Review Article

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Molecular Nexus between Insulin-like peptides and Downstream Kinases Regulate Glucose Homeostasis, Cell Survival and Growth in *Drosophila*

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Abstract

Drosophila is a versatile model organism to study metabolic disorders, one such being diabetes mellitus. Eight insulin-like peptides (ILPs) have been identified in *Drosophila*. ILPs are produced from paired Insulin-producing cells present in brain ganglia. Another protein called adipokinetic hormone (AKH) is homologous to mammalian glucagon and is released from the corpora cardiaca. Synergistic action of ILP and AKH maintains sugar homeostasis in *Drosophila*. ILP binds with insulin receptors on the adipocytes and trigger autophosphorylation and dimerization. The activated receptors then initiate a downstream signaling by various modulators to phosphorylate Akt (protein kinase B, a serine-threonine-specific protein kinase). Akt, when activated, targets multiple signaling molecules including Target of Rapamycin (TOR) that participates in glucose metabolism, protein synthesis, cell proliferation, neuroendocrine signaling, and stress response. Akt also phosphorylates transcription factor FOXO that promotes cell survival by up-regulating TRAIL, a pro-apoptotic protein. High lipid accumulation in the fat body is linked with insulin resistance in *Drosophila*. *Drosophila* reared on high lipid diet shows up-regulation in protein kinase C (PKC). PKC is known to antagonize insulin signaling in fruit flies. A clear concept regarding the complex process of glucose homeostasis can be generated through further investigations. Since *Drosophila* has several advantages over vertebrate models, it can be used to identify additional modulators of insulin biology and metabolism.

Keywords: Akt, *Drosophila,* FOXO, Insulin-like Peptides, TOR

1. Introduction

Insulin is a well-studied peptide hormone having an extensive network of signaling. It is crucial for regulation of glucose homeostasis in the body. But recent studies have suggested its involvement in other conserved processes like reproduction and determination of life span[1]. Dysfunction in insulin signaling is associated with metabolic disorders like diabetes and cancer[2]. Insulin executes its role via insulin receptors (IRs) of family tyrosine kinases in target tissues, mainly liver. Insulin signaling exists in all metazoans. Insect insulin-like peptides (ILPs) are homologs to mammalian insulin and are encoded by multi-gene families that are expressed in various tissues, particularly brain and fat body. ILPs are known to bring about their roles through single IRs. But, till date, it is not known as to how one common receptor can mediate several functions. Hence, ILP offers a new and interesting

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area of research to understand molecular interactions and signaling in insects. ILPs are elaborately studied in the fruit fly *Drosophila melanogaster* because of their short life span; fully known genome sequence; ease in genetic manipulation and laboratory culture of large population. *Drosophila* insulin-like peptides (DILPs) share sequence, structural and functional similarities with vertebrate insulin-like growth factors. Their interactions with insulin, regulating both growth and glucose homeostasis, are also comparable. When *Drosophila* is fed a high sugar diet, DILPs are released in response to high levels of circulating sugar to facilitate glucose uptake by adipocytes. Interestingly, in response to low levels of hemolymph sugar, adipokinetic hormone (AKH) is released[3]. Both ILP and AKH act synergistically to maintain glucose homeostasis in the fruit flies. ILPs are released from one pair of insulin producing cells (IPC) present in the brain ganglia whereas AKH is released from the corpora cardiaca[4]. Studies have documented that perturbation in DILPs/AKH functions affect growth and reproduction, and lead to diabetes-like phenotypes in fruit flies[5,6]. Furthermore, altered expression of genes encoding ILP modulates metabolism and longevity in fruit flies[7].

In this review, we aim to present the recent progress in ILP-mediated signaling that governs several physiological traits in *Drosophila*.

2. Insulin-like peptides (ILPs) in *Drosophila*

Insulin-like peptides (ILPs) act as neurotransmitter and hormone in insects to regulate nutrient intake, growth and reproduction. ILPs are encoded by distinct loci in the genome and activate insulin receptors (InR) at the plasma membrane of target cells. This triggers a series of proteins that cross-talk with each other to regulate metabolism and growth of the organism.

In *Drosophila*, genes encoding eight ILPs (DILP 1-8) have been characterized which are presumably ligands of *Drosophila* insulin receptors (InR)[8]. Fly DILPs are the homologs of the mammalian insulin and insulin-like growth factors.

