



Review

Nutrient sensing and metabolic decisions

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Abstract

Cells have several sensory systems that detect energy and metabolic status and adjust flux through metabolic pathways accordingly. Many of these sensors and signaling pathways are conserved from yeast to mammals. In this review, we bring together information about five different nutrient-sensing pathways (AMP kinase, mTOR, PAS kinase, hexosamine biosynthesis and Sir2), highlighting their similarities, differences and roles in disease.

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Keywords: AMPK; AMP kinase; Hexosamine; *O*-GlcNAC; mTOR; PASK; PAS kinase; Sir2

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Abbreviations: PAS, Per-Arnt-Sim; TOR, target of rapamycin; mTOR, mammalian target of rapamycin; AMPK, 5' -AMP-dependent protein kinase; AICAR, aminoimidazole-4-carboxamide ribonucleoside; ZMP, AICAR monophosphate; GlcNAc, *N*-acetylglucosamine; HBP, hexosamine biosynthetic pathway.

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1. Introduction

All organisms appear to have the ability to sense the available nutrients on a moment-to-moment basis and adjust flux through metabolic pathways and networks accordingly. It has long been appreciated in single-celled organisms that certain nutrients, or metabolites of these nutrients, can regulate their own uptake, synthesis and utilization (e.g. the tryptophan operon in *Escherichia coli*). By contrast, the conventional wisdom for higher eukaryotes has been that altered nutrient availability affected the metabolism in tissues primarily through endocrine and neuronal signals (e.g. insulin, glucagon, epinephrine, etc.). However, research performed in the past decade has shown that many types of mammalian cells can directly sense changes in the levels of a variety of nutrients and transduce this sensory information into changes in flux through metabolic pathways. These signal transduction pathways appear to operate both independently from and coordinately with the hormonal pathways. Since several of these pathways are conserved from the unicellular yeasts to mammals, they must have originally evolved independent of hormonal control. This conservation has proven extremely useful in delineating these pathways.

In this review, we discuss only the sensing and response to macronutrients, particularly glucose, amino acids and fatty acids, or the products of their catabolism. Five pathways were chosen: AMP kinase, mTOR, PAS kinase, hexosamine and Sir2. With the potential exception of Sir2, each of these homeostatic regulators somehow senses nutrient levels and/or cellular energy status and promotes an anabolic phenotype in times of plenty and/or a catabolic phenotype in times of deprivation. Surprisingly, in several cases, the response pathway has been fairly well delineated but the actual sensor remains a mystery. The focus is primarily on mammalian cells, with results found in other model systems discussed in light of their impact on understanding the mammalian systems. Our intent is not to be exhaustive in review of each field, but instead to bring together information and ideas from these separately studied areas. We finish by describing the connections between these pathways and human disease, particularly diabetes and cancer.

2. AMP-activated protein kinase: a metabolic stress sensor

One of the key functions of catabolic metabolism is to maintain high levels of ATP. Cells must be able to rapidly respond to any stress that threatens to lower ATP levels by

arresting nonessential ATP-utilizing functions and stimulating available ATP-generating pathways. The fact that the ADP/ATP ratio in many cell types is maintained within a narrow range regardless of nutrient availability, hypoxia or cellular activity indicates the existence of a very effective and responsive regulatory system. A central player in this system is the AMP-activated protein kinase (AMPK).

In this complex field, one aspect of AMPK regulation makes straightforward, intuitive sense: as its name implies, AMPK is stimulated by elevated AMP/ATP ratios. Since all eukaryotic cells have sufficient levels of adenylate kinase to keep the reaction $2\text{ADP} \leftrightarrow \text{ATP} + \text{AMP}$ close to equilibrium, the AMP/ATP ratio varies approximately with the square of the ADP/ATP ratio (Hardie and Hawley, 2001). Therefore, the AMP/ATP ratio is an exquisitely sensitive indicator of cellular energy status. The finding that essentially all eukaryotic cells have a sensor for the AMP/ATP ratio validates the “adenylate charge hypothesis” for the metabolic coupling of anabolic and catabolic pathways made by Atkinson (1970) over 30 years ago.

AMPK was first identified as an inhibitory activity in preparations of both acetyl CoA carboxylase (Carlson and Kim, 1973) and 3-hydroxy-3-methylglutaryl- (HMG-) CoA reductase (Beg et al., 1978). When both activities were shown to derive from the same AMP-stimulated kinase, it was renamed AMPK (Carling et al., 1987). Once the enzyme was purified from porcine liver, its structural and functional relationship to the yeast Snf1 kinase became apparent (Mitchelhill et al., 1994; Stapleton et al., 1994). Since the Snf1 kinase was already known to be required for the expression of glucose-repressible genes in yeast (Celenza and Carlson, 1986), the idea arose that this subfamily of protein kinases might represent an ancient mechanism for sensing metabolic stress. In fact, AMPK is found even in the very primitive eukaryote *Giardia lamblia*, which lacks mitochondria (Hardie et al., 2003).

