13 *Drosophila* Models to Investigate Insulin Action and Mechanisms Underlying Human Diabetes Mellitus

Yoshihiro H. Inoue, Hiroka Katsube, and Yukiko Hinami

Abstract

Diabetes is a group of metabolic diseases in which the patient shows elevated levels of blood sugar. In healthy condition, there is the regulatory system that maintains constant glucose levels in blood. It is accomplished by two hormones, insulin and glucagon acting antagonistically. Insulin is produced in β cells in pancreas and secreted to blood. It specifically binds to its receptors on plasma membrane and activates the intracellular signaling pathways. At the end, glucose in blood are taken into the cells. The diabetes is classified into two types. In type 1 diabetes (T1D), patients' pancreas fails to produce sufficient insulin. Hence, in type 2 diabetes (T2D), the target cells of insulin fail to respond to the hormone. The metabolic syndrome (MS) is characterized as a prediabetes showing lowered responsiveness to insulin. *Drosophila* has been expected to be a usefulness model animal for the diabetes researches. The regulatory system maintaining homeostasis of circulating sugar in hemolymph is highly conserved between *Drosophila* and mammals. Here, we summarize findings to date on insulin production and its acting mechanism essential for glucose homeostasis both in mammals and *Drosophila*. Subsequently, we introduce several *Drosophila* models for T1D, T2D, and MS. As a consequence of unique genetic approaches, new genes involved in fly's diabetes have been identified. We compare their cellular functions with those of mammalian counterparts. At least three antidiabetic drugs showed similar effects on *Drosophila*. We discuss whether these *Drosophila* models are available for further comparative studies to comprehend the metabolic diseases.

Keywords

Drosophila · Glucose homeostasis · Insulin-like peptides · Diabetes · Type 1 diabetes models · Type 2 diabetes models · Metabolic syndrome models

13.1 Introduction

Diabetes mellitus is a group of metabolic diseases in which the patient shows increased levels of blood sugar. The number of people with diabetes has risen four times for the past three decades. The global prevalence of diabetes among adults over 18 years has almost doubled for the periods. In 2015, an estimated 1.6 million deaths were directly caused by diabetes in the world. The WHO estimated that diabetes will be the seventh

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leading cause of death in 2030 (WHO 2016). The metabolic diseases have been considered to be basically caused by disturbance of sugar homeostasis in our bodies. Normally, there is a sophisticated regulatory system that maintains constant levels of glucose in blood. It is accomplished by two hormones acting, insulin and glucagon acting antagonistically (Aronoff et al. 2004). Both hormones are secreted from different specialized cells in Langerhans islet of pancreas. Insulin produced in pancreatic β cells are secreted to blood, and it specifically binds to its receptors on plasma membrane of target cells. The hormone activates the intracellular signaling pathway. At the end, the signal transmitted to a glucose transporter. Then, glucose existing in blood are taken through the transporter into the cells.

Diabetes are basically caused by a perturbation of somewhere in the process. The inefficient glucose uptake resulted in tissue dysfunction and/or infertility in adults. A development of various tissues and organs is also affected in fetal and neonatal diabetes. The hyperglycemia is caused by either insulin production or its action (David and Mervyn 2009). Therefore, diabetes is classified into two types. In type 1 diabetes (T1D), patients' pancreas fails to produce sufficient insulin. On the other hand, in type 2 diabetes (T2D), the target cells of insulin fail to respond to the hormone. T1D which were previously known as insulin-dependent, juvenile, or childhood-onset is characterized by deficient insulin production. Recently, lowered responsiveness to insulin (insulin resistance) has also caught a great deal of attention as a hallmark of a lifestyle diseases called metabolic syndrome. It is quite important for us to understand onset and pathogenesis of diabetes. Identification of novel genes involved in the processes provides great opportunity for development of therapeutic targets. Furthermore, understanding of basic mechanisms of the insulin production, secretion, and its action contributes to the advances of cell and development biology.

For the purpose, simple animal models allow us to proceed the biological studies faster and more conveniently. As described in other chapters of this book, *Drosophila* has been used as a quite useful animal model for medical studies on various human diseases. For the diabetes research, it has great advantages. The regulatory system that maintains homeostasis of circulating sugar in hemolymph is highly conserved between *Drosophila* and mammals (Baker and Thummel 2007; Haselton and Fridell 2010). In addition to the most sophisticated techniques of genetic analyses, a large amount of information on developmental biology and physiology is available in *Drosophila*. In this chapter, we, firstly, would like to summarize findings to date on insulin production and its acting mechanism essential for glucose homeostasis, remarkably conserved in mammals and *Drosophila*. Subsequently, we introduce several *Drosophila* models for T1D, T2D, and the metabolic syndrome considered as an initial stage of T2D, which have been so far established. And we describe new genes involved in fly's diabetes, identified using the diabetes models. Finally, we try to evaluate whether they are useful as animal models for diabetes studies. Furthermore, we discuss whether these *Drosophila* models are available for large-scale screens to develop new antidiabetic medicines (Pandey and Nichols 2011).

13.2 Glucose Homeostasis in Mammals and *Drosophila*

Before introducing *Drosophila* diabetes models and discussing their availability as human diseases models, we firstly compare regulatory systems to maintain glucose homeostasis in between mammals and *Drosophila* (Fig. 13.1). In mammals, insulin is a unique hormone that plays an indispensable role that controls glucose hemostasis in mammals (Wilcox 2005). The hormone is produced and secreted from β cells existing in the islet of Langerhans in pancreas. Another hormone, glucagon which acts antagonistically to insulin, is also secreted from α cells of the islet. A balance between these two hormones maintains a constant of circulating glucose in blood. The insulin gene encodes the insulin precursor, called preproinsulin, composed of signal peptides, A-chain, C-peptides, and B-chain in this order.

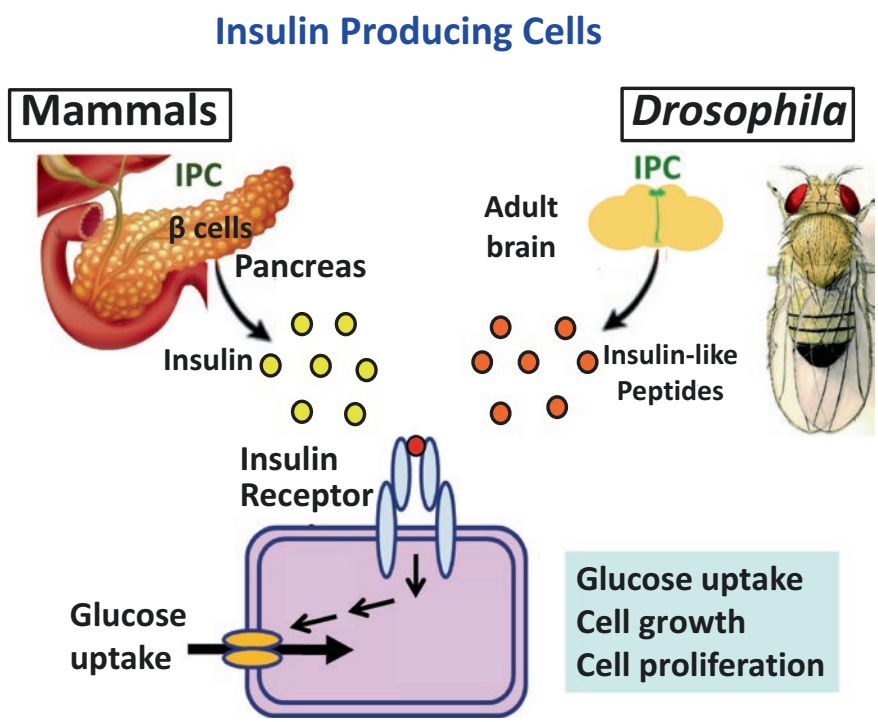

Fig. 13.1 Production, secretion, and signaling of insulin-like peptides and their effects to stimulate glucose uptake in *Drosophila*