DILPs are released from two groups of neurons in the brain containing insulin-producing cells (IPC)[4]. DILP1-7 are secreted from IPCs and released into the hemolymph to reach their target sites. DILP1-7 bind with their target InR and activate signaling cascades that induce glucose uptake into the tissues from the surrounding hemolymph. DILP8 codes for insulin-like relaxin peptide that regulates growth and development[9].

In *Drosophila*, almost every tissue shows expression of DILP though the maximum expression is tissue-specific. DILP2, DILP3, and DILP5 manifest maximum expression in adult brain ganglia. Expression of DILP6 and DILP7 is highest in thoracic and abdominal ganglia of the fly.

3. Spatial and Temporal Expression of DILP

DILPs show differential spatial expression during different developmental stages. During developmental period, *dilp2, dilp4* and *dilp7* mRNA transcripts were reported in mesoderm and mid-gut during late-stage embryogenesis[10]. High expression of *dilp2* was detected in each brain hemisphere and salivary gland whereas little expression was observed in imaginal discs of larvae[10]. Transcription of *dilp5* was turned on in 2nd stage of larvae whereas same of dilp3 was detected in mid- to late-stage third instar larva[8]. Larval fat body showed high expression of *dilp6* but little expression was reported in gut and brain[11]. Larval imaginal discs were also observed to express *dilp8*[9]. In adult *Drosophila*, IPC showed distinct expression of *dilp2*, *3* and *5*[4]. Expression of *dilp6* mRNA was most abundant in adult fat body compared to brain ganglia[7]. Finally, high levels of mRNA transcripts of dilp7 were detected in specific neurons of the ventral cord (dMP2) and several neurons in the brain[12].

A pictorial representation of the expression of DILPs in various tissues of both larva and adult is presented in Figure 1.

Insulin-like peptides in *Drosophila* (DILP) are localized in various tissues of both larval and adult forms. In larval stage, DILP2 are concentrated in brain, salivary glands, mid-gut and imaginal discs. DILP4 are released mainly from mid-gut. Fat body shows maximum expression of DILP 6 within its vicinity. DILP7 is produced by brain and mid-gut. In adults, corpus cardiacum releases adipokinetic hormone (AKH). Insulin-producing cells (IPC) in brain are responsible to produce DILP2, DILP3 and DILP5. After production, these DILPs are released into circulation so to reach their target sites. DILP6 and DILP7 are secreted from both thoracic and abdominal ganglia.

