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Extracellular and Subcellular Regulation of the PI3-kinase/Akt Cassette:

New Mechanisms for Controlling Insulin and Growth Factor Signalling

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Abstract

The PI3-kinase/Akt signalling cassette plays a central role in the response to growth factors, particularly insulin-like molecules, and its misregulation is a characteristic feature of diabetes and many forms of human cancer. Recent molecular genetic studies initiated in the fruit fly, *Drosophila melanogaster*, have highlighted two new cell type-specific mechanisms regulating PI3-kinase/Akt signalling and its downstream effects. First, the cellular response to this cassette is modulated by several classes of cell surface transporters and sensors, suggesting an important role for extracellular nutrients in insulin sensitivity. Second, various cell types show a markedly different subcellular distribution of the activated kinase Akt, influencing the cellular functions of this molecule. These findings reveal new mechanisms by which processes like growth, lipogenesis and insulin resistance can be differentially regulated and may suggest novel strategies for treating insulin-linked diseases.

Keywords

cancer; diabetes; cell polarity; lipogenesis; nutrient sensing; *Drosophila*

For some years, the PI3-kinase/Akt signalling cassette has been recognized as a major regulatory pathway for growth and metabolism, which is involved in human diseases such as cancer and diabetes. However, the recent molecular genetic dissection of this highly evolutionarily conserved cassette in invertebrates has highlighted its importance in events controlled by nutrient-regulated insulin-like molecules. In addition, work in *Drosophila* has revealed a remarkably diverse range of global and cell type-specific functions, including the control of cell growth and polarity, developmental timing, fertility and lifespan [1,2], and linked insulin-like molecules and PI3-kinase/Akt to the TOR (target of rapamycin) kinase signalling cascade, a pathway that also responds to local nutrient levels and energy status of the cell (Figure 1) [1,2]. But how are the effects of PI3-kinase/Akt modulated by nutrients and how does this cassette perform its diverse cellular functions?

Nutrient Sensing and Insulin Sensitivity

TOR and its downstream target S6 kinase (S6K) have been shown to negatively regulate insulin signalling and PI3-kinase/Akt activity in both flies and mammals through a feedback mechanism (Figure 1) [3]. In fact, reducing the activity of this negative feedback pathway through *S6K* mutation can suppress cellular insulin resistance and protect against age- and

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diet-induced obesity [4]. Hence, developing a better understanding of TOR regulation may have important implications in determining how the PI3-kinase/Akt cassette is modulated in insulin-linked disease. Studies in yeast and mammalian cell culture have indicated that TOR can respond to intracellular levels of nutrients, particularly amino acids [5]. However, some whole organism studies, where *in vivo* conditions can be better modelled, have indicated an important role for extracellular amino acids. e.g., [6].

Genetic studies in flies have now identified multiple amino acid transporters (AATs) that modulate growth and TOR signalling. The cationic amino acid transporter (CAT) Slimfast (Slif) plays a particularly important role in the fat body, an endocrine organ with properties related to mammalian liver and white adipose tissue [7]. Here it appears to act through TOR to control the secretion of one or more hormones that modulate global sensitivity to insulin-like molecules. A heterodimeric AAT subunit, Minidiscs (Mnd), also regulates fat body activity, although its amino acid specificity is unknown and it has not yet been shown to modulate TOR [8].

In a screen for AATs that promote insulin- and TOR-dependent growth when overexpressed in peripheral tissues, two members of a completely different class of transporters, the proton-assisted amino acid transporters (PATs), were highlighted [9]. One of these molecules, CG1139, shows very similar transport properties to two characterized mammalian PATs, but the second, called PATH, is several hundred fold less efficient as a transporter, while having a substrate affinity, which is hundreds of fold higher than these other transporters. Despite these differences, both CG1139 and PATH have similar growth regulatory properties, suggesting that bulk amino acid transport is not an essential part of the signalling mechanism.

Thus, different classes of AAT can modulate insulin sensitivity and Akt activity in cell type-specific ways, through mechanisms that are presumably dependent on local levels of extracellular nutrients.