When AMPK is activated, it controls many metabolic processes: it stimulates fatty acid oxidation and glucose uptake, while inhibiting protein, fatty acid, glycogen and cholesterol synthesis. It functions both acutely by directly phosphorylating enzymes involved in these pathways and more long-term by regulating the expression of numerous genes. Its central role as a metabolic fuel gauge/glucose sensor is illustrated by recent studies showing that mice lacking one of the AMPK isoforms have abnormal glucose tolerance and are insulin-resistant (Viollet et al., 2003b). Interest in this enzyme has also increased since the realization that it is a major target of the anti-diabetes drugs metformin and rosiglitazone (reviewed in Rutter et al., 2003).

2.1. Regulation of AMPK

AMPK is a heterotrimer composed of an α -subunit (GenBank: Q13131, P54646) with β - (GenBank: NP006244) and γ - (GenBank: NP997626) regulatory subunits. Mammals express several isoforms of each subunit, and different combinations produce enzymes with differing tissue and subcellular localization, as well as altered sensitivity to AMP (see [Hardie, 2003](#) for a review of the known differences). However, all of the known AMPK complexes are activated both by phosphorylation by an upstream kinase and allosterically by AMP. Phosphorylation of a threonine residue within the activation loop of the α -subunit (T172) is required for kinase activity ([Hawley et al., 1996](#); [Stein et al., 2000](#)). For example, in resting muscle, the enzyme is present in the inactive dephospho-T172 form that is converted to the activated, phosphorylated form following exercise ([Park et al., 2002b](#)). Once AMPK is phosphorylated at T172, its activity can be further activated (between 1.3- and 5-fold, depending on the isoform) by AMP binding to the γ -subunit ([Cheung et al., 2000](#), [Adams et al., 2004](#)). Half-maximal activation of AMPK requires ~ 2 – 10 μ M AMP ([Frederich and Balschi, 2002](#)). High concentrations of ATP abrogate the activation by AMP in vitro, potentially by competitive binding to the allosteric site, in support of the idea that AMPK senses the AMP/ATP ratio as opposed to solely the AMP concentration ([Adams et al., 2004](#); [Hardie et al., 1999](#)). AMP also affects the level of phosphorylation at T172, both by promoting the phosphorylation of AMPK ([Hawley et al., 1995](#)) and by inhibiting its dephosphorylation ([Davies et al., 1995](#)). The combination of these mechanisms is thought to make AMPK highly sensitive to changes in AMP levels (summarized in [Fig. 1](#)).

Any stress that raises the cellular AMP/ATP ratio, either by increasing the utilization of ATP or inhibiting its production, stimulates the AMPK system. In pancreatic β -cells, the same low glucose levels that inhibit insulin secretion also activate AMPK ([da Silva Xavier et al., 2000](#); [Salt et al., 1998](#)). In muscle, the level of activation of AMPK depends on both the duration and intensity of the work ([Winder and Hardie, 1996](#); [Rasmussen and Winder, 1997](#), [Stephens et al., 2002](#)). Pathological stresses, such as hypoxia, ischemia, metabolic poisoning and heat shock, also stimulate the kinase by increasing the AMP levels ([Corton et al., 1994](#); [Kudo et al., 1995](#); [Marsin et al., 2000](#)). In contrast, AMPK is inhibited by relatively high levels of the rapidly mobilized energy stores phosphocreatine and glycogen ([Ponticos et al., 1998](#); [Polekhina et al., 2003](#); [Wojtaszewski et al., 2002](#)).

In addition to the intracellular stresses described above, AMPK activity is also stimulated by the adipocytokines leptin ([Minokoshi et al., 2002](#)) and adiponectin ([Yamauchi et al., 2002](#)), as well as the widely used anti-diabetic and anti-obesity drugs metformin and rosiglitazone ([Fryer et al., 2002b](#); [Zhou et al., 2001](#)). The mechanism by which leptin stimulates AMPK activity is complex, involving both