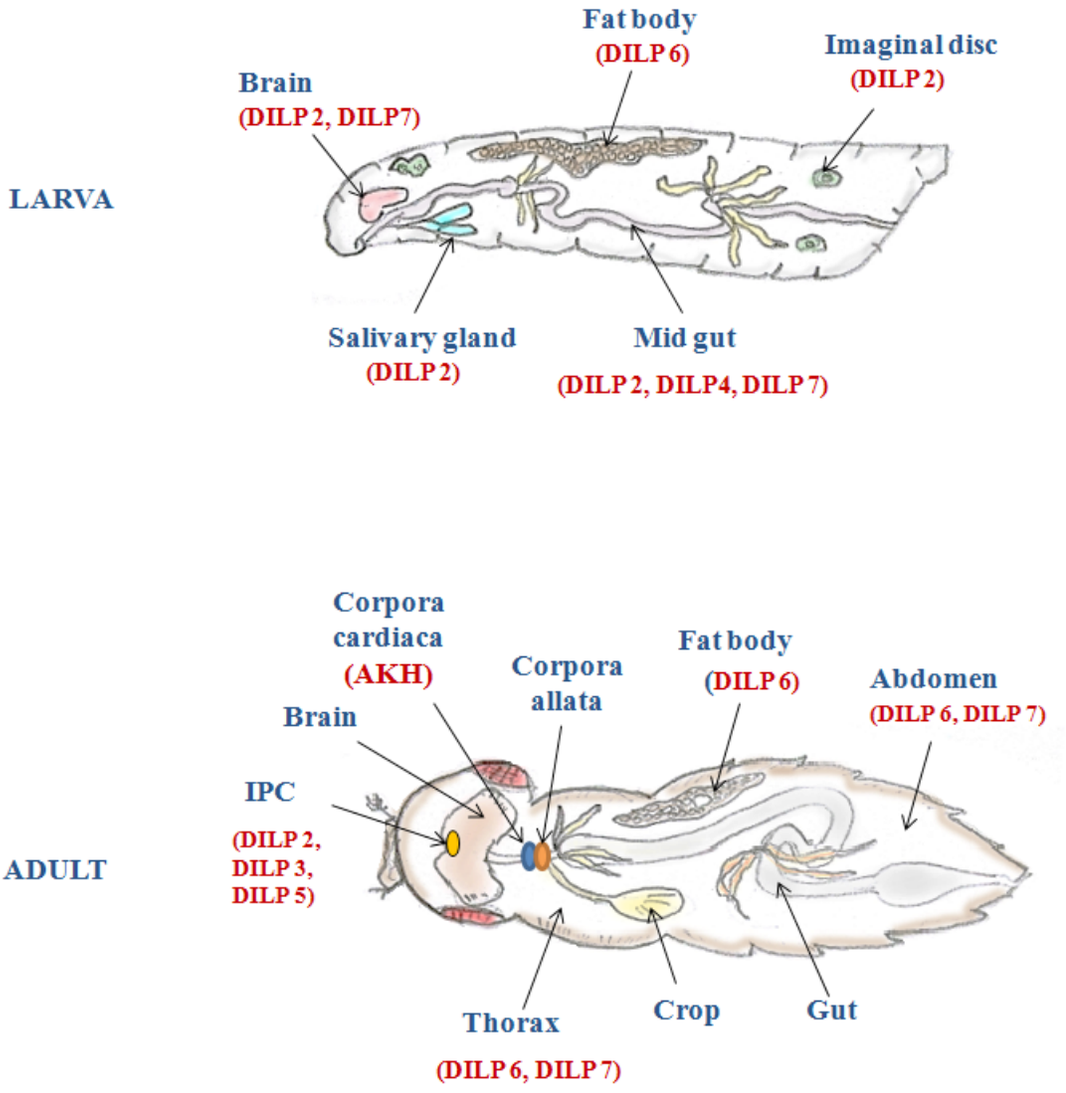

Figure 1. Spatio-temporal localization of DILP in larva and adult of *Drosophila*.

4. ILPs in Other Insects

ILPs are characterized in arthropods and other invertebrates. First confirmation of ILP expression came from silk worm *Bombyx mori* with 38 *ilps*. Later, ILPs were evident in other lepidopteran insects such as *Samia cynthia* and *Agrius convolvuli*[13,14]. In the case of *Anopheles gambiae* (an African malaria mosquito) and *Aedes aegyptii*, five and eight ILPs, respectively, have been identified[15,16]. Similarly, *Apis mellifera* was reported to express two ILPs[17]. The beetle *Tribolium castaneum* contains four *ilp* genes. The pea aphid *Acyrthosiphon pisum* has 10 *ilp* loci[18]. More recently, two *ilps* were identified in *Spodoptera littoralis*[19] and one in *Lonomia obliqua*. Single *ilp* locus is known for two orthopteran species, *Locusta migratoria*[20] and *Schistocerca gregaria*[21].

5. DILP-Induced Signaling Pathway

In response to high sugar intake, DILP is released from neurosecretory cells of the brain ganglia of the fly and binds to insulin receptors (InRs). InRs oligomerise

and cross-phosphorylate at the tyrosine residues. Phosphorylated InRs recruit an adaptor scaffold protein called insulin receptor substrate (IRS). In *Drosophila*, two IRS viz. Chico (an orthologue of mammalian IRS1) and Lnk (an ortholog of the SH2B adaptor protein **1)** have been identified[22,23]. Chico interacts with downstream proteins having src-homology 2 (SH2) domains.[24] One such protein having SH2 domain is phosphatidylinositol 3 kinase (PI3K). Chico binds with the regulatory subunit (p60) of PI3K and activates its catalytic subunit (p110). PI3K in turn phosphorylates membrane-bound phosphatidylinositol 4,5-bis phosphate (PIP2) to generate phosphatidylinositol triphosphate (PIP3). Proteins consisting pleckstrin homology (PH) domain interacts with PIP3 and promotes their membrane localization and interaction with other membrane-anchored proteins. Two proteins, viz. Akt (also known as protein kinase B) and PDK1 (3-phosphoinositide-dependent protein kinase-1), with PH domain, have been demonstrated in *Drosophila*. PIP3 binds with PDK1 which in turn recruits and phosphorylates Akt. Phosphorylation of Akt occurs at two sites. PDK1 phosphorylates Akt at Tyr340 whereas rapamycin-insensitive companion of TOR kinase (Rictor) in the TOR-complex 2 (TOR-C2) phosphorylates Akt at Ser505 residue[24].