After the signal peptide of the preproinsulin is cleaved, the polypeptide folds into the endoplasmic reticulum (ER), forming proinsulin. After the protein folding, the proinsulin is transported to the trans-Golgi network where immature granules containing proinsulin are formed. The polypeptide undergoes maturation into active insulin. After cleavage of the C-peptide located in the central portion of proinsulin, separated the B- and A-chains linked together again by two disulfide bonds, consists of matured insulin. Upon sensing increase of blood glucose and stimuli that promote secretion, secretary vesicles containing matured insulin releases from β cell by exocytosis that are mediated by SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) (Jewell et al. 2011). Insulin in blood binds to insulin receptor of target cells at high affinity, while it can also bind to IGF-1R (insulin-like growth factor 1 receptor) and IGF-2R (insulin-like growth factor 2 receptor) at a lower affinity (Jones and Clemmons 1995; Nakae et al. 2001). Both IGF-1 and IGF-2 play a role as growth factors that promote cell proliferation as well as cell growth, rather than hormones stimulating glucose uptake. These overlapped signaling is referred as the insulin-insulin-like growth factor signaling (IIS). The glucose transporters Glut1 and Glut3 in target tissue cells are involved in sensing of circulating glucose level (Joost and Thorens 2001; Rutter et al. 2015). According to the sensing, the insulin is subsequently released from pancreatic β cells, and it also stimulates glycolysis. This eventually results in stimulation of ATP synthesis in mitochondria, leading to inactivation of ATP-gated potassium channels (K_{ATP}), which allows plasma membranes of the β cell to depolarizing. Then, fusion of the plasma membranes and the insulin-containing vesicles and subsequently insulin secretion takes place (Kreneisz et al. 2010). Insulin secreted from the pancreatic β cells binds

to the extracellular α subunit of the InR, activating tyrosine kinase domain intrinsic to its β subunit (Lee and Pilch 1994). Binding of insulin to the α subunit induces a conformational change, resulting in the autophosphorylation of several tyrosine residues in the β subunit (Van Obberghen et al. 2001). These phosphor-amino acid residues are recognized by phosphotyrosine-binding domains of adaptor proteins such as the insulin receptor substrate family (IRS1~4) (Saltiel and Kahn 2001; Lizcano and Alessi 2002). The phosphotyrosine residues on IRS proteins are recognized by the SH2 domain of the p85 regulatory subunit of PI_3-kinase. The catalytic subunit of the kinase, p110, then phosphorylates PIP_2 converting to the formation of PIP_3. A downstream effector of PIP_3 is another kinase, AKT, which is recruited to the plasma membrane. Activation of the AKT requires the protein kinase 3-phosphoinositide-dependent protein kinase-1 (PDK1). Once AKT is activated, it catalyzes phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3). A major substrate of GSK3 is glycogen synthase, an enzyme that catalyzes the critical step in glycogen synthesis. Phosphorylation of glycogen synthase by GSK3 inhibits glycogen synthesis; therefore, the inactivation of GSK3 by AKT promotes energy storage as glycogen. These protein kinases are responsible for mediating many of the ultimate metabolic actions of insulin, including translocation of GLUT4 in mammals, activation of glycogen synthesis, and suppression of gluconeogenesis by inhibiting transcription of the gene encoding phosphoenolpyruvate carboxykinase (Brady et al. 1998; Lochhead et al. 2001).

Whereas, in *Drosophila*, sugars in diet are taken from the digestive duct and transported into the fat body (FB), which acts as a homologous tissue of both the liver and adipose tissue in insects, respectively. They are converted to trehalose in FB. The disaccharide is once converted to glycogen and stored in the FB. And it is released into the hemolymph when needed, while glucose is only contained less than one-hundredth of trehalose (Nation 2002; Ugrankar et al. 2015). The sugar levels in hemocytes are antagonistically controlled by *Drosophila* insulin-like peptides (DILPs) and the glucagon-like peptide, adipokinetic hormone (AKH) (Ikeya et al. 2002; Rulifson et al. 2002; Kim 2004; Lee and Park 2004). The DILPs that belong to the insulin-relaxin superfamily are composed of eight members involved in Dilp1~8 (Fig. 13.2). (Wu and Brown 2006; Colombani et al. 2015). But no orthologues of genes encoded by mammalian IGF peptides have been found in *Drosophila* genome. Dilp8 is divergent from other members to some extent and bind to relaxin-type membrane receptor, Lgr3 (Colombani et al. 2015; Vallejo et al. 2015). The DILPs except Dilp8 binds to a single receptor, termed the insulin receptor homolog (InR) on the plasma membrane of their target cells (Nishida et al. 1986; Fernandez et al. 1995).

The Dilp binding leads to activation of tyrosine kinase domain furnished in the receptor and recruiting of docking protein, Chico/IRS orthologue, and Lnk (Fig. 13.2). Though the docking protein binds the p60 regulatory subunit of PI_3K (Pi3K92E), the InR recruits the protein together with the catalytic subunit (Pi3K21B) to the plasma membrane. The active PI_3K converts PIP_2 to PIP_3 in the plasma membrane. A formation of the PIP_3 is negatively regulated by the PTEN protein, a phosphatase that dephosphorylates PIP_3 and coverts it to PIP_2. The signaling molecule, PIP_3, recruits two kinases, PDK1 (Pdk1) and AKT (dAkt1), to the plasma membrane, enabling the PDK1 to phosphorylate AKT. Akt1 is the core kinase component of the insulin/insulin-like growth factor signaling (IIS) pathway. It functions downstream of Pi3K92E and is activated by phosphatidylinositol binding and phosphorylation. It mediates versatile signaling pathways essential for cell growth and survival. The AKT phosphorylates several substrates including to the transcription factor, Foxo (forkhead box protein O), a critical transcription factor for metabolism and stress responses. It activates another kinase, glucose synthase kinase 3 (GSK-3/Sgg). And it results increased glucose uptake and fatty acid synthesis at the end. A con-