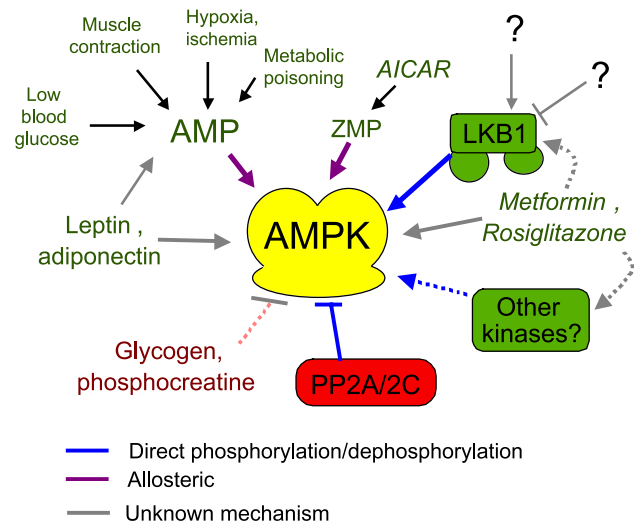


Fig. 1. AMPK inputs. AMPK agonists are emphasized in green, while antagonists are in red. Blue lines indicate direct phosphorylation/dephosphorylation, purple indicates allosteric interactions and gray indicates an unknown mechanism. Dashed lines indicate potential interactions. Pharmacological agents are distinguished by italics.

[AMP]-dependent and -independent components ([Minokoshi et al., 2002](#)). Activation of AMPK by metformin occurs by a mechanism that involves phosphorylation by an upstream kinase, but not AMP/ATP ratios ([Hawley et al., 2002](#); [Fryer et al., 2002b](#)).

Many studies of AMPK activation have utilized the adenosine analog 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR). AICAR is rapidly transported into cells and phosphorylated to form the AMP mimetic ZMP, presumably without changing the levels of AMP or ATP ([Hardie et al., 1998](#)). ZMP activates AMPK and treatment of cells with AICAR causes many of the same phenotypes as exercise, glucose deprivation and hypoxia. However, while AICAR is an important tool, it is not specific in its action ([Rutter et al., 2003](#)). Therefore, several recent studies have used AICAR in combination with either metformin (which activates AMPK by an AMP-independent mechanism; [Hardie et al., 2003](#)) or a dominant-negative, kinase-inactive mutant of AMPK ([Mu et al., 2001](#)) to argue that the observed effects directly involve AMPK.

The utility of studying the homologous yeast Snf1 kinase alongside AMPK again became apparent recently in the discovery of at least one of the major upstream activating kinases. While the AMPK kinase activity had been partially purified from rat liver for several years ([Hawley et al., 1996](#)), its identification remained elusive. Large-scale protein interaction studies were used to identify two related protein kinases that interact with the regulatory subunits of Snf1 ([Gavin et al., 2002](#); [Ho et al., 2002](#)). Knocking out the genes for these two proteins, along with the gene for a third related kinase, prevents yeast from growing on nonglucose carbon sources and prevents phosphorylation of Snf1 on the activation loop threonine ([Hong et al., 2003](#)). [Hardie's](#) lab

came to the same conclusion using a separate yeast-based approach: they searched a library of strains that express 119 yeast protein kinases as GST fusions and identified one that could activate Snf1 and AMPK in vitro (Sutherland et al., 2003). Again, they found that three related yeast kinases had to be knocked out to produce the *snf*-phenotype. Both groups noted that these three yeast kinases are related to the mammalian kinase LKB1 (GenBank: Q15831), a previously known tumor suppressor. LKB1, in complex with two other proteins, activates AMPK in vitro (Hawley et al., 2003; Woods et al., 2003). HeLa cells, which do not express LKB1 and are unable to activate AMPK, do activate AMPK once LKB1 is expressed (Hawley et al., 2003). As further proof, these authors went on to show that AMPK is activated normally in wild-type mouse embryo fibroblasts, but not in LKB1^{-/-} cells. At least in these cells, LKB1 seems to account for all of the upstream kinase activity acting on AMPK (Hawley et al., 2003). With the discovery that LKB1 is the upstream activating kinase comes the question of how LKB1 is regulated. Is it regulated by one or more as yet unidentified pathways? Or is it constitutively active, allowing the AMP/ATP ratio to be the major regulator of AMPK activity?

2.2. Downstream effects of AMPK activation

2.2.1. Regulation of glucose transport, glycolysis, liver glucose output and general glucose sensing

Activated AMPK increases glucose transport into skeletal and cardiac muscle independent of insulin action (Goodyear and Kahn, 1998) (Hayashi et al., 1998). A combination of increased GLUT4 (Kurth-Kraczek et al., 1999; Mu et al., 2001; Russell et al., 1999; Ojuka et al., 2000) and GLUT1 (Abbud et al., 2000; Fryer et al., 2002a) translocation to the plasma membrane and increased transcription of the GLUT4 gene (Zheng et al., 2001) all may contribute to the increased transport. Results from a transgenic mouse that overexpresses a dominant-negative, kinase-dead AMPK in muscle suggest that AMPK signaling is essential for hypoxia- and AICAR-induced GLUT4 translocation, but only partially responsible for the increased translocation induced by exercise (Mu et al., 2001) (summarized in Fig. 2).

