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Meeting Report

Integrating Insulin Signaling and Stress Responses

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Energy is the central currency of all biological processes, from molecules to ecosystems. Recent years have seen great interest in broad ecological patterns of organismal energetics, spanning all phyla.[1-3] At the other end of the spectrum, the molecular, cellular and tissue-level regulation of energy acquisition, storage, and utilization have received even more attention. This interest was evident at the 48th Annual Drosophila Research Conference, recently held in Philadelphia (March 7–11, 2007) amidst freezing weather and flight delays, but great food. Highlighted in the meeting was a growing body of research in understanding the control and consequences of nutrient allocation to growth, reproduction and longevity (Fig. 1).

Central to our understanding of how energy input is allocated at the tissue and cellular level are the highly conserved Insulin and Target of Rapamycin (TOR) signaling pathways. Together, these regulate the physiology of the cell by responding to nutrient signals (insulin, amino acids, and growth factors) to control metabolic homeostasis and organismal growth, primarily through controlling protein synthesis (Fig. 2). In Drosophila activation of the Insulin pathway is mediated by insulin-like peptides (Dilps), which function as the equivalent to mammalian insulin and insulin-like growth factors. Dilp binding to the insulin receptor (InR) initiates intracellular signaling primarily through a phosphorylation cascade to regulate glucose, protein, and lipid metabolism. A central player in Insulin signaling is TOR, which responds to and can integrate signals not only from growth factors (insulin), but also nutrients (amino acids), cellular energy levels (AMP:ATP) and stress (hypoxia) to regulate growth. An important question is how Insulin/TOR integrates the overall metabolic status of the organism to control different cellular responses in different tissues.

During the course of an animal's lifespan the consequences of Insulin and TOR signaling change based on cellular and organismal needs. In Drosophila, larval development is characterized by rapid growth and the acquisition of sufficient energy stores to fuel pupal development and the non-feeding immature adult. Here, a balance must be met to up-regulate general macromolecular synthesis (primarily via TOR signaling) for immediate growth by increasing cell mass and cell number, while at the same time storing triglyceride and glycogen for future needs (primarily via insulin signaling). Ultimately, a critical mass and sufficient nutrient accumulation must be achieved to trigger åmetamorphosis and support the successful transition from the larva to the adult. In contrast, in the adult, where the size of the animal is already established, Insulin/TOR signaling controls aging and impacts on reproduction.

In Drosophila, there are seven Dilp genes, but only one insulin receptor. Dilps-1,-2, -3 and -5 are produced in paired insulin-producing cells (IPC) located in the *pars intercerebralis* of the brain. The remaining Dilp genes are active in other larval tissues (imaginal discs, gut or ventral nerve cord cells) but none is expressed in the larval fat body.[5] Although there is evidence of redundancy between the Dilps, systematic loss-of-function analyses have not yet been carried out. In mammals, circulating insulin-like growth factor-1 (IGF-1) is complexed with IGF-binding protein-3 or -5 and acid labile subunit (ALS), which serves as a large scaffolding protein. In Drosophila a homolog of ALS (dALS) has been identified[6] and an Inducible Membrane-bound Polysomal-L2 (IMP-L2) protein has been previously shown to have insulin and IGF-binding activity.[7] Work presented by Nathalie Arquier from the Leopold lab (University of Nice-Sophia Antipolis, France) describes a model for the stabilization and sequestration of circulating Dilp-2 implicating dALS and Imp-L2 that shares features with the control of postnatal circulating mammalian Insulin-like growth factors. Interestingly, the *Imp-L2* gene encodes a secreted member of the immunoglobulin (Ig) superfamily and was previously identified as a direct target of Ecdysone signaling. Currently, it is not clear how or whether other functions of Imp-L2 fit into Insulin signaling, but an intriguing possibility is that the Ecdysone regulation of

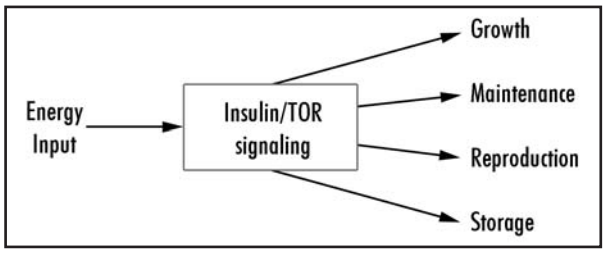

Figure 1. Insulin and TOR signaling mediate allocation of energy among tissues and biological processes.