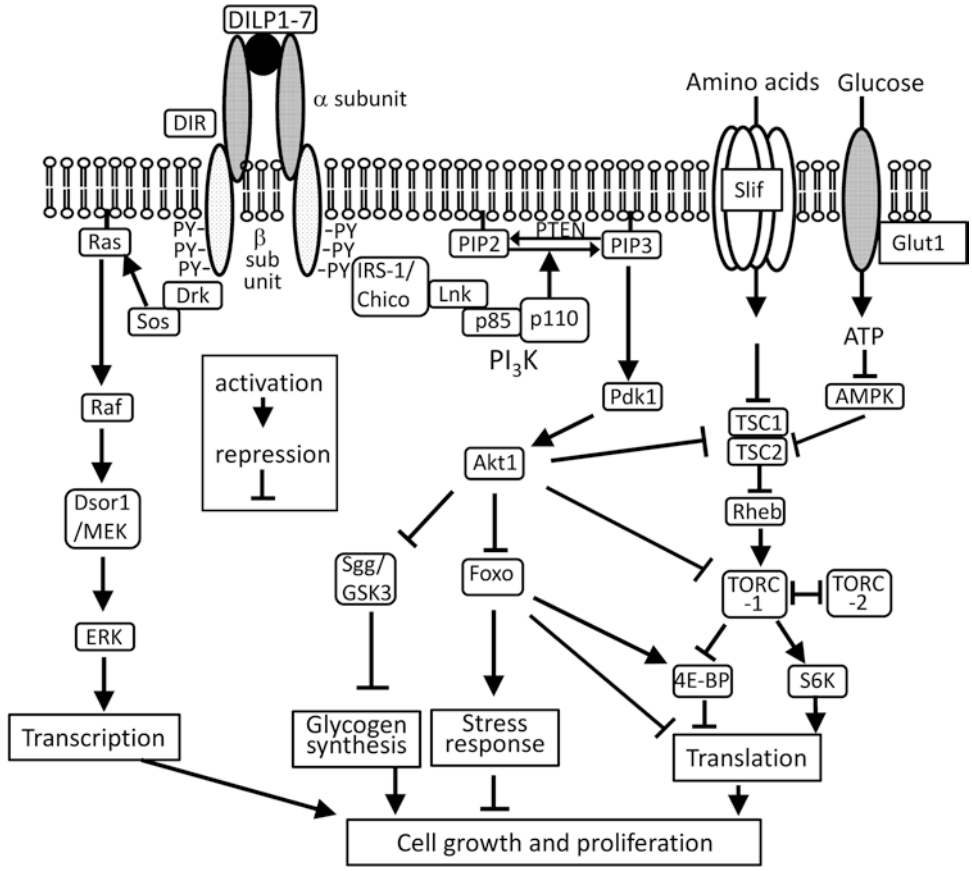

Fig. 13.2 Signal transduction pathway of DILPs and a transport of glucose and amino acids in *Drosophila*. The insulin-like peptides (Dilp1-7) (black sphere) bind to the extracellular α subunit of their specific receptor, DIR, on the plasma membrane. The binding activates the tyrosine kinase in the β subunit and signaling pathways locating downstream. The binding leads to activation of tyrosine kinase domain furnished in the receptor and recruiting of docking protein, Chico/IRS orthologue, and Lnk. Though the docking protein binds the p60 regulatory subunit of PI_3K, the DIR recruits the protein together with the catalytic subunit (p110) to the plasma membrane. The active PI_3K converts PIP2 to PIP3 in the plasma membrane. A formation of the PIP3 is negatively regulated by the PTEN protein, a phosphatase that dephosphorylates PIP3 and coverts it to PIP2. The signaling molecule, PIP3, recruits two kinases, Pdk1 and Akt1, to the plasma membrane, enabling the PDK1 to phosphorylate AKT. Akt1 is the core kinase component of the insulin/insulin-like growth factor signaling (IIS) pathway. It functions downstream of PI_3K and is activated by phosphatidylinositol binding and phosphorylation. It mediates versatile signaling pathways essential for cell growth and survival. The Akt1 phosphorylates several substrates including to the transcription factor, Foxo, a critical transcription factor for metabolism and stress responses. The kinase phosphorylates and inhibits the Tsc1/Tsc2 complex, which is an inhibitor of the Tor signaling pathway, an essential regulator of growth and metabolism. The Akt1 also activates another kinase, Sgg/GSK-3 essential for inhibition of glycogen synthesis. A concentration of glucose in the hemolymph is sensed by GLUT1 and the glucose uptake occurs through the glucose transporter, associated with the insulin signal transduction

served mechanism to sense the glucose concentration in hemolymph is also present in *Drosophila* (Rulifson et al. 2002; Kim 2004; Kreneisz et al. 2010). In conclusion, as insulin peptides and the IIS pathway are well conserved between *Drosophila* and mice, *Drosophila* can be used as one of animal models for studies on insulin production, secretion, and glucose homeostasis.

13.3 Insulin-Like Peptides that Control Growth and Proliferation as well as Sugar Metabolism in *Drosophila*

A series of genetic studies on mutants for DILPs and signaling factors of the IIS pathway have revealed that they play essential roles in cell growth and proliferation of various tissues during *Drosophila* development (Fig. 13.4) (Brogiolo et al. 2001; Hsu and Drummond-Barbosa 2009; Kannan and Fridell 2013). There are some differences in action of insulin-like peptides between *Drosophila* Dilps and mammalian insulin. In mammals, insulin and IGFs basically divide respective role, insulin for glucose uptake and IGFs for cell proliferation and growth. On the other hand, in *Drosophila*, DILP1~7 have both growth factor function and metabolic function to maintain sugar homeostasis (Fig. 13.1). Furthermore, these functions are different from each other in between larval stage and adult stage. DILPs regulate growth of all tissues in both somatic and germline cells, whereas their effects in adults are predominantly restricted to metabolite homeostasis, stress response, fecundity, and longevity (Broughton et al. 2005; Gronke et al. 2010). Insulin is known to play an essential role in glucose uptake in *Drosophila* cells (Ceddia et al. 2003), and seven genes encoding insulin-like peptides (ILPs) have been identified in the *Drosophila* genome. These peptides are synthesized in clusters of medial neurosecretory cells in the *Drosophila* brain (Rulifson et al. 2002). The InR and its downstream signaling cascade are well conserved in *Drosophila* (Fernandez et al. 1995; Bohni et al. 1999). It has been reported that InR and its signaling cascade can stimulate both cell proliferation and growth in cultured *Drosophila* cells as well as in larval imaginal cells (Chen et al. 1996; Brogiolo et al. 2001).

In addition to proliferation and growth of somatic cells, Dilps also play a critical role in cell growth, proliferation, and maintenance of germline cells during gametogenesis. Division of germline stem cells is a critical step that determines the numbers of germ cells. Tissue-extrinsic signals that reflect the nutrient condition of the organisms influences stem cell proliferation and their maintenance in both females and males (Hsu and Drummond-Barbosa 2005; Hsu et al. 2009; Ueishi et al. 2009). Hypomorphic *InR* mutant females exhibit infertility, and the number of cysts produced from female GSCs decreased. Furthermore, growth of nurse cells was also inhibited in the mutant ovaries (LaFever and Drummond-Barbosa 2005). This result suggests that the IIS signaling is required for female GSC division and cell growth of their progenies within the egg chambers (Hsu and Drummond-Barbosa 2009). *Drosophila* oogenesis is dependent on environmental nutrient conditions (Fuller and Spradling 2007). Therefore, it is reasonable to assume that both cell number and growth in egg chambers are directly regulated by hormonal control via DILPs. Furthermore, maintenance of the stem cells depends on local signals provided by niches, in which the stem cells reside (Fuller and Spradling 2007). In addition to common regulatory factors required for maintaining stem cells, Drummond-Barbosa's group showed that insulin signaling integrates the effects of nutrient and age on germline stem cell (GSC) maintenance. This is mainly regulated by Notch signaling mediated by interaction between GSC and niche cells, called cap cells, sending the signal that maintain GSC, mediated by E-cadherin (Inaba et al. 2010). The authors also reported that the loss of GSC and niche cell occurs with age and that the age-dependent impairment can be suppressed by increased levels of Dilp2. These results indicate that the Dilp signal plays an important role in the regulation of stem cell niches and, thereby, of stem cell numbers.

Wheares, Spermatogenesis in *Drosophila* commences with cell division of GSCs to produce male germline cells at the tip of the testis. The study of spermatogenesis in *Drosophila* can aid in understanding the regulatory mechanisms underlying cell proliferation and growth during development. In young adult *Drosophila* males, 5

to 8 GSCs are usually present at the tip of the testis. To maintain their multipotential stem cell characteristics, GSCs receive signals from the adjoining hub cells. Both a ligand encoded by the unpaired gene and the JAK-STAT signaling cascade are involved in this signal transfer (Yamashita 2008); (Tulina and Matunis 2001). The proximal cell of the two daughter cells derived from asymmetric division of a stem cell exclusively receives the unpaired signal and becomes a self-renewed GSC. For self-renewal and differentiation of GSC daughters, it is crucial to set up cell division axis perpendicular to a cluster of the hub cells (Yamashita et al. 2003). The distal daughter cell leaves the niche and differentiates as a spermatogonium, which then undergoes cell division four times to produce a 16-spermatocyte cyst. Ueishi and colleagues found that inhibition of insulin production and insulin signaling mutations resulted in decreased numbers of germline cells in *Drosophila* testes. GSC numbers were maintained in young mutant males, with a gradual decrease in abundance of GSCs with age. Furthermore, in mutants, a lower frequency of GSC division was seen. Insulin signaling was found to promote cell cycle progression of the male GSCs at the G2/M phase. The spermatocytes differentiated from a progeny of GSC enter a growth phase during which they increase remarkably in volume by up to 25-fold. This is the largest extent of cell growth that proliferative cells can accomplish. Although the extracellular signal and the signaling cascade that maintain GSC numbers have been partially identified (Fuller and Spradling 2007); Inoue et al. 2012), the signals and regulatory factors that allow the spermatocytes to increase up to such a remarkable extent before meiotic initiation had been identified.

The *Drosophila* premeiotic spermatocytes have achieved most distinctive cell growth up to 25 times after premeiotic DNA replication. Ueishi and colleagues reported that a loss of DILPs by specific apoptosis induction to insulin-producing cells interfered growth of spermatocytes, suggesting that the spermatocyte cell growth is required for DILPs (Ueishi et al. 2009). They further showed that an accumulation of active Akt form phosphorylated by its upstream factor, PDK1, in the growing spermatocytes. A diameter of spermatocytes from mutant males for InR or IRS orthologue encoded by *chico* decreased in size. We further showed that the expression of constitutive active form of PI_3-kinase catalytic subunit significantly stimulated the spermatocyte growth (Ogata and Inoue unpublished). These genetic data strongly suggest that the ILPs and its signaling cascade through PI_3-kinase to Akt play a role in induction of the remarkable cell growth in *Drosophila*. As mammalian insulin can also activate the Ras-MAP kinase cascade after the insulin receptor (as a review, (Avruch 1998)), we further showed that $Ras85D^{v12}$, a constitutively activated mutation for *Ras85D* (Kim et al., 2006) also induced approximately 10 % increase of cell diameter in length (Ogata, Azuma and Inoue, unpublished). These genetic data suggest that both PI_3K-Akt cascade and Ras-MAP kinase cascades acting downstream of InR are essential for induction of the premeiotic spermatocyte growth. The *InR* mutations also interfered cell growth within egg chambers in ovaries without affecting cyst morphology and cell numbers (LaFever and Drummond-Barbosa 2005). Taking together, these findings indicate that the IIS plays a critical role in both oogenesis and spermatogenesis in *Drosophila*.