Following increased glucose uptake into cells, AMPK may increase flux through the glycolytic pathway while inhibiting glycogen synthesis. In ischemic cardiac muscle, AMPK phosphorylates and activates 6-phosphofructo-2-kinase (PFK-2) (Marsin et al., 2000). The resulting increased fructose 2,6-bisphosphate levels should activate phosphofructokinase-1, increasing glycolytic flux. The inducible isoform of PFK-2, like the cardiac but unlike the liver and skeletal muscle isoforms, has potential AMPK phosphorylation sites. This isoform is important for the survival of tumor cells, and AMPK has been proposed to play a role in protecting tumors from hypoxic stress (Kato et al., 2002). Additionally, increased flux through glycolysis

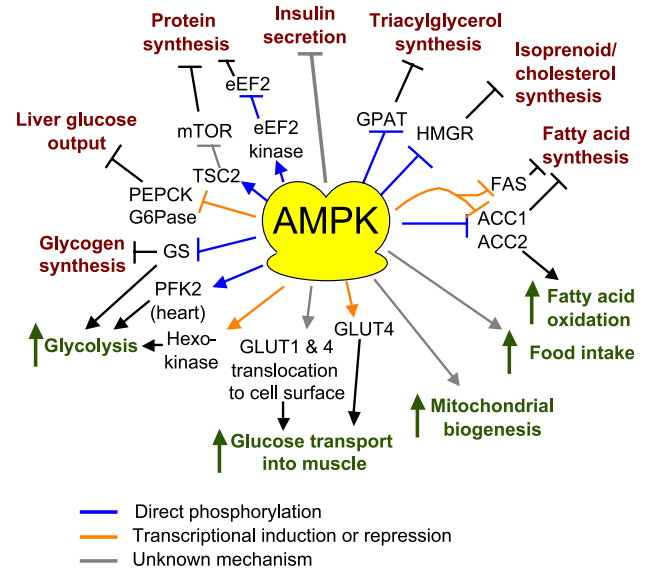


Fig. 2. AMPK outputs and outcomes. A subset of the proteins affected by AMPK is given. Blue lines indicate direct phosphorylation targets, orange lines indicate an effect on the transcriptional induction or repression of the target and gray lines indicate an unknown mechanism. Green lettering is used to emphasize outcomes that are increased upon AMPK activation and red lettering is used to emphasize outcomes that are decreased upon AMPK activation. PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose 6 phosphatase; TSC2, tuberous sclerosis component 2; mTOR, mammalian target of rapamycin; eEF2, eukaryotic elongation factor 2; GPAT, glycerol phosphate acyl transferase; HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; FAS, fatty acid synthase; ACC, acetyl CoA carboxylase; PFK2, 6-phosphofructo-2-kinase; GLUT1 and -4, glucose transporters 1 and 4; GS, glycogen synthase.

may also result from inhibiting glycogen synthesis. In support of this notion, treatment of a muscle cell line with AICAR leads to a reduction in the active, dephosphorylated form of glycogen synthase (Halse et al., 2003).

Mitochondrial biogenesis in muscles is stimulated by both chronic energy deprivation and long-term exercise training as an adaptive response to increase the oxidative metabolic capacity of the cells (Zong et al., 2002; Putman et al., 2003). By using transgenic mice expressing a dominant negative mutant AMPK, Shulman's lab showed that AMPK is required for increased muscle mitochondrial biogenesis in response to chronic energy deprivation (Zong et al., 2002). They treated both wild-type and transgenic mice with an analog of creatine that increased the intramuscular AMP/ATP ratio; AMPK activity and mitochondrial biogenesis were stimulated in only the wild-type mice. Using Sprague–Dawley rats and daily AICAR injections, Putman et al.'s (2003) results also support the hypothesis that AMPK at least partially regulates the increase in mitochondrial volume associated with chronic exercise training.

Liver has a unique role in glucose homeostasis in mammals, largely due to its ability to produce and export glucose. Both AICAR and metformin suppress glucose output from hepatocytes, and the effects of both of these agents are completely repressed by a specific AMPK inhibitor (Zhou et al., 2001). Expression of the genes for

the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase in liver is reduced by activation of AMPK both in cell culture using AICAR (Lochhead et al., 2000) and in vivo using adiponectin (Yamauchi et al., 2002). Together, these data suggest that activation of AMPK inhibits hepatic glucose output in an insulin-independent manner.