Akt mediates most of insulin's metabolic effects, regulating glucose transport, lipid synthesis, gluconeogenesis, and glycogen synthesis. Akt also targets multiple signaling molecules that participate in protein synthesis, cell proliferation, neuroendocrine signaling, and stress response[24]. For instance, Akt phosphorylates downstream proteins glycogen synthase kinase-3 (GSK-3) and the *Drosophila* ortholog shaggy (Sgg), which regulates glycogen synthesis[25] as well as translocation of glucose transporter GLUT4 to plasma membrane[26].

6. Akt Up-regulates Target of Rapamycin (TOR) Signaling

TOR is a kinase that regulates metabolic homeostasis in the body. TOR integrates signals from nutrients present in the environment and mitogens to control cellular growth by targeting protein synthesis. In the signaling cascade induced by DILP, TOR lies downstream of PI3K and Akt/PKB. Akt is a negative regulator of tuberous sclerosis complex (Tsc1/2). Tsc1/2 in active form inhibits TOR kinase pathway[27]. Upon phosphorylation and inactivation of Tsc1/2 by Akt, TOR signaling pathway is up-regulated[24]. Both ribosomal protein S6K and eIF4E-binding proteins are targets of TOR kinase. TOR phosphorylates S6K and eIF4E-binding proteins and promotes synthesis of various proteins that regulate cell division, growth and metabolism. Downstream signaling induced by TOR is inhibited by rapamycin. Rapamycin forms a complex with FKBP12 and interacts with TOR kinase. This results in arrest of cell division at G1 phase and causes dephosphorylation of ribosomal protein S6K and eIF4E-binding proteins. In the presence of rapamycin or a nutrient-deficient environment, TOR signaling is interrupted and, as a result, ILP signaling is blunted. Mutations in *Drosophila* TOR have been reported to exert important impacts on cell growth, survival and proliferation[28,29].

7. Akt Regulates FOX Signaling in *Drosophila*

FOX (Forkhead box) proteins are a family of transcription factors that control cell survival, growth, proliferation and longevity. It also promotes glucose catabolism. FOX proteins are categorized under helix-turn-helix class of proteins. They are the major targets of Akt. Insects and mammals have multiple FOX proteins encoded by more than 16 genes in *Drosophila* and 43 genes in human[30]. Members of the class O (FOXO) proteins participate in transcription of negative regulators of cell-cycle, such as retinoblastoma-related protein p130 and CDKI $p27^{Kip1}$. Therefore, FOXO group of transcription factors mediate cell cycle arrest, DNA repair and apoptosis[31]. Akt phosphorylates and inhibits translocation of FOXO into the nucleus and blocks its activity. Akt phosphorylation of FOXO promotes cell survival since FOXO also up-regulates the pro-apoptotic protein TRAIL. A pictorial view of DILP signaling is presented in Figure 2.

ILP released from the neurosecretory cells of the brain ganglia in *Drosophila*, binds to insulin receptors (InRs). InRs oligomerise and cross-phosphorylate at the tyrosine residues. Phosphorylated InRs recruit Chico and Lnk. Chico interacts with phosphatidylinositol 3 kinase (PI3K). Chico binds with the regulatory subunit (p60) of phosphatidylinositol 3 kinase (PI3K) and activates its catalytic subunit (p110). PI3K in turn phosphorylates membrane-bound phosphatidylinositol 4,5-bis phosphate (PIP2) to

generate phosphatidylinositol triphosphate (PIP3). PIP3 promotes membrane localization of Akt and PDK1. PIP3 binds with PDK1 and phosphorylates Akt. Akt mediates most of insulin's metabolic effects, regulating glucose transport, lipid synthesis, gluconeogenesis, and glycogen synthesis. Akt phosphorylates the downstream protein glycogen synthase kinase-3 (GSK-3) which regulates glycogen synthesis. Akt is a negative regulator of tuberous sclerosis complex (Tsc1/2). Tsc1/2 in active form inhibits TOR kinase pathway. Upon phosphorylation and inactivation of Tsc1/2 by Akt, TOR signaling pathway is up-regulated. Ribosomal protein S6K and eIF4E-binding proteins both are targets of TOR kinase. TOR phosphorylates S6K and eIF4E-binding proteins and promotes synthesis of various proteins that regulate cell division, growth and metabolism. Akt phosphorylates and inhibits translocation of FOXO into the nucleus and blocks its activity as negative regulator of cell cycle proteins.