As other elucidated roles of the Dilps in adult stage, they are involved in resistance to various stresses such as starvation stress and oxidative stress and lifespan (as a review, (Owusu-Ansah and Perrimon 2014)). The peptides are also involved in regulations of adult vision, behavior, and their appetite. The specific neurons producing Dilp7 stimulates the adult intestine as to promote their appetite (Miguel-Aliaga et al. 2008; Cognigni et al. 2011). The Dilp7 regulates adult female behavior to decide egg-laying (Sousa-Nunes et al. 2011; Bai et al. 2012). This is the reasonable regulation that allows female flies to coordinate their food uptake with promotion of fecundity.

13.4 Human Diabetes: Type 1, Type 2, and Metabolic Syndrome

Human diabetes is a group of metabolic diseases defined by the increased levels of blood sugar, termed hyperglycemia. They are generally classified into either since the pancreas fails to produce sufficient insulin (T1D) or since the target cells of insulin fail to respond to the hormone (T2D) (Katsarou et al. 2017; DeFronzo et al. 2015). T1D account for an estimated less than 10% of all diabetes cases. This type disease had been previously considered to be induced by an autoimmune condition in which the immune system is activated to destroy the IPC in the pancreas (Hanafusa and Imagawa 2007). Alternatively, another class of T1D that the β cell loss arises from unknown causes, without autoantibodies, has been reported. It has been suspected whether inflammation, various cell stresses, and insulin resistance that take place in the insulin-producing cells would lead to the cell death (Eizirik et al. 2009; Bluestone et al. 2010; Atkinson et al. 2011; Katsarou et al. 2017). Particularly, it has been attracted interest that the endoplasmic reticulum (ER) stress is involved in the β cell loss, which might result from continuously enhanced insulin production in the insulin resistance condition. Before the cell death has seriously occurred in cells where ER stress has accumulated, reduction and/or loss of cell activity has been commonly observed in many cases (Sreenan et al. 1999; Ferrannini 2010). Because of that, the patients with this form of diabetes failed to produce or secrete sufficient insulin.

On the other hand, T2D is defined as a long-term metabolic disorder that is characterized by high blood sugar (hyperglaycemia), insulin resistance, and relative lack of insulin response. The insulin resistance is commonly observed from earlier stage of T2D. This class accounts for about 90% of cases of diabetes, with the other 10% due primarily to T1D 1 and gestational diabetes. One of the most characteristic properties of T2D is the impaired response of target organs to insulin, called insulin resistance (Weyer et al. 1999; Kahn et al. 2014). The primary causes of insulin resistance have not yet been clarified in most of the cases. As the primary causes of the resistance, one can simply speculate that impairment of insulin production and secretion, such as downregulation of a IIS factor, takes place. It has been shown that mutations in the insulin gene and InR gene were responsible for sever hyperglycemia syndromes associated with insulin resistance, such as type A insulin resistance and the Rabson-Mendenhall syndrome (Liu et al. 2015; Meur et al. 2010; Jiang et al. 2011). Some mutations decrease the amount of insulin receptors localized on the cell surface. Other mutations impair the functions of the insulin receptor (refer https://www.ncbi.nlm.nih.gov/gene/3643). Patients carrying mutations at both alleles display more severe phenotype than are patients heterozygous for the mutation. And furthermore, if a non-cell autonomous factor, which prevent insulin from binding to its receptor, is expressed, the IIS is certainly inhibited. To compensate elevated glucose levels due to the resistance, organisms try to raise insulin secretion. This counteracts the insulin sensitivity of the target tissues, leading to rather worsen the symptom (Kasuga 2006; Kahn et al. 2014). Due to inadequate compensation, the glucose intolerance arises from the combination of insulin resistance and deficiency of functional insulin.

Metabolic syndrome (MS) is a collection of risk factors that includes glucose dysregulation, central obesity, dyslipidemia, and hypertension. There are multiple definitions that have been described regarding the criteria of the syndrome. This clustering of risk factors is obviously linked to an increased risk of developing T2D. The MS is also characterized by insulin resistance. Prediabetes, which is a combination of insulin resistance and excess body fat, is considered an underlying cause of MS. Therefore, similar biomedical and genetic studies to those having been performed to clarify the T2D pathogenesis can be applied to studies on onset and development of the MS. For the purpose, diabetes models representing the characteristics of T1D, T2D, and MS, have been individually established in *Drosophila*.

13.5 *Drosophila* Models for Type 1 Diabetes

For understanding mechanisms that the insulin-producing cells (IPC) are lost by the cause other than autoimmune condition as observed in T1D patients, experimental animals that allow us to induce cell death in the insulin-producing cells might provide a valuable suggestion. Here, we would like to describe some *Drosophila* models reproducing the IPC loss and phenotype of human T1D (Figs. 13.3a and 13.4a). Immunostaining experiments with anti-Dilps antibodies revealed that three major Dilps (Dilp2, 3 and 5) among seven Dilp members are synthesized, stored, and secreated from specialized neuronal cells, named insulin-producing cells in brains (Ikeya et al. 2002; Broughton et al. 2005). Although the IPCs correspond to only 14 median neurosecretory cells in the *Drosophila* central nervous system, these cells can play an equivalent role as IPCs to the β cells in mammals. Thus, if cell death exclusively occurs in these 14 of IPC cells, this can reproduce T1D-like phenotypes in the *Drosophila* (Fig 13.4a). To generate *Drosophila* lacking of IPC, genetic ablation of IPC was achieved by ectopic induction of apoptosis exclusively in the cells using Gal4/UAS system. As mentioned in previous chapter, the Gal4/UAS system allows us to carry out ectopic induction of any genes located under the UAS sequences (see Chapter 1, (Phelps and Brand 1998)). Using the system, Rulifson et al. induced apoptosis specifically in larval IPC neurons by ectopic expression of pro-apoptotic gene, *reaper* (Rulifson et al. 2002) (See Figs. 13.3a and 13.4b, c). The genetic ablation of the larval IPC resulted in the elevated circulating sugar levels in the larval hemolymph. And the hyperglycemia phenotype resulted in developmental delay and growth retardation at larval stage. Eventually smaller adult flies emerged (Rulifson et al. 2002). These phenotypes are reminiscent of hallmarks of T1D, a loss of pancreatic β cells, and undernutrition. Another experiment that carried out the ablation of IPCs using a different procedure provided the same phenotypes in both larvae and adults. A genetic ablation of IPCs was performed in adults and compared their phenotypes with those seen in larvae (Haselton et al. 2010). For ablation of IPC cells at adult stage, a modified Gal4/UAS system called the GeneSwitch was used. This is based on a GAL4-progesterone receptor chimera that is hormone inducible, which is specifically activated after binding of the activator RU486, which is fed to flies (Osterwalder et al. 2001). Normal adults contain circulating sugars in hemolymph at a lower concentration during and

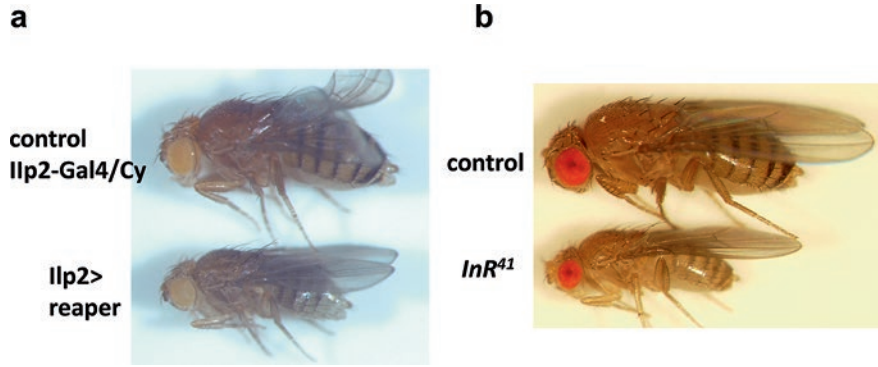

Fig. 13.3 A *Drosophila* type 1 model having genetic ablation of the insulin-producing cells (IPCs) and a model fly showing insulin resistance which is a hallmark of type 2 diabetes. (**a**) (upper) A control fly (*InR /TM3, Sb*). carrying a balancer chromosome carrying Cy (*ilp2-Gal4/Cy*). (lower) A fly derived from larvae expressing pre-apoptotic gene, *reaper*, exclusively in IPCs by Gal4/UAS system (*ilp2>reaper*). (**b**) (upper) A control fly *(InR41/TM3, Sb)*. (lower) A fly homozygous for a hypomorphic *InR* mutation (InR^{41}), displaying insulin resistance due to a reduced expression of *InR* gene. Note that both models show significantly smaller than their sibling controls, indicating that a growth retardation has occurred during larval and pupal stages in both cases