The global involvement of AMPK in glucose sensing is presently being studied in mice with either of the two catalytic subunits disrupted (AMPK α 1^{-/-} or AMPK α 2^{-/-}) (Viollet et al., 2003a,b; Jorgensen et al., 2004). The double knockout of both AMPK α isoforms caused early embryonic lethality, while mice with a single isoform knockout are viable (Viollet et al., 2003b). As assessed by glucose tolerance testing, AMPK α 1^{-/-} mice show no defect in glucose homeostasis while AMPK α 2^{-/-} mice show clear glucose intolerance as well as reduced insulin sensitivity (Jorgensen et al., 2004; Viollet et al., 2003a,b). The AMPK α 2^{-/-} mice also display higher free fatty acid levels than controls in both the fasted and fed states (Viollet et al., 2003b). Overall, these researchers found that AMPK α 2^{-/-} mice, but not their isolated organs, exhibited defects in glucose sensing. These findings, together with prior work showing high expression levels of AMPK α 2 in neurons and activated astrocytes (Turnley et al., 1999), led to the suggestion that a lack of AMPK α 2 could disrupt sympathetic effects on energy metabolism. Indeed, they found that AMPK α 2^{-/-} mice excrete increased levels of catecholamines (Viollet et al., 2003b). Direct interpretation of these knockout mice results is obviously complicated by the fact that the mice adapt to living without the AMPK isoforms; not only do the AMPK α 2^{-/-} mice excrete higher levels of catecholamines than normal, but also their muscles, and perhaps other organs, increase expression of AMPK α 1 (Viollet et al., 2003b). The future development of regulated, tissue-specific knockouts of the specific AMPK isoforms may help to address these issues of adaptation.

2.2.2. Regulation of fatty acid synthesis and oxidation

Acetyl CoA carboxylase (ACC) was one of the first known substrates of AMPK (Carlson and Kim, 1973). This enzyme produces malonyl-CoA, both an intermediate in de novo fatty acid synthesis and a potent inhibitor of carnitine-palmitoyl-CoA transferase I (CPT I). Since CPT I controls the entry of long-chain fatty acids into the mitochondrial matrix, the rate-limiting step of β -oxidation, high levels of malonyl-CoA inhibit fatty acid oxidation. Several groups have shown that AMPK phosphorylates and inactivates both isoforms of ACC (Hardie et al., 1998). As a result, activated AMPK lowers malonyl-CoA levels, which simultaneously activates fatty acid oxidation and inhibits fatty acid synthesis. Cholesterol and triacylglycerol synthesis are also reduced via phosphorylation/inactivation of HMG-CoA reductase and glycerol phosphate acyl transferase, respectively (Beg et al., 1978; Park et al., 2002a). Additionally, activation of AMPK results in the down-regulation of the

ACC1 and fatty acid synthase genes in liver (Leclerc et al., 1998; Foretz et al., 1998).

2.2.3. Regulation of transcription and protein synthesis

AMPK activation alters the expression of many genes at both the transcriptional and translational levels. In mice expressing the dominant negative AMPK in muscles, the transcription of hundreds of genes was found to be either up- or down-regulated by more than two-fold in comparison with control mice (Mu et al., 2003). One subset of these genes, including GLUT4, hexokinase and mitochondria enzymes, is up-regulated both by AMPK activation and by endurance training (Hardie et al., 2003). In liver, AMPK activation causes the phosphorylation of the carbohydrate-response-element-binding protein (ChREBP), thereby inhibiting the glucose-induced gene expression of liver pyruvate kinase, fatty acid synthase and ACC (Kawaguchi et al., 2002). Activated AMPK also suppresses expression of the sterol-regulated-element binding protein-1 (SREBP-1), an important lipogenic transcription factor (Zhou et al., 2001). HNF4 α , a member of the steroid hormone receptor superfamily that regulates expression of several key genes in both liver and pancreatic β -cells, is also a target of AMPK (Leclerc et al., 2001). In addition to the transcriptional regulation of specific genes, AMPK appears to have a global effect on protein synthesis. Translational elongation is acutely blocked through the activation of elongation factor-2 kinase and phosphorylation/inactivation of eEF2, allowing protein translation to pause until ATP levels are restored (Horman et al., 2002). The eEF2 kinase may be a direct substrate of AMPK (Horman et al., 2003). AMPK activation also blocks translation initiation by inhibiting the mTOR signaling pathway (see below) (Bolster et al., 2002; Dubbelhuis and Meijer, 2002; Krause et al., 2002).

2.2.4. AMPK activators as anti-diabetes and anti-obesity drugs

Since activating AMPK increases glucose uptake and fatty acid oxidation, and inhibits hepatic glucose output, many have assumed that AICAR analogs would make useful drugs for treating type II diabetes. Indeed, the commonly used drugs metformin and rosiglitazone function primarily by stimulating AMPK activity. However, one must remember that many cell types contain AMPK, and in each, the stimulation of the kinase signals nutrient starvation. As described below, stimulation of AMPK in pancreatic β -cells and in the hypothalamus may not result in an output that is beneficial to curing diabetes or obesity.

