Control of Metabolism and Growth Through Insulin-Like Peptides in *Drosophila*

Charles Géminard, Nathalie Arquier, Sophie Layalle, Marc Bourouis, Maija Slaidina, Renald Delanoue, Marianne Bjordal, Mickael Ohanna, May Ma, Julien Colombani, and Pierre Léopold

Insulin signaling is a conserved feature in all metazoans. It evolved with the appearance of multicellularity, allowing primordial metazoans to respond to a greater diversity of environmental signals. The insulin signaling pathway is highly conserved in insects and particularly in *Drosophila*, where it has been extensively studied in recent years and shown to control metabolism, growth, reproduction, and longevity. Because misregulation of the insulin/IGF pathway in humans plays a role in many medical disorders, such as diabetes and various types of cancer, unraveling the regulation of insulin/IGF signaling using the power of a genetically tractable organism like *Drosophila* may contribute to the amelioration of these major human pathologies. *Diabetes* 55 (Suppl. 2):S5–S8, 2006

Extensive study of mammalian insulin/IGF biology has established that the insulin/IGF system (IIS) is split into two complementary and interacting subsystems that govern growth, metabolism, reproduction, and longevity (1). Insulin, which is secreted from the pancreatic β-cells in response to elevated glucose and amino acid levels, primarily regulates anabolic metabolism in the classic insulin-responsive tissues such as adipose, muscle, and liver. Insulin, IGF-I, and IGF-II, all of which possibly act through the insulin receptor, also regulate reproductive physiology by modulating gonadotropin production in the pituitary and sex steroid production in the ovary. IGF, as a major downstream target of growth hormone, is essential for regulating growth and body size both prenatally (IGF-I and IGF-II) and postnatally (IGF-I). Despite this knowledge about the physiological roles of the IIS, our current understanding of how the insulin/IGF system regulates, coordinates, and integrates these processes remains incomplete. Recent advances in the elucidation of this conserved pathway in simpler organisms that are amenable to efficient genetics, such as *Drosophila*, appear more and more to represent interesting synergistic approaches that promise to allow a better understanding of this orchestration.

From the Centre National de la Recherche Scientifique/University of Nice-Sophia Antipolis, Unité Mixte de Recherche 6543, Nice, France.

Address correspondence and reprint requests to Pierre Léopold, CNRS/University of Nice-Sophia Antipolis, UMR 6543, Parc Valrose, 06108 Nice cedex 2, France. E-mail: leopold@unice.fr.

Received for publication 6 April 2006 and accepted in revised form 15 May 2006.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

20E, 20-hydroxyecdysone; ALS, acid labile subunit; APC, AKH-producing cell; AKH, adipokinetic hormone; FB, fat body; IIS, insulin/IGF system; IPC, insulin-producing cell; IRS, insulin receptor substrate; JH, juvenile hormone; PI, phosphatidylinositol; TOR, target of rapamycin.

DOI: 10.2337/db06-S001

FLY INSULINS, GLUCAGON, AND METABOLIC CONTROL

The functional separation of IGF and insulin signaling that is seen in mammals dates to approximately 600 million years ago, as the two distinct types of molecules are already present in the lower metazoan tunicate phylum. Insects have a single insulin/IGF system that may correspond to the ancestor of the dual insulin/IGF system. While insects do not have a tissue, such as the pancreas, that is specialized in carbohydrate homeostasis, *Drosophila* do have a group of insulin-producing cells (IPCs) that are located in the brain and constitute an endocrine organ for the regulation of growth and sugar metabolism (2,3). These 14 neuroendocrine cells, which are organized into two clusters located symmetrically in the pars intercerebralis, project their axons toward two main targets: the aorta, a primitive circulatory system allowing recycling of the hemolymph in the anterior part of the larva, and the corpora cardiaca (CC), a neuroendocrine tissue producing a glucagon-like hormone (see below). After their release into the open hemolymph circulation, the *Drosophila* insulin-like peptides are thought to reach their target tissues and receptors and elicit their biological functions. A total of seven different *Drosophila* insulin-like peptide (Dilp)-encoding genes are found in the *Drosophila* genome. Three distinct *dilp* genes are expressed in the IPCs, and four more are expressed in several additional larval tissues (imaginal discs, gut, or ventral nerve chord cells) (2). Significantly, no *dilp* expression is detected in the fat body (FB), an important metabolic tissue that carries out important nutrient storage and secretion functions analogous to those of the vertebrate liver and adipose tissue (see below). Although loss-of-function analysis is still lacking that would allow distinct roles to be assigned to the different *dilp* genes, gain-of-function experiments suggest a certain level of redundancy, as the effects of the genetic ablation of the IPCs, which express the *dilp2*, *-3* and *-5* genes, can be efficiently rescued by the generalized expression of *dilp2* (3).