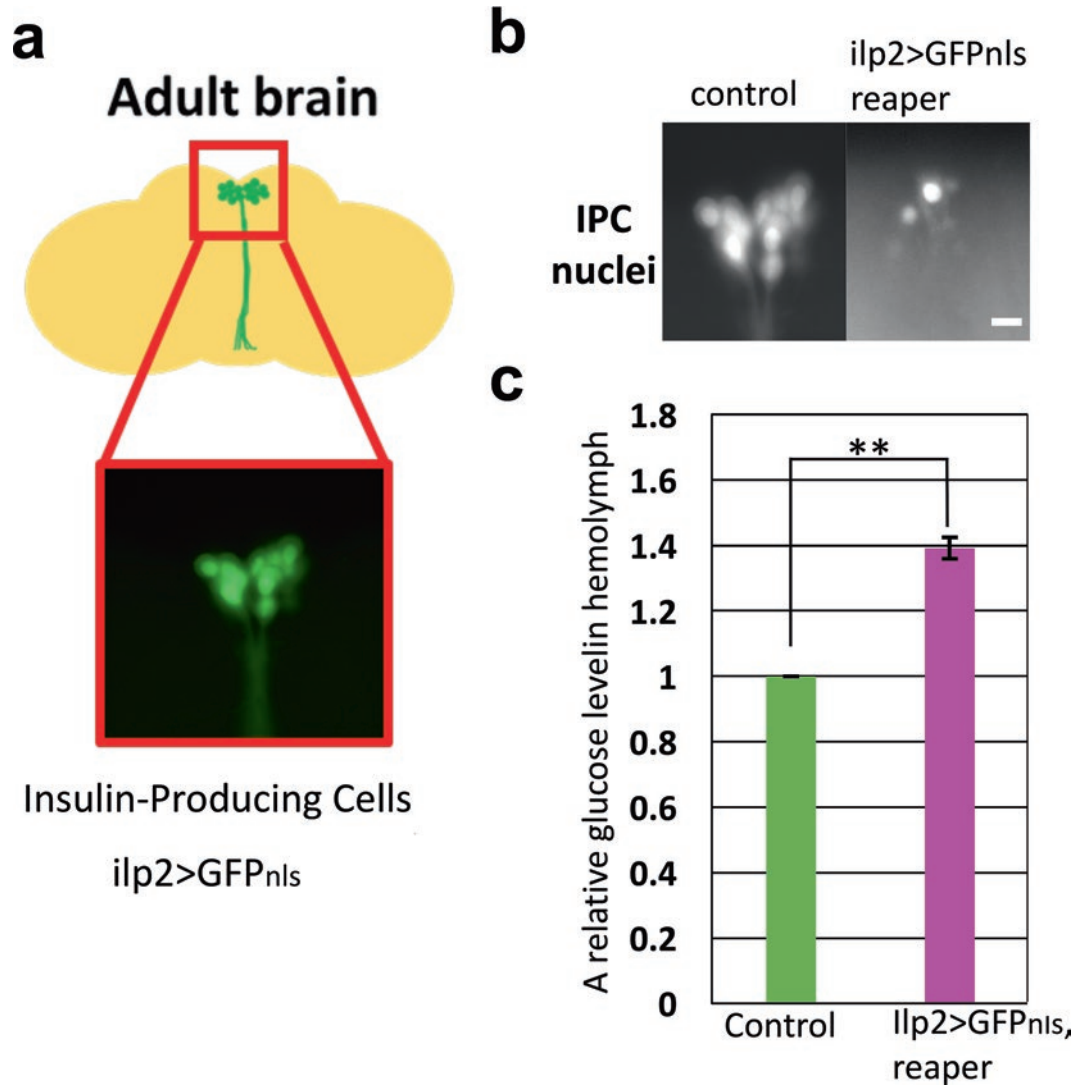

Fig. 13.4 A visualization of IPC in adult brains and genetic ablation of the cells by ectopic induction of apoptosis. (**a**) A illustration of insulin-producing cells in an adult brain. The producing cells can be visualized by the IPC-specific expression of GFP having nuclear localization signal sequences using Gal4/UAS system (ilp2>GFPnls). (**b**) Observation of adult IPC. Note that a distinctive decrease of the cells was observed in the adult brain expressing the preapoptotic gene, reaper (middle). (**c**) Quantification of circulating glucose levels in larval hemolymph. A significant elevation of the levels is seen in the IPC-ablated flies, compared with those of control flies

after fasting (glucose clearance). Once the flies resumed feeding, the sugar level immediately increased. On the other hand, adults lacking IPC by the genetic ablation at adult stage displayed higher sugar levels and slower glucose clearance. Interestingly, the flies lacking of the IPCs displayed extended adult lifespan as an effect of calorie restriction (Haselton et al. 2010). The glucose intolerance seen in the flies was rescued by injection of mammalian insulin, indicating that the flies did not show the strong insulin resistance. The adult stage-specific ablation of the

insulin-producing neurons modulates glucose homeostasis and extends lifespan without insulin resistance. Regarding to the hyperglycemia phenotype, the effects of the IPC ablation are consistent between larval stage and adult stage. Its influence is largely restricted to metabolic homeostasis, resistance to stress, fecundity, and lifespan rather than growth aspects in adults (Owusu-Ansah and Perrimon 2014). Both glucose feeding and fasting experiments more easily manipulate in adults rather than in larvae. It is more difficult to create fasting condition in larvae. Similar to glucose clearance in mammals, wild-type adults displayed the rapid response to decline glucose levels in hemolymph after fasting. Whereas, the IPC-ablated flies showed higher sugar levels than control adults did (Haselton et al. 2010). These evidences suggest that one can obtain more reliable results regarding on regulation of glucose homeostasis by experimental systems using adults rather than larvae.

Among known eight Dilps in *Drosophila*, Dilps1-7 play positive roles essential for cell growth and cell prolifeation. They are also required for glucose homeostasis or roles related to the issues (Brogiolo et al. 2001; Ikeya et al. 2002; Rulifson et al. 2002; Broughton et al. 2005; Gronke et al. 2010; Yang et al. 2008; Veenstra et al. 2008; Okamoto et al. 2009; O'Brien et al. 2011; Bai et al. 2012), In contrast, the Dilp8 has a role in regulation of adaptive development in a response to tissue damages (Colombani et al. 2012; Garelli et al. 2012). Genetic interaction exists among genes required for sugar homeostasis. There is a functional redundancy rescued by another member(s) of DILPs. Thus, a deletion of each *Dilp* gene has no phenotypes in every case. Therefore, their functions have been speculated by mainly expression patter and dominant phenotypes by overexpression experiments (Owusu-Ansah and Perrimon 2014). Three Dilps, Dilp2, 3, and 5 expressing in the IPCs, play a central role in glucose homeostasis. Dilp2 presents highest sequence homology with human insulin. These three Dilps play a central role in glucose homeostasis as well as cell growth and proliferation. Dilp1 is also produced in the IPCs and involved in body size determination, while Dilp4 expressing in larval midgut is involved in larval growth. Dilp6 and Dilp7 expressing in other tissues than the IPCs are more related to adult activity such as growth after diet feeding and egg-laying behavior, respectively (Brogiolo et al. 2001; Yang et al. 2008; Okamoto et al. 2009; Bai et al. 2012; Yang et al. 2008). Homozygous mutants for five *Dilps1-5* genes showed a sever growth defects and developmental delay, quite similar to those seen in the IPC ablation (Zhang et al. 2009). Some of homozygous flies deficient for five Dilps genes (*Dilp1-5*) can survive until adult stage, although many of them died during development. The survivors showed the small fly phenotype, indicating a strong growth retardation. Phenotypes of homozygous mutants for five *Dilps* (*Dilp1-5*) genes were mostly overlapped with those generated from the IPC ablation as described above. Diabetes symptoms, growth defects, and development delay have also been observed in the larvae deficient for major *Dilps* genes (Zhang et al. 2009). The authors also reported that the animals contained increased sugar levels in hemocytes; instead they had reduced triacylglycerides (TG) which is a major fat stored in their bodies in amount and reduced heat production. This is a reflection of lowered metabolic activity. These phenotypes are all reminiscent of T1D hallmarks.

