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Amino acid transporters and nutrient sensing mechanisms:

new targets for treating insulin-linked disorders?

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Abstract

The insulin/IGF signalling (IIS) cascade has an important role in regulating normal development and physiology, as evidenced by its effects in a host of major human diseases including cancer, Type II diabetes and neurodegeneration. Recently it has become clear that multiple types of local nutrient sensing mechanism impact on cellular insulin sensitivity through the downstream kinase TOR (target of rapamycin). *In vivo* analysis in flies has surprisingly highlighted proton-assisted amino acid transporters as having a uniquely potent role in regulating IIS/TOR activity and growth, potentially via a novel signalling mechanism. Other molecules such as the heterodimeric amino acid transporter, CD98, which provides the principal route for cellular uptake of leucine, an amino acid implicated in regulating TOR, also appear to have important effects. As our understanding of how nutrient sensing impacts on IIS/TOR increases, novel targets to modulate aberrant IIS in disease are likely to emerge, which could complement current strategies designed to block kinases in this pathway.

Keywords

cancer; nutrient sensing; transceptor; transporters; *Drosophila*

Nutrient sensing modulates the insulin/insulin-like growth factor signalling cascade

The central importance of the insulin/IGF signalling (IIS) cascade in regulating not only metabolism, but also other key physiological processes, such as growth and cell survival has become increasingly clear over the last few years. In fact defective IIS has emerged as a major player in a wide range of human diseases including cancer, diabetes and neurodegeneration [1,2]. Genetic dissection in the fruit fly *Drosophila melanogaster* has greatly helped to advance our understanding of the IIS pathway [2]. In particular it revealed the link between endocrine IIS and the evolutionarily ancient nutrient sensing cascade, involving the kinase, TOR (target of rapamycin), through the G protein Rheb and the Tuberous Sclerosis proteins 1 and 2 (Tsc1 and Tsc2; see Figure 1). TOR is regulated by extracellular nutrients, most notably amino acids. However, hypoxia and other nutrients such as glucose can modulate TOR via the energy sensor AMP-activated protein kinase (AMPK) [3], thus modulating cellular insulin sensitivity [4]. This finding has been pivotal in the development of novel strategies to treat IIS-related diseases by drugging this nutrient sensing mechanism. Rapamycin, an inhibitor of TOR, is currently in Phase III clinical trials to treat specific growth disorders, including renal tumours and pulmonary

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lymphangiomyomatosis (LAM), a genetic disorder characterised by the abnormal growth of smooth muscle-like cells in the lungs [5]. It also blocks neurodegenerative processes in flies (see [3]).

How do amino acids control TOR? Recent studies in several different systems have indicated a complex mode of TOR regulation involving several potential amino acid 'sensors' and signalling cascades.

The proton-assisted amino acid transporter family as nutrient sensors

Amino acid transporters (AATs) would seem likely to be involved in amino acid sensing mechanisms. *In vivo* analysis of the effects of overexpressing a wide range of AAT classes under physiologically relevant conditions in flies has surprisingly highlighted the proton-assisted amino acid transporter (PAT/SLC36) family [6] as having a uniquely potent effect on IIS activity and growth [7]. Why might the PATs have these properties? Transport studies in *Xenopus* oocytes suggest that PATs may use a signalling rather than a transport-dependent mechanism to regulate TOR, acting as so-called 'transceptors'. Such transporter-like receptors have been identified in yeast [8] and more recently in mammals (the sodium-dependent neutral amino acid transporter 2 (SNAT2/SLC38A2; [9])). Interestingly, one human PAT, PAT4, does not seem to transport the characterised amino acid substrates used by other PATs, opening up the possibility that hPAT4 may also act as a transceptor.

Therefore, the identification of PATs as growth regulatory nutrient sensors in flies not only emphasises the importance of testing AATs in an *in vivo* setting where cells are exposed to a normal physiological environment, but also suggests that these transporters may be able to signal in response to interacting amino acids that are not then transported into cells [7]. We have recently found that the human PATs promote growth in flies (DCIG, unpublished observation). Furthermore, several studies indicate that PAT expression is upregulated in cancer (see <http://www.oncomine.org/main/index.jsp>), suggesting these transporters as potential anti-cancer targets.