When the IPCs are genetically ablated in the brain of *Drosophila* larvae, emerging adults are small and have high circulating carbohydrate levels (2,3). This suggests that the fly insulins carry out both the growth-promoting functions of the vertebrate IGFs and the metabolic functions of vertebrate insulin. The study of the insulin signaling pathway in flies has so far focused on its manifest role in controlling body size; surprisingly, despite the availability of numerous genetic tools, the ability of this pathway to control carbohydrate metabolism has not yet been fully evaluated.

Metabolic changes have also been observed in flies with

reduced IIS. Flies mutant for the insulin receptor substrate (IRS)-encoding gene *chico* have almost double the normal total lipid content per fresh weight, a phenotype reminiscent of the hypertriglyceridemia seen in IRS-1 mutant mice. Interestingly, no changes in protein or glycogen content are observed in these mutants (4).

Adipokinetic hormone (AKH) is another important metabolic hormone in insects that shows some structural similarities to vertebrate glucagon (5). AKH-producing cells (APCs) are located in the so-called corpora cardiaca (CC) of the ring gland, and constitute a small population of neuroendocrine cells that oppose the action of the IPCs (2,6). These two neuroendocrine cell populations differentiate during embryogenesis and project axons that contact the open hemolymph circulation as well as each other. Together, they comprise a specialized network for controlling metabolism and growth that likely shares a common evolutionary ancestry with the α- and β-cells of the vertebrate endocrine pancreas (3). Interestingly, during normal larval development, a remarkable accumulation of Dilp2 is observed in CC cells, which do not express the *dilp* genes (3). This suggests either a possible neuro-mediator function for the *Drosophila* insulin-like peptides or a neurohemal function for the CC cells.

More recently, the function of the APCs has been directly addressed using a genetic cell ablation approach (6). Interestingly, APC-ablated animals survive to adulthood without presenting any growth defects, although they are severely hypoglycemic. A careful study of the glucagon-producing cells reveals that, contrarily to the IPCs, they express *Drosophila* cognates of the Ir and Sur subunits of ATP-sensitive K^+ channels. Furthermore, larvae treated with tolbutamide (a sulfonylurea that promotes closure of ATP-sensitive K^+ channels, depolarization of the cells, and increased secretion) show a 40% increase in the level of circulating glucose that is suppressed when the APCs are ablated. This indicates that the ATP-sensitive K^+ channels function in glucagon-secreting cells to regulate the levels of circulating glucose in the animal. It also suggests, more generally, that the main factor controlling acute glycemic changes in the fly might be glucagon and not insulin. This is reminiscent of the situation in birds, where total pancreatectomy causes fatal hypoglycemia associated with the disappearance of circulating glucagon (7).

In addition to these hyperglycemic effects during larval development in *Drosophila*, AKH also has a strong hyperlipidemic effect that was first demonstrated in long-distance flying insects. This effect further extends the parallel with the lipid remobilization function of vertebrate glucagon. Indeed, AKH plays a major role in releasing stored carbohydrates and lipids from the FB in the form of trehalose and diacylglycerol, the two main energy sources for the flight muscles during the extremely energy-costly long-distance flights of migratory insects (5). A bona fide AKH G coupled–protein receptor has been identified in several insects, including *Drosophila* (8,9). Interestingly, this protein is structurally related to the vertebrate gonadotropin-releasing hormone receptor. When AKH peptide is applied to FB cells, it induces a strong intracellular accumulation of cAMP and ultimately increases the activity of glycogen phosphorylase, which is responsible for glycogen remobilization. In response to AKH, FB stores of triacylglycerol are converted into diacyglycerol, implying the activity of the FB triacylglycerol lipase, an enzyme that is functionally related to the vertebrate hormone-sensitive lipase.