IMP-L2 might allow the concentration and availability of circulating Dilps to be developmentally controlled by Ecdysone. It also remains to be seen how the localized release of Dilps might be temporally and spatially regulated. Two possibilities come to mind: localized tissue-specific proteolytic degradation, or the equilibration of the tertiary complex with individual components in the hemolymph.

In a series of elegant experiments, Eric Rulison (University of California, San Francisco) has traced the origin of the insulin-producing cells (IPC) and the adipokinetic hormone-producing (AKH) neurosecretory cells. AKH is a metabolic hormone with glucagon-like functions in mobilizing both glucose and triglycerides. Evidence was presented that showed a single pair of neural stem cells (neuroblasts) gives rise to the brain IPCs, which are analogous to islet β-cells, and a second pair of neuroblasts engenders the AKH-producing cells (APCs) that are located in the *corpora cardiaca*. These progenitors of IPCs and APCs arise as near neighbors from a domain that expresses genes whose orthologs are also active in vertebrate hypophyseal placode, the source of endocrine anterior pituitary and neurosecretory hypothalamic cells. These data suggest that the brain endocrine axis was present in the common bilaterian ancestor, where it orchestrated islet endocrine functions with insulin and glucagon-like hormone producing cells. The fact that the insulin and glucagon-secreting cells are specified from a common *anlage* in both flies and vertebrates suggests that there are evolutionarily conserved cell specification mechanisms for brain endocrine cells and pancreatic islet cells.

Work from the Wilson (University of Oxford, England) and Pichaud (University College London, England) labs reveals a role for subcellular localization of Akt (also known as protein kinase B) in effecting different metabolic and developmental responses to insulin. The insulin signaling cascade is activated when Dilps bind to the InR, leading to recruitment of phosphoinositide 3-kinase (PI3K) to the cell surface. Membrane bound PI3K then converts phosphatidylinositol-4,5-bisphosphate (PIP2) to the 3,4,5-phosphorylated form PIP3. Modulation of membrane PIP3 levels is controlled by activity of PI3K and the phosphoinositide phosphatase PTEN, a major human tumour suppressor. PIP3 co-recruits Akt and 3-phosphoinositide-dependent protein kinase (PDK1) to the plasma membrane, where PDK1 (and a second kinase known as PDK2, which is possibly TORC2) activates Akt (P-Akt). P-Akt accumulates at the cell surface, where it is believed to promote growth and anabolism in most cell types, functions that are globally disrupted in mutants with reduced insulin signaling.

In nutrient-storing nurse cells of the Drosophila ovary, triglycerides are stored in small lipid droplets. The Wilson lab has shown that loss of PTEN in these cells leads to an increase in P-AKT not specifically at the cell surface, but throughout the cytoplasm. These mutant nurse cells accumulate enlarged lipid droplets and up-regulate LSD-2, a perilipin homologue preferentially associated with the

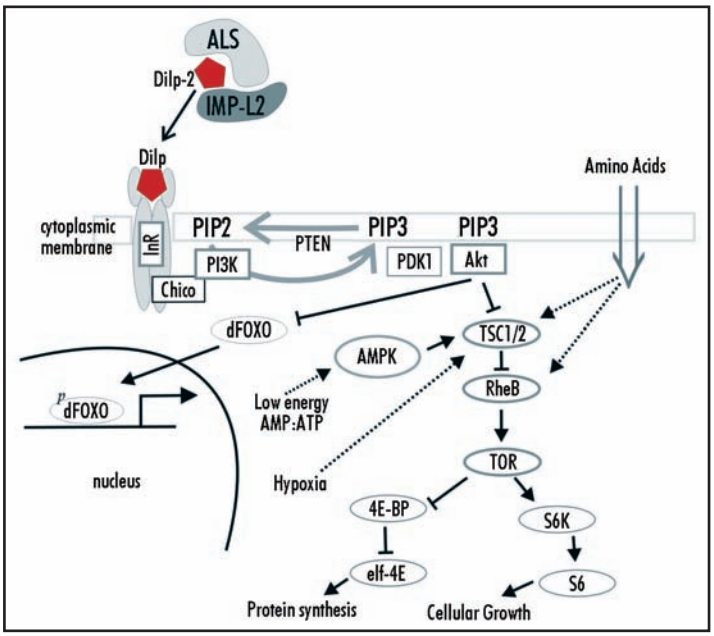

Figure 2. Insulin and TOR signaling in Drosophila. Insulin signaling promotes growth through the control of protein synthesis via TOR to upregulate S6K and suppress 4E-BP, and through regulating carbohydrate and lipid metabolism by mechanisms that have yet to be defined. The response to energetic stress and hypoxia lowers protein synthesis and requires the TSC complex to down-regulate S6K.