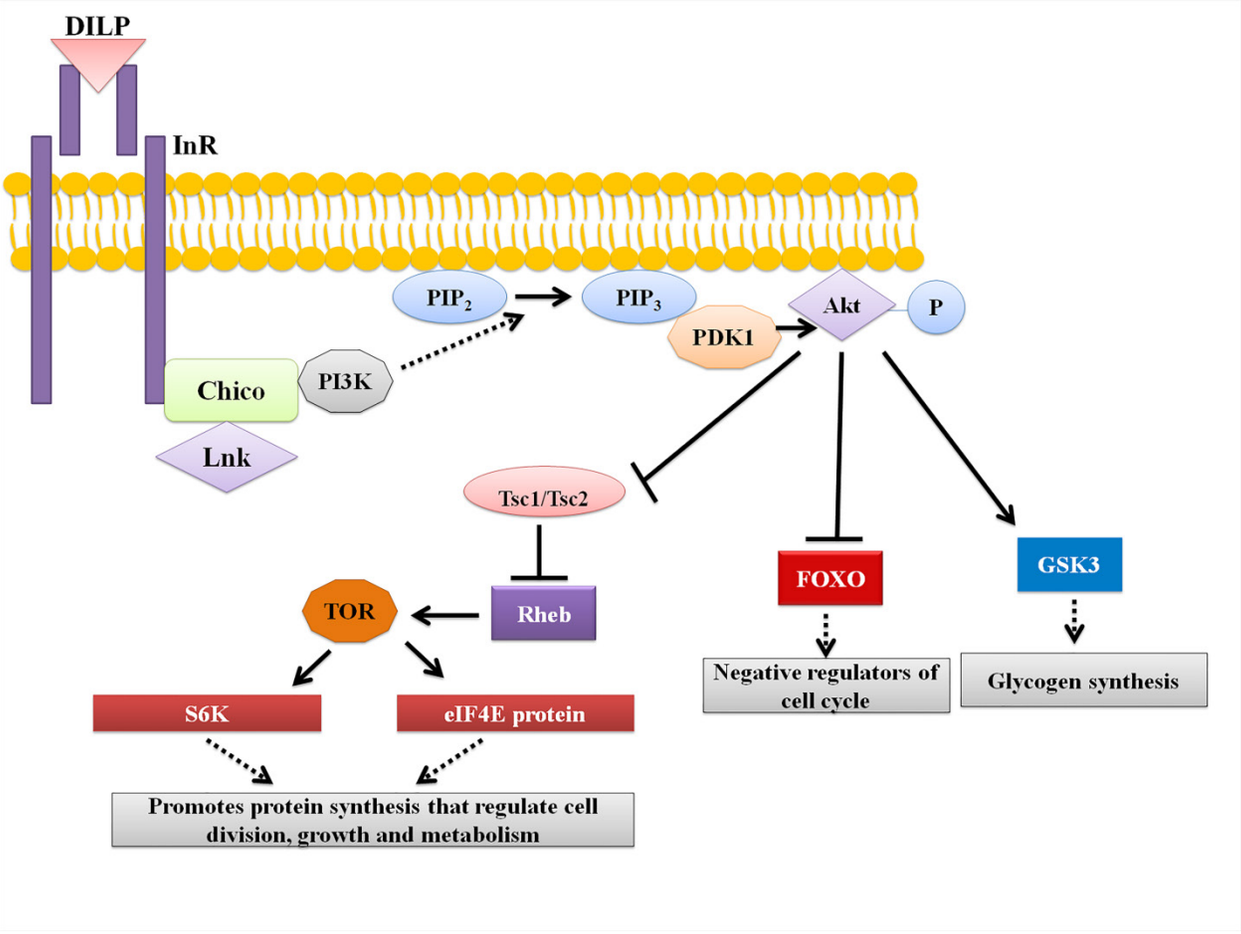

Figure 2. Cross talk between DILP, TOR and FOXO-mediated signaling in *Drosophila*.