Together, these data reveal several remarkable similarities in the endocrine regulation of carbohydrate homeostasis between insects and vertebrates and also highlight the specific prevalence that the IGF-like function has over insulin-mediated metabolic control in *Drosophila*. In developing flies, both the growth and the metabolic functions of insulins are mediated through the activation of a single insulin receptor tyrosine kinase called InR. In view of this, uncoupling circulating sugar levels from insulin production and release in the brain IPCs might be advantageous for preserving the ability to regulate the accumulation of circulating insulins in developing larvae, as required for the control of organismal growth during the juvenile stages. How this hierarchy is conserved in the adult after growth has stopped needs to be better understood.

The question remains, then, of to what signals do these insulin- and glucagon-producing neurosecretory cells in the insect brain respond? Experiments involving the targeted deprivation of basic amino acids in the IPCs suggest that amino acid levels inside the cells might not regulate *dilp* gene expression nor protein accumulation in the IPCs (10; C.G., P.L., unpublished observations). Alternatively, the absence of Sur/Kir subunit expression in these cells suggests that they do not respond directly to glucose levels. In any case, these questions await more direct experimental treatment.

FLY INSULINS AND GROWTH CONTROL

The pictures of dwarf *Drosophila* obtained by genetic manipulation of IIS have undoubtedly contributed to the rapid recognition of this animal model in the field of growth control. A flurry of articles has demonstrated the existence of a bona fide *Drosophila* insulin receptor signaling pathway that controls both cellular and organismal growth (11). The *Drosophila* insulin receptor (InR) is surprisingly similar in structure to the vertebrate insulin receptor, with a marked extension of the COOH-terminal β-chain domain that is suspected to serve as a direct docking site for the downstream phosphatidylinositol (PI) 3-kinase. This extended COOH-terminal domain might thus allow the receptor to bypass the absolute need for IRS-mediated transduction of the insulin signal. Accordingly, flies lacking the unique IRS ortholog, *Chico*, are viable and small, a phenotype definitely less severe than the embryonic and early larval lethality of InR and PI 3-kinase loss-of-function, respectively (4). Genetic data support the fact that in *Drosophila*, most, if not all, InR signaling is mediated by the PI 3-kinase pathway, through the conserved cascade involving PDK1 and AKT/PKB (protein kinase B) membrane relocalization and activation. Cell-autonomous growth defects are observed upon inactivation of the pathway, demonstrating that it mainly regulates cell growth. The demonstration that this pathway systemically controls animal size was made by either reducing or increasing circulating levels of insulin ligands: the genetic ablation of the brain IPCs leads to a proportionate reduction in animal growth during larval development and eventually to small adults; conversely, targeted overexpression of Dilp2 in the IPCs during larval development augments the final size of the adult (T. Ikeya, E. Hafen, J.C., M.B., P.L., personal communication).