In pancreatic β -cells, stimulating AMPK suppresses glucose-induced insulin secretion by inhibiting glucose metabolism and ATP production (da Silva Xavier et al., 2003). Additionally, there is evidence that AMPK activation may even prompt apoptosis specifically in this cell type (Kefas et al., 2003a,b). AICAR and metformin treatments both decrease circulating plasma insulin levels in humans (Iglesias et al., 2002; Wu et al., 1990), but most researchers

have assumed this is due to a reduction in blood glucose. More studies will be required to determine whether these and related agents cause an undesirable decrease in β -cell viability or insulin secretion.

Activation of AMPK in the hypothalamus may stimulate appetite. In fact, it again makes intuitive sense that elevated AMP levels might signal a requirement for an increased uptake of nutrients. Minokoshi et al. (2004) recently showed in mice that AMPK activity is inhibited in particular regions of the hypothalamus by leptin and in other regions by insulin, high glucose and refeeding. Additionally, expression of a dominant negative AMPK mutant in the hypothalamus was sufficient to reduce food intake and body weight, while constitutively active AMPK increased both (Minokoshi et al., 2004). In fact, direct pharmacological activation of AMPK in the hypothalamus is sufficient to increase food intake (Andersson et al., 2004). Therefore, drugs that activate AMPK may increase obesity by increasing appetite, the opposite of the desired effect.

3. mTOR

A second well-studied and evolutionarily conserved intracellular nutrient-sensing pathway is that involving the mTOR protein kinase. This kinase is a central component in the control of cell size; inhibiting the pathway decreases cell size and causes G1-phase cell-cycle arrest (Harris and Lawrence, 2003). mTOR is involved in regulating many aspects of cell growth including translation initiation and elongation, ribosome biogenesis, autophagy, protein kinase C signaling, cell-cycle progression and transcription.

The target of rapamycin (TOR) was originally identified in *Saccharomyces cerevisiae* as a gene that conferred susceptibility to the immunosuppressive and antifungal agent rapamycin (Heitman et al., 1991). The homologous mammalian TOR protein (mTOR, also previously called FRAP, RAFT1 and SEPT) (GenBank: P42345) was identified shortly thereafter (Sabatini et al., 1994; Brown et al., 1994; Sabers et al., 1995). It is ubiquitously expressed in all tissues with the highest levels in muscle and brain (Harris and Lawrence, 2003). Rapamycin inhibits TOR signaling in yeast, humans and other organisms, and closely related homologs of TOR have been identified throughout eukarya (Crespo and Hall, 2002). In contrast to AMPK, the mTOR pathway is activated by nutrient-rich conditions, particularly by high levels of amino acids and by insulin. Also, unlike AMPK, the actual molecule(s) being sensed by the mTOR pathway remain(s) a mystery.

Metazoans all have a single TOR gene that encodes a large (~300 kDa) multidomain protein containing a serine/threonine protein kinase domain of the phosphatidylinositol 3-kinase-related family (Brunn et al., 1997b; Burnett et al., 1998; Abraham, 2001). Immediately N-terminal to the kinase domain is the FKBP12-rapamycin binding (FRB)

domain. Structural studies have indicated that the FKBP12 protein is required to present rapamycin in the appropriate conformation to the FRB domain of TOR (Choi et al., 1996). C-terminal to the kinase domain is a putative regulatory domain that is phosphorylated in response to insulin and growth factors (Scott et al., 1998; Nave et al., 1999; Sekulic et al., 2000). Additionally, almost half of the protein is composed of 20 HEAT repeats, which form repeating stacks of α helices that create surfaces for potential protein–protein interactions (Groves and Barford, 1999). There are at least two other intrinsic and essential components of the mTOR signaling complex: raptor and mLST8 (reviewed in Harris and Lawrence, 2003). While the mechanistic roles of raptor and mLST8 are presently being debated, at least raptor appears to modulate the choice of substrates by mTOR (Kim et al., 2002).

3.1. Activation of mTOR

3.1.1. Amino acids

Amino acids are positive regulators of mTOR signaling. In most studies using mammalian cells, leucine is the most effective stimulator of the pathway (Jefferson and Kimball, 2003). TOR apparently senses intracellular amino acid pools, as opposed to tRNA charging status or external amino acids (Beugnet et al., 2003; Dennis et al., 2001; Pham et al., 2000). Additionally, leucine signals to mTOR independent of insulin and growth factor pathways (Proud, 2002; Patti et al., 1998; Jefferson and Kimball, 2003; McDaniel et al., 2002; Harris and Lawrence, 2003). Studies by Kim et al. (2002) demonstrated that the raptor–TOR interaction qualitatively changes in response to nutrient conditions. The authors, in fact, provide evidence for the existence of at least two raptor–TOR conformations that are separable by mutagenic alteration of raptor. These various raptor–TOR conformations may help regulate which potential substrates are actually phosphorylated by TOR under a given set of conditions. However, the basic mechanism of amino acid sensing as relates to mTOR activation (e.g. what actually binds amino acids) still remains unknown and even whether mTOR becomes phosphorylated in response to amino acids remains controversial (see Fig. 3 for an overview).

