECOLOGY OF INSECTS, <i>Martha R. Weiss</i>	635
PLANT-MEDIATED INTERACTIONS BETWEEN PATHOGENIC MICROORGANISMS AND HERBIVOROUS ARTHROPODS, <i>Michael J. Stout, Jennifer S. Thaler, and Bart P.H.J. Thomma</i>	663
INDEXES	
Subject Index	691
Cumulative Index of Contributing Authors, Volumes 42–51	717
Cumulative Index of Chapter Titles, Volumes 42–51	722

## ERRATA

An online log of corrections to *Annual Review of Entomology* chapters may be found at <http://ento.annualreviews.org/errata.shtml>