There is a bit difference in phenotypes induced by the insulin depletion between *Drosophila* models and mouse mutants. The knockout mouse deficient for the insulin genes in both alleles results in lethal at neonatal stage (Duvillie et al. 1997). Mouse has two insulin genes, *Ins1* and *Ins2*. Double homozygous mutant pups displayed severe growth retardation. They did not show any glycosuria at birth. But soon after suckling, they developed diabetes with ketoacidosis and died within 48 h. The insulin deficiency did not preclude pancreas organogenesis and the appearance of the various cell types of the endocrine pancreas. Although some of homozygous flies deficient for five Dilps genes (*Dilp1-5*) were viable, many of them died during development. The survivors showed the small fly phenotype indicating a strong growth retardation. In conclusion, both

Drosophila T1D models generated from genetic ablation of the IPCs and those homozygous for mutations of major *Dilp* genes appear to be suitable for genetic studies on T1D.

13.6 *Drosophila* Models for Human Type 2 Diabetes

As we described previous sections, one of the most characteristic properties of T2D is the insulin resistance. This symptom has been considered as earlier stage of the T2D pathogenesis. To gain insight into mechanism by which insulin resistance occurs and progress into T2D, simple model organisms, particularly *Drosophila*, have been considered to be more suitable for the purpose, because they are capable of performing precise genetic analyses.(Fig. 13.3b).

High-sugar diet models: A simple *Drosophila* model for studies on diet-induced T2D was initially established (Musselman et al. 2011). The authors fed wild-type larvae on high-sugar diet (HSD) containing seven times higher sucrose than control diet. The larvae raised on HSD had increased levels of both circulating glucose and trehalose in the larval hemolymph. The larval development of the larvae delayed significantly, and the fat accumulation increased, as observed in T1D fly models. Injection of mammalian insulin to the larvae failed to restore the impaired insulin response only partially as speculated by levels of phosphorylated Akt in IIS. This suggests that insulin resistance occurred as seen in T2D patients. Expression of Dilp2 peptide has risen in the larvae adapting to a prolonged higher level of glucose. This phenotype corresponds to a human hyperinsulinemia ((DeFronzo et al. 2015)). And furthermore, microarray analysis to identify genes which transcription has changed demonstrated that target genes of the Foxo transcription factor were upregulated in the models. This is consistent with observations in gluconeogenic livers of insulin-resistant mice (Michael et al. 2000) and T2D patients (O'Brien et al. 2011). The insulin resistance seems to occur by evolutionarily conserved mechanisms, because the transcriptional effects of high-sugar feeding was observed commonly in mouse, human, and *Drosophila* insulin resistance. From studies using another model of the HSD-feeding larvae established independently, same conclusions were obtained. Continuous feeding of HSD for a longer period resulted in hyperglycemia, growth retardation, hyperinsulin induction, and excess accumulation of fat (Pasco and Leopold 2012). Similarly, adults raised on HSD also displayed a diet-dependent weight gain, metabolic dysfunction, elevated Dilp mRNA, and decreased activity of insulin signaling as far as examined in fat body cells. These phenotypes indicate that insulin resistance, which is a hallmark of human T2D, can be reproduce in *Drosophila* larvae and adults raised on HSD (Morris et al. 2012). Another HSD-induced adult model also displayed hyperglycemia, insulin resistance, increased fat accumulation, and shortened lifespan (Na et al. 2013). The HSD-induced models have an advantage that more sever hyperglycemia phenotype can be observed rather than in flies possessing IPC ablation (Rulifson et al. 2002; Song et al. 2010).

The insulin resistance eventually resulted in induction of the target genes of the stress JNK cascade (Musselman et al. 2011). This result suggested that there is a genetic interaction between the insulin signaling pathway and the stress MAP kinase pathway. Pasco and Leopold further obtained interesting genetic results that the HSD-triggered insulin resistance was suppressed by ectopic overexpression of a *Drosophila* orthologue of lipocalin, Nlaz. Expression of the *Nlaz* gene increased in larvae raised on HSD (Pasco and Leopold 2012). It had previously been known that lipocalin 2, a small extracellular protein, can modulate of diabetes phenotype in mouse (Gavi et al. 2007; Kim et al. 2012). In *Drosophila* larvae, *Nlaz* mutants or restricted depletion of the gene in fat body can rescue metabolic disorders seen in HSD-induced larval models. These genetic data from mouse and *Drosophila* models suggest that there is a therapeutic potential to rescue diabetes type 2 patients. These genetic evidences derived from *Drosophila* diabetes models can contribute to mammalian studies to verify mechanism as well as to develop therapeutic protocol.

Other *Drosophila* T2D models generated by genetic modifications have also been established. For example, heterozygotes for an *InR* mutation exhibited reduced the receptor activity, and the insulin signaling must be impaired in the mutant flies (Fig. 13.4b) (Tatar et al. 2001). As a result, the DILP secretion was enhanced. The FB-specific depletion of InR reproduced the hyperinsulin production and insulin resistance (Park et al. 2014a). These fly phenotypes are reminiscent of hyperinsulinemia in live-specific gene disruption of InR gene in mouse (Michael et al. 2000).

To understand the mechanisms underlying T2D pathogenesis, it is essential to identify new molecular markers for gene diagnosis and targets for therapeutic intervention of T2D. To achieve the purpose, genome-wide association studies (GWAS) have been carried out in T2D patients. Over 90 disease-associated SNP loci associated with human T2D have been identified (Renstrom et al. 2009; Dimas et al. 2014). However, it is not certain whether these genetic loci are really involved in pathogenesis of insulin resistance and H2D. If this is the case, for understanding molecular mechanism underlying the T2D pathogenesis, it is important to clarify the role of individual genes in the disease. *Drosophila* is one of the most suitable animals for the genetic analyses. For example, one of GWAS candidate loci for T2D encodes a transcription factor, GLIS3 (Yang et al. 2009). Its *Drosophila* orthologue is *lmd* (*lame duck*). Depletion of the gene in *Drosophila* IPC by induction of dsRNA against its mRNA was carried out (Park et al. 2014a). The depletion resulted in hyperglycemia phenotype. And it resulted in a significant decrease of *Dilp2* mRNA, indicating that the lmd protein is required for transcription of the *Dilp* gene. It is consistent with results from the mammalian studies that the GLIS3 is required for the insulin gene expression. The genetic results proposed that the human *GLIS3* locus is associated with susceptibility for T2D (Dupuis et al. 2010; Nogueira et al. 2013). Similar genetic approaches seem to be quite effective to elucidate gene function and examine whether these genes are required for T2D pathogenesis. In addition, the approaches did not only allow us to clarify gene functions of individual genes but also to discover the gene network in which the gene is involved in.

Drosophila models to investigate metabolic syndrome: Metabolic syndrome is one of lifestyle diseases relevant to T2D. Currently, it has drawn worldwide attention. As patients suffering from it also display insulin resistance, the disease is placed as a pre-stage of type 2 diabetes. The syndrome is associated with the risk of developing type 2 diabetes and cardiovascular disease. Not only insulin resistance but also several overlapping aspects have been pointed out between metabolic syndrome and prediabetes. Insulin signals also regulate energy storage in fat body. Therefore, disruption of IIS eventually results in lipid metabolic disorder, that is, the metabolic syndrome. One of conserved factors crucial for metabolism of lipids and glucose is AKH in *Drosophila*. This peptide is secreted from specialized cells called corpora cardiaca (CC) of the ring gland. AKH stimulates lipolysis in fat body. Subsequently, TAG breakdown to free fatty acid (FFA). Glycogen breakdown is also stimulated, as AKH activate glycogen phosphorylase. Accordingly, trehalose is released from fat body to hemolymph. Protein kinase A is involved in the process. FFA moves to oenocytes which is a counterpart of adipocytes in mammals to produce energy. Thus, by lowering the IIS pathway, not only hyperglycemia but lipid accumulation in fat body is also promoted. The accumulation eventually results in insulin resistance. Next, regarding insulin resistance, it is possible to speculate the following hypothesis about the process toward onset of the insulin resistance. The lipid accumulation in fat body activates TORC1 in TOR pathway (Gutierrez et al. 2007). The TORC1 can activate S6 kinase. The protein kinase phosphorylates and inhibits a InR substrate, IRS-1, encoded in *chico* gene in *Drosophila*. Once the adaptor protein is phosphorylated, this form of the protein cannot transduce the IIS signal substantially. At the end, a responsiveness to insulin becomes lowered.