3.1.2. Cellular energy status

Conditions and treatments that activate AMPK (see above) promote the dephosphorylation of the well-characterized mTOR substrates 4E-BP1 and S6K1 (the roles of these two substrates in protein synthesis are described below in Section 3.2) (Bolster et al., 2002; Kimura et al., 2003; Krause et al., 2002; Dennis et al., 2001; Dubbelhuis and Meijer, 2002). For example, AICAR reduces the phosphorylation of S6K1. However, in cells with a rapamycin-resistant form of S6K1, AICAR has no effect (Kimura et al., 2003). Together, the evidence supports the model that activated AMPK can inhibit mTOR function.

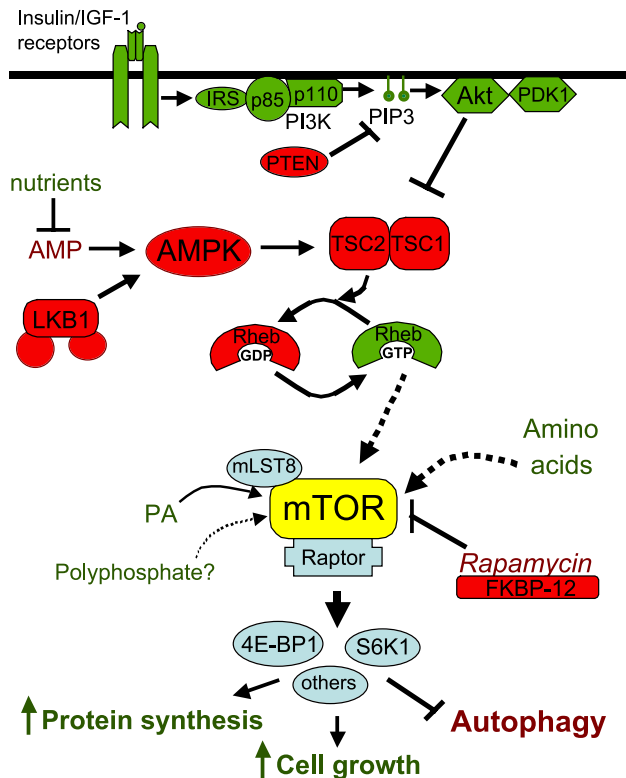


Fig. 3. Hypothetical TOR signaling pathway. This figure is adapted from that of Harris and Lawrence (2003). See text for description. Green is used to emphasize components that lead to the activation of mTOR, while red indicates components that cause inactivation of the kinase. Dashed lines are used to indicate unknown and potentially indirect interactions.

The recent discovery that AMPK phosphorylates and activates TSC2 (Inoki et al., 2003b), an inhibitor of mTOR function, provides one potential mechanism by which decreased cellular energy levels could rapidly inhibit mTOR activity.

Increased cellular energy status may activate mTOR by amino acid-independent ways. Polyphosphate, an evolutionarily conserved high-energy polymer, stimulates mTOR in vitro (Wang et al., 2003). These researchers went on to show that expression of a yeast polyphosphatase, an enzyme that specifically degrades polyphosphates, in a human carcinoma cell line (MCF-7) resulted in reduced phosphorylation of 4E-BP1 in response to either amino acids or insulin (Wang et al., 2003).

3.1.3. Hormonal stimulation of mTOR

In addition to being a component of the nutrient sensory pathway leading to phosphorylation of 4E-BP1 and S6K1, mTOR is also necessary for the hormonally induced phosphorylation of these two proteins. Insulin, for example, causes the phosphorylation of both of these two proteins in a rapamycin-sensitive fashion (reviewed in Harris and Lawrence, 2003). Cells must somehow integrate the information coming from hormonal signals with the cell-autonomous nutrient and energy signals to appropriately phosphorylate these key proteins. The cell-autonomous input seems to be

at least partially dominant; cells that have been starved for amino acids or energy are not competent to respond properly to hormonal signals (Dennis et al., 2001; Hara et al., 1998).