Subcellular Regulation of the PI3-kinase/Akt Cassette

Although altered PI3-kinase/Akt signalling most obviously affects growth, other more subtle phenotypes are also observed. For example, photoreceptors mutant for the PI3-kinase antagonist PTEN (phosphatase and tensin homologue deleted on chromosome 10; Figure 1) show altered morphology in apical light-sensing structures called rhabdomeres. This phenotype is linked to selective activation of Akt in the apical region, caused by loss of a specific isoform of PTEN that localizes to focal adhesion structures flanking this domain [10].

A more recent analysis of cultured kidney epithelial cells has suggested that differential regulation of PI3-kinase (and potentially Akt) signalling in apical/basolateral domains is important in other types of epithelial cells and that restricting the activity of this cassette is critical for normal apical morphology [11]. Interestingly, both of these studies suggest that the signalling cassette is controlling shuttling of proteins to and from the apical surface, in a process reminiscent of this pathway's well-documented role in glucose transporter shuttling. Importantly, some cells use asymmetric activation of the PI3-kinase/Akt cassette to drive migration [12]. Unravelling the protein shuttling and actin remodeling events involved in all these processes is now paramount, particularly since these events may be important in linking PI3-kinase/Akt to the activity of other membrane proteins, such as the AATs discussed above.

Activated Akt can diffuse from the cell surface into the cytoplasm and nucleus, but the functions of these latter pools have only recently started to be subjected to genetic analysis.

Drosophila nurse cells in the ovary store nutrients, which are ultimately pumped into the oocyte through linking ring canals. In these cells, activated Akt is not surface-localised, but distributed throughout the cytoplasm. *PTEN* mutant nurse cells have greatly upregulated levels of cytoplasmic activated Akt and accumulate highly enlarged lipid droplets of up to 15 μm diameter [13]. This phenotype, which partially resembles the appearance of an adipocyte, is not produced by cell surface-activated Akt, suggesting it is specifically linked to cytoplasmic Akt activity.

Interestingly, in response to insulin/Akt, both adipocytes [14] and nurse cells increase levels of perilipin-like molecules, which promote lipid droplet formation, indicating that this function for cytoplasmic signalling in lipid storage is conserved. The recent discovery in flies of another conserved regulator of insulin-dependent lipid metabolism called Melted, which sequesters inhibitory insulin signalling components to the cell surface [15] suggests that subcellular control of this system is a widespread and critical aspect of its function.

It will now be essential to determine how these different subcellular pools of activated Akt are regulated and to examine how they modulate signalling activity. Moreover, the progress in dissecting conserved mechanisms of PI3-kinase/Akt cassette regulation in flies highlights the importance of continuing to study these events in an *in vivo* genetic system, where multiple cell types can be analysed in their normal extracellular environment.

Abbreviations used

AAT	amino acid transporter
CAT	cationic amino acid transporter
InR	insulin receptor
P-Akt	phosphorylated activated Akt kinase
PAT	proton-assisted amino acid transporter
PI3-kinase	phosphoinositide 3-kinase
PTEN	phosphatase and tensin homologue deleted on chromosome 10
S6K	S6-kinase
TOR	Target of rapamycin

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Figure 1. PI3-kinase/Akt and the insulin signalling cascade in *Drosophila*

Genetic analysis of growth regulation in flies has linked PI3-kinase/Akt and the insulin receptor (InR) signalling cascade (orange) to the much more ancient local nutrient sensing system involving TOR kinase. TOR activity appears to also be controlled by a number of cell type-specific amino acid transporters (AATs) or sensors, which may act in transport-dependent or -independent ways. Furthermore, the subcellular localization of activated Akt (P-Akt; detected by an antibody to phospho-Ser-505 in fly Akt) varies in different cell types. Specific cell surface domains may express altered levels of P-Akt in epithelial cells. In other cells, cytoplasmic P-Akt (pink) may be produced, which modulates lipid droplet formation in nutrient-storing nurse cells. Since P-Akt can negatively feedback via at least two mechanisms to suppress insulin sensitivity (green), high levels of cytoplasmic P-Akt could make cells resistant to cell surface-induced insulin-dependent functions.