TARGET OF RAPAMYCIN (TOR), IIS, AND NUTRITIONAL SENSING

Recently, several links have been established in both vertebrates and invertebrates between IIS and nutrient availability. In vivo studies in flies have demonstrated that, during larval growth, PI 3-kinase activity downstream of InR requires the presence of amino acids in the food (12). These results support the idea that the insulin pathway might coordinate tissue growth with nutritional conditions. At the same time, neither PI 3-kinase nor AKT/PKB activities are downregulated upon amino acid withdrawal in mammalian or insect cells in culture, suggesting that this pathway does not directly participate in a nutrient checkpoint (13). By contrast, the implication of target of rapamycin (TOR) kinase in a conserved nutrient checkpoint has been clearly established in both yeast and mammalian cells (14). In metazoans, TOR is part of a complex signaling pathway comprising other components like the tuberous sclerosis complex (TSC) tumor suppressor that consists of a TSC1 and TSC2 heterodimer (TSC1/2), as well as the recently identified small GTPase named Rheb. In each individual cell, this pathway links the external nutritional status to intracellular metabolism. At present, we understand little about how these cell-intrinsic mechanisms function to connect growth with nutrient levels in multicellular organisms, in which humoral controls are believed to buffer variations in nutrient availability. Recent work has explored the possibility that specific organs could function as growth sensors that induce the nonautonomous modulation of insulin signaling in response to changes in nutrient levels. The insect FB has important storage and humoral functions that are comparable to those of the vertebrate liver and adipose tissue, raising the possibility that it participates in a nutrient-sensing mechanism. By using a genetic tool allowing tissue-specific amino acid deprivation through the downregulation of an amino acid transporter, Colombani et al. (10) demonstrated that the FB operates as a nutritional sensor, coordinating organismal growth with nutritional status. The sensor mechanism is triggered in FB cells through the downregulation of the TOR signaling pathway. This in turn induces general growth inhibition via a remote downregulation of insulin signaling in peripheral tissues. This study provides in vivo evidence for a central sensor mechanism that controls organismal growth and delineates the respective contributions of the TOR and insulin signaling pathways in the control of tissue growth (10). How the insulin signal is modulated by the FB sensor, and, in particular, what the nature of the humoral link is between the FB sensor and the insulin-producing cells in the brain, are among the questions that remain to be addressed.

The modulation of IIS in peripheral tissues that is observed upon the activation of the FB sensor suggests that either circulating levels of insulins or their bioactivity could be modified. Recent experiments in our laboratory suggest that insulin release by the brain IPCs could be controlled by nutrition (J.C., C.G., P.L., unpublished data). Further proof for this regulation awaits a sensitive detection assay for circulating insulins in the fly hemolymph.

Mammalian IGF functions, on the other hand, are controlled by binding partners that modulate both the stability of IGF and its availability for receptor binding. Could the binding partners of Dilp be involved in regulating its functions in *Drosophila*? Most circulating mammalian IGFs are part of a 150-kDa complex comprising IGF-I, different IGF-BPs, and a large scaffold protein called IGFBP-ALS (acid labile subunit) (15). The formation of the 150-kDa complex creates a reservoir of circulating IGF, due to the strong stability of the complex and to its inability to cross the endothelial barrier and reach target tissues. Imp-L2, a candidate *Drosophila* IGF-BP homolog, was recently identified in a genetic screen. Imp-L2 shares a low level of homology with human IGF-BP7, a distantly related member of the IGF-BP family, and was shown to directly bind mammalian IGF-1 and IGF-2 in vitro (16). Both gain- and loss-of-function studies in the fly indicate that Imp-L2 may counteract the growth-promoting effects of the *Drosophila* insulin-like peptides, a growth-inhibitory function shared by several of the mammalian IGF-BPs (B. Honegger, E. Hafen, personal communication). Our laboratory has identified a putative ortholog of ALS, called dALS, which shows specific expression in the larval IPCs and FB. Interestingly, dALS expression is severely suppressed in starved animals, resembling the control of mammalian ALS expression by nutrition. It remains to be determined whether dALS, *Drosophila* insulin-like peptides, and Imp-L2 can form circulating complexes in vivo, and in what stoichiometry. Ongoing genetic analysis of dALS function will also tell us more about their possible role in nutrition-regulated Dilp function.