Other amino acid transporters as TOR regulators

While the PATs have a powerful effect on growth *in vivo*, other amino acid transporters are also likely to impact on IIS in growing cells. Work in cell culture has suggested that leucine as a key regulator of IIS/TOR, see [10]. The principal mechanism for cellular leucine import involves the heterodimeric amino acid transporter CD98 [11], components of which have themselves recently been linked to growth regulation in cancer, eg LAT1. Studies in *Drosophila* have also identified tissue-specific roles for AATs in TOR regulation. In particular, the putative CD98 light chain, Minidiscs and the cationic amino acid transporter, Slimfast (Slif; discussed in [7]) have both been implicated in regulating the endocrine activity of the fat body, potentially via TOR.

Linking amino acid transporters to IIS

Integrating local nutrient regulation of TOR into the control of IIS-mediated growth might present some challenges to a multicellular organism. While growth of many cells may need to be co-ordinated during normal development primarily through endocrine insulin-like molecules, responses of other tissues like the endocrine fat body probably need to be controlled more by local nutrient levels. The presence of high affinity AATs, such as the fly PAT, Pathetic (Path) [7], suggests a mechanism that might reduce local nutrient-dependence in growing cells. Path should be fully saturated even under starvation conditions and therefore will continue to provide a basal signal to TOR in this environment. This could allow the endocrine input to IIS to predominate in growth regulation, while permitting other

amino acids transporters to more subtly enhance signalling to TOR in more plentiful nutrient conditions.

Another important aspect of TOR regulation is the ability of this molecule to control AAT activity and therefore feed back on its sensors. Under conditions favourable for growth in flies, TOR has been shown to regulate bulk endocytic uptake, while promoting surface accumulation of specific nutrient importers such as Slif [12]. Interestingly, work in yeast has also shown that the subcellular localisation and activity of different AATs is regulated by orthologues of components within the upstream IIS pathway, e.g. Tsc and Rheb (discussed in [7]). Trafficking is likely to modulate the growth-promoting functions of the PATs, since studies in human cells have shown that the PATs are expressed both at the cell surface and in the lysosome [6] and may function in both these compartments. A recent report in *Drosophila* cell culture has indicated that increasing TOR signalling does not affect bulk amino acid import [13]. However, the different mechanisms involved in TOR regulation discussed above, which may not all require amino acid transport, suggest that this issue will need to be re-evaluated by testing specific AAT classes individually for their effects TOR activity and growth in different conditions.

Even if we understand how AATs are regulated and signal, a big challenge is to bring the bits of the puzzle together and identify the signalling cascades that link to TOR. Work in cell culture has suggested at least two independent systems that might be involved. One of these is MAP4K3 [14] that has been shown to activate S6K in a amino acid-dependent manner. In addition, the class III PI3 kinase, Vps34 [15], also appears to regulate TOR in response to amino acids. It will now be important to see whether *in vivo* studies confirm these molecules as components of nutrient sensing mechanisms and whether the PATs, CD98 transporters and/or other AATs lie upstream.

As our understanding of the local nutrient sensing mechanism regulating IIS signalling activity grows, novel strategies to drug these pathways should emerge, which may selectively complement current TOR and PI3-kinase inhibitors in specific tumour types, discussed in [1].

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Abbreviations used

IGF	insulin-like growth factor
IIS	insulin/IGF signalling
AAT	amino acid transporter
PAT	proton-assisted amino acid transporter (SLC36)
SNAT2	sodium-dependent neutral amino acid transporter 2 (SLC38A2)
TOR	Target of Rapamycin
PTEN	phosphatase and tensin homologue deleted on chromosome 10
S6K	S6 kinase
Slif	Slimfast
LAM	lymphangiomyomatosis

AMPK AMP-activated protein kinase
Path Pathetic

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Figure 1. Regulation of the insulin/IGF signalling (IIS) pathway by local nutrient sensing mechanisms

IIS has a key role in the normal development and physiology of multicellular organisms. Downstream of the insulin receptor (InR) or IGF receptor, the major human suppressor gene PTEN antagonises the effects of PI3-kinase on phosphatidylinositol 3,4,5-trisphosphate (PIP3) to reduce the activity of the serine threonine kinase, Akt (pathway shown in blue). Akt regulates TOR kinase, which itself can feed back to negatively regulate Akt activity (pathway shown in pink). Cellular sensitivity to IIS is controlled by local nutrient sensing mechanisms acting on TOR that appear to involve a range of amino acid transporters (AATs), such as the proton-assisted amino acid transporters (PATs) and the CD98 family of heterodimeric amino acid transporters. Effective drugging of this pathway may have important therapeutic benefits in diseases such as cancer, Type 2 diabetes and neurodegenerative disorders.