surface of lipid droplets that modulates lipid storage. Surprisingly, selective activation of Akt at the cell surface of nurse cells does not produce the same phenotype,[8] suggesting that the cytoplasmic pool of P-AKT specifically affects lipid droplet storage. In photoreceptor cells, the Wilson lab found that the boundaries of the apical membrane are flanked by a specific isoform of PTEN, and loss of PTEN disrupts apical morphology. The importance of subcellular localization of Akt and its localized activation in this process was highlighted by the Pichaud lab's observation that apical membrane morphogenesis of photoreceptor cells is dependent upon localized activation of Akt downstream of apically-localized PIP3.[8] These data suggest that different modes of Akt activation, perhaps via localized regulation of PIP3, by the kinases PDK1 and PDK2 or protein phosphatases might well be involved in the selective accumulation of cell-surface vs. cytoplasmic P-Akt, and thus might play a role in directing different biological responses to insulin signaling.

In addition to the role of Insulin/TOR signaling in size control and lipid metabolism, these pathways are directly involved in stress responses. This makes perfect sense. Although it has been argued that, at the organismal level reduced metabolism is beneficial under stressful conditions,[10] at the cellular level energy is required to mount changes in cellular processes, e.g., synthesis of stress proteins, to counteract stress. Central to integrating an organism's response to stress is the TSC1/2 complex, which integrates upstream signals from the Insulin pathway via PI3K/Akt and the cellular energy sensing AMP-activated kinase (AMPK) pathway. Signaling through PI3K/Akt activates inhibitory phosphorylation of TSC2 while AMPK stimulates TSC2 activity. Activation of TSC1/2 complex leads to a decrease in TOR activity via the GTPase-activating protein domain of the TSC1/2 complex, which converts active Rheb-GTP to Rheb-GDP. Thus, P-Akt down-regulates TSC1/2 activity and leads to increased TOR activity, while up-regulation of AMPK in response

to the AMP:ATP ratio leads to a decrease in TOR activity and thus a decrease in protein synthesis.

One stress in which a role for Insulin signaling is now evident is hypoxia (low oxygen). In the absence of oxygen, cells can not metabolize lipids and must rely on glycolysis and other anaerobic pathways to generate ATP. A classic example is the Pasteur effect in yeast, in which glucose metabolism increases dramatically to compensate for the low energetic efficiency of anaerobic ethanol production. Thus, it seems evident that TOR/Insulin signaling should be affected by hypoxia signaling. Work presented by the Wappner lab (Instituto Leloir, Buenos Aires, Argentina) has the set the stage for a detailed understanding of the cellular response to hypoxia by carrying out a genome wide RNAi screen for regulators of hypoxia-dependent transcription. This work was presented by Andres Dekanty, who received first prize for his poster presentation. The response to hypoxia is controlled by the binding of HIF-1 complex to HIF response elements (HRE) to provoke a transcriptional response. In Drosophila, HIF-1 is composed of constitutively expressed Tango and the oxygen-regulated protein, Similar (Sima). Steady-state levels of Sima protein is determined by Fatiga, a prolyl-4-hydroxylase that targets Sima degradation under normoxic conditions.

The Wappner lab[11] has previously shown that induction of HRE dependent transcription can be mediated by Insulin/PI3K/Akt pathway to activate TOR to increase Sima translation. In addition, Akt and PDK1 also appear to be involved in the subcellular localization of Sima. Over-expression of Sima protein in normoxic embryos can swamp the normal degradation process of Sima. Under these conditions, Sima protein accumulates and is localized to the cytoplasm. Over-expression of Akt and PDK1 in normoxic embryos shifts the localization of Sima to the nucleus and thus mimics the hypoxic response. Dekanty's RNAi screen for genes required for HRE-mediated transcription has lead to the identification of more than 100 genes fulfilling different cellular functions, as well as components of Insulin/TOR signaling, thus further emphasizing the integration of stress responses with the growth of organisms.

In summary, the parallels between specification of vertebrate and fly neurosecretory cells involved in modulating energy reservoirs, and the similarities between the regulation of circulating dILPs and insulin-like growth factors are striking. Drosophila research continues to provide insights into the cellular mechanisms underlying both organismal and cellular responses to changes in energy needs.

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