CROSS TALK BETWEEN IIS AND OTHER INSECT HORMONAL SYSTEMS FOR THE CONTROL OF ANIMAL GROWTH

In addition to IIS, there is accumulating evidence that several other insect hormones participate in the control of tissue growth in *Drosophila*. Two important hormones determine the nature and timing of the developmental transitions in insects: the sesquiterpenoid juvenile hormone (JH) and the steroid hormone 20-hydroxyecdysone (20E) (17). In particular, the end of juvenile growth and development and the transition to the pupal stage and maturation is marked by a drop in JH titers and a peak in 20E. Thus, final individual size mainly depends on two parameters: the speed of growth, or growth rate, which is primarily controlled by IIS, and the extent of growth period, which is limited by the arrival of the larval-pupal transition, itself timed by peaks of ecdysone secretion. Our recent work has pointed to an unexpected role of 20E in opposing the action of insulins and negatively controlling the animal growth rate. Indeed, the genetic manipulation of 20E basal levels during larval development is sufficient to induce changes in the size of larvae at the pupal transition, without modifying the timing of larval development. Increased circulating levels of 20E inhibit the larval growth rate and, conversely, their reduction allows faster growth. This growth inhibition ultimately targets dFOXO function, the unique inhibitory transcription factor that acts downstream of InR/PI 3-kinase/AKT in *Drosophila*. In addition, inhibiting ecdysone signaling in the FB induces a systemic growth increase similar to what is observed when hormone signal is generally downregulated in all tissues, thus revealing a key role for the FB in relaying ecdysone-dependent growth control signals (18).

Together with previous work (10), these data suggest that various inputs such as nutrition and ecdysone converge on this important regulatory organ, which then uses control mechanisms involving IIS to modulate organismal growth.

Interestingly, the specific activation of IIS in the ecdysone-producing tissue specifically induces ecdysone-dependent growth phenotypes, suggesting that the IIS pathway might itself control ecdysone synthesis. A similar direct activation has been reported in the adult ovaries of other insects (19,20). IIS activity may thus constitute a specific input for the regulation of basal ecdysteroid synthesis, allowing the growth program or nutritional information to be coupled with ecdysteroid production.

How then is growth connected to developmental timing? In insects, a molecular coupling could exist between organismal growth and the series of endocrine events leading to the end of larval development. Our finding that 20E can modulate growth rates in addition to developmental transitions places this hormone in a central position for coordinating these two key processes and controlling organismal size.

IIS CONTROL OF LONGEVITY AND REPRODUCTION

In *Drosophila*, as in many other species, the impairment of IIS in adulthood is associated with increased longevity, increased stress resistance, and reduced reproduction, a set of phenotypes quite similar to what is seen in diapausing animals (21). JH could play a major role in relaying these effects, since InR mutants are deficient in JH production, and the development of nonvitellogenic ovaries in InR mutant females can be restored by administering the JH analog methoprene (22). Dietary restriction (DR) is also an important regulator of lifespan and fecundity. As described above, IIS is responsive to nutrients during development, suggesting that it might relay dietary restriction effects for lifespan and fecundity control. The mode of action of this pathway in regulating lifespan and fecundity in response to dietary restriction remains to be established (23).

CONCLUDING REMARKS

The use of *Drosophila* genetics has allowed accelerated progress in assigning new functions to the invertebrate insulin/IGF system. Within a very limited time, IIS has been established as a master controller of energy metabolism and growth, reproduction, and longevity, showing a remarkable series of functionally conserved features with mammalian insulin/IGF signaling.

Will *Drosophila* prove in the near future to be a useful model for diabetes studies? Skeptics may think that flies are distant enough from humans to preclude all medical applications, especially considering that even the use of mammalian models is sometimes criticized. Nevertheless, what flies have always been useful for is the possibility of setting up rapid genetic screens to decipher conserved pathways and identify new components that could be future targets for drug design. The recent demonstration that *Drosophila* responds to an anti-diabetes medication also suggests that it could possibly become an attractive system for screening new anti-diabetes compounds.

ACKNOWLEDGMENTS

The research in our laboratory is supported by grants from the Programme National de Recherche contre le Diabète, the Fondation pour la Recherche Médicale, the Fondation de France, the Institut National de la Santé et de la Recherche Médicale, and the CNRS.

We are very grateful to Gisèle Jarretou and Aurore Dussert for technical help in the course of our experiments and to Peter Follette for comments on the manuscript.