A continuous feeding of high-fat diet containing saturated fatty acids (HFD) bring about increased glucose levels in adult flies (Birse et al.

2010). Fat deposition was observed in both adipose tissues, fat body, and other tissues such as the midgut in the flies raised on the HFD. This is reminiscent of a common symptom seen in metabolic syndrome and T2D patients. Conversely, flies fed on HSD indeed increased fat deposition in the fat body and displayed severely lowered responsiveness to insulin, insulin resistance (Musselman et al. 2011). These are evidences indicating that there is a close relationship between fat and glucose metabolism. In the flies, it was also reported that insulin signaling was substantially suppressed in fat body. Instead, the stress-responsible pathway mediated by JNK was upregulated. The JNK phosphorylates and inhibits a *Drosophila* lipocalin orthologue, NLaz which we described at previous section. The downregulation of NLaz impaired responsiveness to insulin in peripheral tissues. Furthermore, reduced insulin signaling resulted in induction of expression of Foxo target genes (Musselman et al. 2011). It leads to insulin resistance.

For a long time, it has not been clarified how downregulation of IIS and upregulation stress signaling pathway in fat body interact with each other to develop insulin resistance and metabolic syndrome. It has been recently argued that the inflammation is associated with obesity and onset of metabolic syndrome in human and model organisms (Hoffmann et al. 2013). *Drosophila* fat body plays a multiple role in maintenance of metabolic homeostasis, stress responses, and production of antimicrobial peptides against infectious microbes (Arrese and Soulages 2010). Therefore, these cells can simultaneously respond to multiple intracellular signals, dependent on the situation. However, it is possible to interpret that the diapause of metabolic signaling was recognized as a sort of inflammation and that it may have resulted in activation of JNK cascade in the fat body. Thus, it is worth to consider the following hypothesis. In a response to inflammation derived from fat accumulation in the fat body, one of inflammation cytokines, TNFα, was possibly induced. It activates the stress JNK pathway. It can phosphorylate IRS-1, which is a substrate of the InR. The phosphorylation interferes the insulin signaling (Boucher et al. 2014). Eventually, it leads to appearance of insulin resistance and hyperglycemia one after another in adult flies fed on HSD or HFD for longer period (Hoffmann et al. 2013).

In conclusion, high-sugar diet or mutations and depletion of IIS factors can reproduce insulin resistance in the fly models. HSD possibly induces inflammation factor, TNFα expression. It resulted in insulin resistance. These MS and T2D models are useful to reveal mechanism underlying T2D pathogenesis.

13.7 Genetic Identification of New Genes Required for Glucose Homeostasis and Diabetes Pathogenesis Using *Drosophila*

It has been considered that complex genetic interaction between genetic loci controlling T2D susceptibility is involved in pathogenesis of human T2D. To identify the susceptibility loci for the disease, human genome-wide association studies (GWAS) have been performed, as described in previous section. However, it is usually difficult to assign specific causative genes, although it can identify a genomic region existing SNP marker linked most closely to susceptible genetic trails associated with the disease. One needs further to identify the causative gene within the region and to evaluate whether these candidate genes are really responsible for the disease. For the purpose, *Drosophila* provides excellent experimental system. Here, we introduce several susceptibility genes identified from different genetic approaches.

For instance, Pendse et al. selected the HHEX gene encoding Hox-class transcription factor, which gene polymorphisms seem to be associated with human T2D (van Vliet-Ostaptchouk et al. 2008). They carried out fat body-specific depletion for its *Drosophila* orthologue, *dHHEX*. The depletion resulted in elevated glucose levels in adult hemolymph and reduced insulin sensitivity (Pendse et al. 2013). Another research group has also carried out IPC-specific depletion of *Drosophila* orthologues for several human candi-

date genes. These genetic analyses revealed that *Imd* (a *Drosophila* orthologue of human *GLIS3* gene) and *CG9650* (a *Drosophila* orthologue of human *BCL11A* gene) are required for Ilp2 production or secretion (Yang et al. 2009; Park et al. 2014b) It is highly likely that these two human orthologue genes are also responsible for T2D.

Large-scale *Drosophila* RNAi screens called glucome screening to find out genes, whose depletion resulted in hyperglycemia of flies have been performed. Fat body-specific and muscle-specific depletion screens of ~1000 genes yielded ~160 candidate genes for hyperglycemia genes (Ugrankar et al. 2015). As one of the candidate genes involved in regulation of glucose metabolism, *CSNK1α1* gene encoding the alpha subunit of Casein kinase 1 were identified. The authors further demonstrated that heterozygous and homozygous mutants for the murine orthologue in their adipose tissue developed diabetes, indicating that the kinase plays a conserved role in glucose metabolism in both *Drosophila* and mouse.

Another type of genetic screens also contributed identification of novel modifiers for insulin signaling in *Drosophila*. Colombani et al. accomplished overexpression screen to identify growth modifiers by *Gal4*-dependent altering expression of each gene. They demonstrated that downregulation of *slimfast* gene specifically in fat body caused a global growth defect due to local repression of PI3 kinase signaling. The gene encodes amino acid transporter (Colombani et al. 2003). Teleman et al. also carried out gain-of-function screen for genes affecting tissue growth. Consequently, they identified a new modulator gene, *melted*, which encodes a PH domain protein that interacts with Tsc1 and FOXO. It can recruit FOXO to the plasma membrane in an insulin-regulated manner (Teleman et al. 2005). To identify new components that express and regulates the production of the Dilps in IPC, Cao et al. isolated mRNA from IPC collected by laser microdissection and performed transcriptome analysis of the mRNAs (Cao et al. 2014). Among those mRNAs expressing abundantly in IPCs, *unc-104* encoding a kinesin 3 family was essential for insulin secretion. Rab protein, which is a key regulator of intracellular vesicle transport, was also required for insulin production or secretion. These two proteins are required for a transport of the vesicles containing Dilps. Other genetic studies have also uncovered several regulators essential for insulin production and/or secretion in *Drosophila*. They include a small GTPase, Steppke, characterized as guanine nucleotide exchange factor (GEF) for ARF, a key regulator of both retrograde and anterograde traffic at the Golgi complex ARF at the Golgi complex. This finding suggests that ARF, a key and its GEF are essential for insulin signaling in larval stage. Another genetic screen to identify regulators of insulin sensitivity revealed that MAPK involves maintenance of glucose in hemolymph at appropriate levels through transcriptional control of InR gene (Zhang et al. 2011).

In addition, it has been uncovered that several miRNAs are involved in insulin production in IPC or its responsiveness in peripheral tissues. miR-278 mutant flies had elevated glucose levels and displayed increased insulin production, indicating that they are insulin resistant. The miRNA contributes to regulate energy homeostasis by regulating insulin responsiveness (Teleman et al. 2006). miR-14 regulates insulin production in IPCs through its target, *sugarbabe* mRNA encoding a predicted transcription factor regulating insulin gene expression (Varghese et al. 2010). Through the negative regulation of insulin gene expression, the miRNA regulates overall glucose metabolism. More recently, Ueda et al. reported that *Drosophila* miR-305, showing high homology with seed sequences of miR-239 in *C. elegans*, is involved in aging (Ueda et al. 2018). They showed that the lifespan of adults overexpressing miR-305 was significantly shorter. Conversely, a reduction in miR-305 expression led to a longer lifespan than that in control flies. miR-305 overexpression accelerated the impairment of locomotor activity and promoted the age-dependent accumulation of poly-ubiquitinated protein aggregates in the muscle, as flies aged. Thus, they concluded that the ectopic expression of miR-305 has a deleterious effect on aging in *Drosophila*. miR-239 in *C. elegans* can activate the IIS pathways (de

Lencastre et al. 2010). It has been reported that the inhibition of these pathways results in lifespan extension (Friedman and Johnson 1988; Clancy et al. 2001; Tatar et al. 2001; Holzenberger et al. 2003; Altintas et al. 2016). RNA-seq analysis to identify target genes of miR-305 demonstrated that the *tobi* mRNA, a target gene for insulin in the brain, and mRNAs for Dilp2 and Dilp5 increased following *miR-305* overexpression. Conversely, mRNAs for other insulin-like peptides, Dilp6 and Dilp8, were somehow downregulated to the controls following *miR-305* overexpression. Dilp6 inhibits *dilp2* expression. *Dilp6* overexpression extended lifespan in flies (Okamoto et al. 2009; Bai et al. 2012). Thus, Ueda et al. speculate that *miR-305* targets and negatively regulates the *dilp6* mRNA, which results in the increased expression of *dilp2*. It has been reported that a reduction in the sugar metabolism eventually leads to an extension in lifespan in *Drosophila* (Huang et al. 2015; Altintas et al. 2016). Another report also demonstrated that the ectopic expression of Foxo, which is negatively regulated by the IIS signaling pathway, resulted in lifespan extension (Demontis and Perrimon 2010). Assuming that *dilp6* is one of the targets of *miR-305*, its downregulation results in an unexpected activation of the IIS pathway. Thus, oxidative phosphorylation in the mitochondria is certainly stimulated, and as a consequence, reactive oxygen species (ROS) production is enhanced. These results are consistent with the observations showing that an increased sensitivity against oxidative stress and the induction of the oxidative stress marker are observed in adults overexpressing *miR-305*. (Ueda et al. 2018).