8. Other Molecules that Regulate ILP Secretion

IPCs in *Drosophila* are under direct or indirect control of several molecules produced from different parts of the body. Serotonin [5-hydroxytryptamine (5-HT)] neurons in fruit fly have been linked with the secretion of DILPs. Loss of nucleostemin family GTPase NS3 in these neurons exhibit insulin deficiency phenotypes in fruit flies[32]. Luo et al.,[33] reported that reduction of Octopamine receptor lowers Ilp3 expression but no effect was observed in carbohydrate metabolism. Studies confirmed that GABAergic neurons exert inhibitory impact on IPCs in brain[34]. Further studies suggested that nutrition-

dependent signals from fat body modulate GABAergic inhibition of IPCs. Fat body secretes leptin-like hormone Unpaired 2 (Upd2) that executes nutrition-dependent signaling[35]. Another study suggests that the peptide hormones short neuropeptide F (sNPF) and corazonin play significant role in modulating DILP production as decrease of these hormones produces hyperglycemia-like symptoms in *Drosophila*[36].

Kwak et al.,[37] reported that *Drosophila* adiponectin receptor (AdipoR) induces secretion of DILPs from IPC. Unger et al.,[38] postulated that gut hormone decretin suppresses insulin secretion in mammals after starvation. *Drosophila* Limostatin (Lst) was recently identified as the first decretin[39]. Levels of Lst increase during fasting in gut-associated cells and this suppresses ILP production and secretion through the G-protein-coupled receptor encoded by *CG9918*[39].

Therefore, it is apparent that secretion of DILPs is a complex process and this involves convergence of a variety of signaling molecules onto the IPCs.

9. Insulin Resistance in *Drosophila*

Several workers around the globe have produced insulin-resistant flies by rearing them on high sugar diet or high fat diet[40-42]. *Drosophila* fed on high sugar diet manifested increase in ILP level and lipid accumulation in fat body. Moreover, the flies developed hyperglycemia-like phenotypes.[40,41] In mammals, lipid accumulation is linked with activation of protein kinase C (PKC) which has been linked with insulin resistance. Study on *Drosophila* S2 cell line has also documented that activated PKC may antagonize insulin signaling[43]. From these findings it can be said that flies treated with high sugar diet manifest lipid accumulation which in turn causes insulin resistance through PKC activation.

Other studies have claimed that activation of transcription factor FOXO leads to lipid accumulation in adipocytes as well as suppression of *Ilp* mRNA level[44]. Further, FOXO induces negative feedback of insulin signaling by lowering the production of mRNA for insulin receptors (InR) as well as downstream signaling mediator Insulin Receptor Substrate (IRS)[45,46]. FOXO is induced in low IIS (Insulin/Insulin like growth factor Signalling) condition and is linked to sensitize insulin responses in *Drosophila* and mammals[46,47]. Again, exposure to excessive sugar diet and overactive InR signaling can reduce response of peripheral tissues towards insulin ligand[46,48].

10. Conclusion

In the contemporary scenario, diabetes has emerged as one of the most familiar lifestyle diseases in man. Cause and symptoms have been characterized in humans though many aspects of molecular interactions at the level of genes and their products are still under investigation. *Drosophila,* compared to other experimental models for diabetes, has clear advantages like simple genetic system, known genome sequence, easy to make genetic manipulations, high evolutionary conservation, characterized insulin pathway and metabolic genes. *Drosophila* has other advantages such as short life cycle, easy to culture, etc. *Drosophila* has different developmental stages which mimic feeding (larva and adult) and fasting (pupa) stages important to study insulin signaling. Interactions between insulin-like peptides, InR, IIS, TOR, Akt and FOXO have been well established in *Drosophila*. But *in vivo* characterization of other diabetes susceptibility genes and their mechanisms of action are yet to be investigated. Studies to understand interaction between individual genes and their target tissues to maintain homeostasis of glucose, lipids and other metabolites need to be carried out in fruit flies. Diabetes seems to be a product of interactions between huge numbers of intracellular molecules. Therefore, *Drosophila* in this case may be beneficial in identification of additional modulators of insulin biology and metabolism. Understanding the nature of gene-environment interactions in diabetes susceptibility is an important goal, and *Drosophila* would obviously be helpful in this context.

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