REFERENCES

1. Nakae J, Kido Y, Accili D: Distinct and overlapping functions of insulin and IGF-I receptors. *Endocr Rev* 22:818–835, 2001
2. Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E: An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. *Curr Biol* 11:213–221, 2001
3. Rulifson EJ, Kim SK, Nusse R: Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* 296:1118–1120, 2002
4. Bohni R, Riesgo-Escovar J, Oldham S, Brogiolo W, Stocker H, Andruss BF, Beckingham K, Hafen E: Autonomous control of cell and organ size by CHICO, a Drosophila homolog of vertebrate IRS1–4. *Cell* 97:865–875, 1999
5. Van der Horst DJ: Insect adipokinetic hormones: release and integration of flight energy metabolism. *Comp Biochem Physiol B Biochem Mol Biol* 136:217–226, 2003
6. Kim SK, Rulifson EJ: Conserved mechanisms of glucose sensing and regulation by Drosophila corpora cardiaca cells. *Nature* 431:316–320, 2004
7. Sitbon G, Laurent F, Mialhe A, Krug E, Karmann H, Gross R, Strosser MT, Cohen L, Jean-Marie P, Foltzer C, Mialhe P: Diabetes in birds. *Horm Metab Res* 12:1–9, 1980
8. Hansen KK, Hauser F, Cazzamali G, Williamson M, Grimmelikhuijzen CJ: Cloning and characterization of the adipokinetic hormone receptor from the cockroach Periplaneta americana. *Biochem Biophys Res Commun* 343:638–643, 2006
9. Staubli F, Jorgensen TJ, Cazzamali G, Williamson M, Lenz C, Sondergaard L, Roepstorff P, Grimmelikhuijzen CJ: Molecular identification of the insect adipokinetic hormone receptors. *Proc Natl Acad Sci U S A* 99:3446–3451, 2002
10. Colombani J, Raisin S, Pantalacci S, Radimerski T, Montagne J, Leopold P: A nutrient sensor mechanism controls Drosophila growth. *Cell* 114:739–749, 2003
11. Hafen E: Cancer, type 2 diabetes, and ageing: news from flies and worms. *Swiss Med Wkly* 134:711–719, 2004
12. Britton JS, Lockwood WK, Li L, Cohen SM, Edgar BA: Drosophila's insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Dev Cell* 2:239–249, 2002
13. Radimerski T, Montagne J, Rintelen F, Stocker H, van der Kaay J, Downes CP, Hafen E, Thomas G: dS6K-regulated cell growth is dPKB/dPI(3)K-independent, but requires dPDK1. *Nat Cell Biol* 4:251–255, 2002
14. Wullschleger S, Loewith R, Hall MN: TOR signaling in growth and metabolism. *Cell* 124:471–484, 2006
15. Boisclair YR, Rhoads RP, Ueki I, Wang J, Ooi GT: The acid-labile subunit (ALS) of the 150 kDa IGF-binding protein complex: an important but forgotten component of the circulating IGF system. *J Endocrinol* 170:63–70, 2001
16. Sloth Andersen A, Hertz Hansen P, Schaffer L, Kristensen C: A new secreted insect protein belonging to the immunoglobulin superfamily binds insulin and related peptides and inhibits their activities. *J Biol Chem* 275:16948–16953, 2000
17. Riddiford LM: Hormone receptors and the regulation of insect metamorphosis. *Receptor* 3:203–209, 1993
18. Colombani J, Bianchini L, Layalle S, Pondeville E, Dauphin-Villemant C, Antoniewski C, Carre C, Noselli S, Leopold P: Antagonistic actions of ecdysone and insulins determine final size in Drosophila. *Science* 310:667–670, 2005
19. Maniere G, Rondot I, Bullesbach EE, Gautron F, Vanhems E, Delbecque JP: Control of ovarian steroidogenesis by insulin-like peptides in the blowfly (Phormia regina). *J Endocrinol* 181:147–156, 2004
20. Riehle MA, Brown MR: Insulin stimulates ecdysteroid production through a conserved signaling cascade in the mosquito Aedes aegypti. *Insect Biochem Mol Biol* 29:855–860, 1999
21. Tatar M, Bartke A, Antebi A: The endocrine regulation of aging by insulin-like signals. *Science* 299:1346–1351, 2003
22. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS: A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292:107–110, 2001
23. Partridge L, Piper MD, Mair W: Dietary restriction in Drosophila. *Mech Ageing Dev* 126:938–950, 2005