13.8 Development of Antidiabetic Drugs Using *Drosophila* Models

As pointed out throughout the book, *Drosophila* is one of the most suitable model animals in deciphering mechanisms of many human diseases as well as in identification of new genes involved in onset and development of the diseases. Many of cell biological, physiological, and neurological properties are conserved between mammals and *Drosophila*. More than 75% of human genes responsible for diseases are conserved and working in the fly. These approaches lead to identify new genes and pathways that could be future targets of drug design. Furthermore, it also has an advantage in the discovery of therapeutic agents (Pandey and Nichols 2011). Drug development process currently commences high-throughput screens for small chemicals or natural resources based on *in vitro* assays using cultured cells or on biochemical assays such as target-to-chemical binding assays. The second steps are usually processes that consume time and costs, as a large number of rodent models are utilized. Nevertheless, the majority of candidates are usually removed out of the selection in this process. If one would incorporate another selection using *Drosophila* models after initial large selections into the therapeutic discovery process, it allows us to proceed the screens more rapidly at lower expenses for the drug discovery.

In reality, it has been demonstrated that several types of known antidiabetic drugs or related substances have similar effects to *Drosophila* individuals. Among them, we introduce examples of three chemical compounds here. Firstly, sulfonylureas have been extensively used for treatment of T2D (Sola et al. 2015). The drugs act by stimulating insulin release from the β cells in the pancreas. Sulfonylureas bind to the specific receptor, blocking the inflow of potassium ion (K^+) through the ATP-dependent channel. The plasma membrane becomes depolarized. This opens voltage-gated Ca^{2+} channels. The rise in intracellular Ca^{2+} leads to increased fusion of vesicles containing proinsulin with the cell membrane and therefore increased secretion of insulin. *Drosophila* corpora cardiaca (CC) cells also express the sulfonylurea receptor and ATP-sensitive K^+ channels regulating release of AKH corresponding to glucagon in mammals. Kim and Rulifson demonstrated that homeostasis of circulating glucose

was significantly impaired by exposure to sulfonylureas (Kim and Rulifson 2004).

Metformin is another antidiabetic drug prescribed most commonly. The drug reduces glucose production in the liver and increases its uptake in muscle and adipose tissues (Bailey and Turner 1996). Metformin inhibits complex I in mitochondria. These effects result in increase of the AMP/ATP ratio in the cells, activating of AMP-activated protein kinase (AMPK) and triggering a cascade that inhibits gluconeogenic gene expression and energy-consuming processes. As it also interferes lipogenesis, it results in inhibition of protein kinase C due to reduction of diacylglycerol, which is an activator of the kinase. As a result, it eventually leads to release the InR from negative regulation by protein kinase C. Thus, it restores insulin resistance. Diet and administration of metformin ameliorated high-fat diet-induced hyperglycemia and obesity phenotypes in *Drosophila* adults. Kim et al. showed that the metformin's effect was entirely dependent on an endosomal Na+/H+ exchanger, a possible molecular target of the drug in both *Drosophila* and *C. elegans* (Kim et al. 2016).

Epigallocatechin gallate (EGCG) rich in green tea extract can extend lifespan of *Drosophila* adults through induction of endogenous antioxidant enzymes (Li et al. 2007). It also affects glucose metabolism and increases fitness. These effects went along an increased expression of Spargel, a *Drosophila* orthologue of mammalian PGC1α essential transcription factor for expression of the genes involved in energy metabolism (Wagner et al. 2015). The EGCG downregulates Dilp5 and phosphoenolpyruvate carboxykinase, major regulators of glucose metabolism, as well as the *Drosophila* homolog of leptin, Upd2. The decrease in glucose metabolism in connection with an upregulated expression of Spargel rather contributes to the lifespan expression in EGCG-fed flies. Taking together, these observations strongly suggest that *Drosophila* is a useful experimental model that allows us to evaluate known antidiabetic drugs and perform low- to high-throughput screens to discover novel antidiabetic drugs.

13.9 Conclusion Remarks and Outlook

Drosophila has a great potential as an experimental model system to study on sugar homeostasis including production and acting mechanism of insulin-like peptides. Previous *Drosophila* studies uncovered that the IIS pathways activated by the peptides control sugar metabolism, organism growth, reproduction, and longevity. The IIS pathways showed many conserved features with the mammalian pathways. Furthermore, a genetic disruption of Dilps or IIS factors resulted in diabetes-like phenotypes. On the basis of these results, it is possible to conclude that *Drosophila* is a suitable animal model for diabetes studies.

Certainly, major circulating sugar of insects is a trehalose, which has a less sugar toxicity than glucose has (Benaroudj et al. 2001). Although physiological harmful influences and developmental defects were observed in many *Drosophila* diabetes models, we should consider more carefully whether findings from *Drosophila* directly can apply to human. Nonetheless, *Drosophila* has great advantages for researchers to set up express genetic approaches that allow them to obtain results more quickly. The *in vivo* characterization using the organism confirmed that candidate human diabetes susceptibility genes suggested from GWAS are required for glucose homeostasis and involved in pathogenesis of diabetes (Pendse et al. 2013; Park et al. 2014b). Although *Drosophila* models were used to validate predicted functions of the human genes in these cases, other distinctive genetic studies by partial genomic RNAi screening called the glucome screening have been also performed to find out new genes required for glucose homeostasis. As consequences of these large screens and accumulation of individual studies, *Drosophila* researchers have so far identified new modulators including some microRNAs (Teleman et al. 2005, 2006; Ueda et al. 2018; Varghese et al. 2010). They are essential modulators for the IIS and TOR pathways. As some of them are novel factors that have been uncovered in mammals, yet they must be good examples verifying that the

Drosophila findings could contribute to mammalian studies in this field.

In addition, the involvement of the IIS and relevant regulatory factors are different from developmental stages and cell types. In larval stage, many imaginal cells are undergoing mitotic divisions, and some of larval cells continue to grow. The Dilps and the IIS play a more important role in stimulation cell growth and proliferation at the stage. Hence, they act on maintaining sugar homeostasis in adults in which fewer proliferative cells except germline cells are contained. However, in gametogenesis, the peptides are important for induction of germline stem cell division and cell growth of premeiotic cells before meiotic division. The Dilps and IIS pathways are critical key factors for cell proliferation and growth in *Drosophila*. Therefore, it is important to investigate their diverged functions and regulations in each tissues and development stage, in addition to understand integrated regulatory system to maintain metabolic homeostasis in whole organisms. Particularly, in mammals, influence of hyperglycemia condition to gametogenesis and fetal development have not been well characterized yet (Nandi et al. 2010). *Drosophila* provide a good experimental model system to investigate the issues.

It has been recently reported that *Drosophila* can respond to some antidiabetic drugs, as human and mammalian models do. In contrast to therapeutic medication for neuro-diseases, studies and trials on the drug discovery using *Drosophila* diabetes models are still developing stages. However, the evidences encourage us to push forward the trials. It is possible to set up experiments that can evaluate more easily effects of candidate antidiabetic drugs by examining alternation of simple phenotype, such as body size change, which diabetes model flies display. Researchers will be able to perform the experiments at a large scale but at considerably lower costs within a limited time. In facts, such antidiabetic drug screens using *Drosophila* models are beginning in earnest in some collaborative research projects and several pharmaceutical companies. Once antidiabetic drugs will have been identified, subsequently trials to look for target proteins or genes for the drugs possibly are carried out using *Drosophila* genetic techniques. These studies make it possible to elucidate modes of drug action. It is reasonable to expect that these efforts result in discovery of new regulatory system that has been uncovered in mammals. It is also promising that comprehensive RNAi screens to isolate *Drosophila* larvae or adults showing diabetes phenotype can find new genes essential for regulation of sugar metabolism.

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