Defining the network involved in integrating the signals from hormones and nutrients to the mTOR complex is a work in progress. The following hypothetical network is adapted from the ideas of others (Harris and Lawrence, 2003; Shamji et al., 2003), and the evidence supporting this network is convincingly documented therein. This proposed network is illustrated in Fig. 3. The activated insulin receptor phosphorylates the insulin receptor substrate-1 (IRS-1) protein. The phosphorylated IRS-1 recruits and activates phosphatidylinositol 3-kinase (PI3K). Other hormones that stimulate PI3K enter the pathway at this point. Active PI3K converts phosphatidylinositol-4,5-phosphate (PIP₂) in the cell membrane to phosphatidylinositol-3,4,5-phosphate (PIP₃). PTEN, the PIP₃ phosphatase, dampens the signal. The Ser/Thr kinases Akt (also called protein kinase B) and PDK1 are recruited to the PIP₃-containing membranes via their plextrin-homology domains, where PDK1 phosphorylates and activates Akt. The next component is hypothesized to be the TSC1–TSC2 heterodimer, which apparently indirectly inhibits the mTOR complex. TSC2 is phosphorylated by Akt in vitro, and the Akt-phosphorylated form of TSC2 has reduced ability to suppress mTOR signaling (Inoki et al., 2002; Manning et al., 2002; Potter et al., 2002). As mentioned above, the inhibition of the mTOR pathway by AMPK may also be mediated by TSC2; in this case, AMPK-dependent phosphorylation activates TSC2. The effect of TSC1–TSC2 on mTOR is apparently indirect and is hypothesized to work through the GTP-binding protein Rheb. TSC2 has homology to GTPase activating proteins (GAPs) and the TSC1–TSC2 complex displays GAP activity towards Rheb (Zhang et al., 2003b; Tee et al., 2003; Inoki et al., 2003a). Considerable work in *Drosophila* has placed Rheb downstream of TSC1–TSC2 and upstream of TOR (Zhang et al., 2003b; Saucedo et al., 2003; Stocker et al., 2003; Patel et al., 2003). It is not yet clear whether Rheb directly activates mTOR. In fact, deleting Rheb from yeast leads to an increase in amino acid uptake (Urano et al., 2000), suggesting that it could indirectly regulate TOR by altering amino acid levels.

Phosphatidic acid (PA), the lipid second messenger produced by phospholipase D in response to multiple growth factors and hormones (Ktistakis et al., 2003), may directly activate mTOR (Fang et al., 2001). These researchers showed that a fragment of mTOR containing the FRB binds PA tightly (Fang et al., 2001). Activation of the mTOR pathway was correlated with the production of PA in HEK293 Cells. Specific mutations in the FRB reduce PA binding without affecting the kinase activity. In vivo, these mutants prevented full activation of S6K1 in response to serum stimulation (Fang et al., 2001). It is not yet clear

exactly how the PA stimulation fits into the entire mTOR regulatory network.

As described above, mTOR is involved in integrating the signals from nutrients and growth factors to control the growth of individual cells. Recent work in *Drosophila* indicates that TOR can also influence peripheral tissue growth via a humoral mechanism (Colombani et al., 2003). The fat body, an insect organ that has endocrine and storage functions similar to the vertebrate liver, coordinates the growth of the organism. When the *slimfast* gene, which encodes an amino acid transporter, is specifically down-regulated within the fat body, the flies suffer a global growth defect similar to that caused by amino acid deprivation. The same effects were seen by inhibiting TOR signaling in the fat body (Colombani et al., 2003). Additionally, over-expressing an activated S6K mutant partially counteracted these effects. These studies indicate that the fat body functions as a TOR-dependent amino acid sensor, which generates an unidentified humoral signal to influence insulin signaling and growth in other tissues (Colombani et al., 2003). It is presently unknown whether mammals utilize the mTOR pathway in an analogous fashion.

3.2. Downstream effects of mTOR activation

The two best characterized targets of mammalian TOR signaling, ribosomal S6 kinase 1 (S6K1) and the eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP1, also called PHAS-I), are both factors that control protein synthesis. Approximately 5% of human caloric intake is expended in the synthesis of proteins (Mathews et al., 2000), so it is not surprising that the level of protein synthesis is substantially modulated by nutrient status. While S6K1 and 4E-BP1 are the best characterized substrates of TOR, there are probably many others, potentially including nPKC δ , nPKC ϵ , STAT3 and p53 (Yonezawa et al., 2004). Additionally, mTOR was very recently shown to regulate the production of ribosomal RNA by modulating the activity of the Pol I transcription factor TIF-IA (Mayer et al., 2004), providing another mechanism for its involvement in ribosome biogenesis. The regulation of the well-characterized mTOR substrates, 4E-BP1 and S6K1, is described below.

The translation of most mRNAs depends on an interaction between eIF4E, eIF4G and the mRNA 5' cap to initiate protein synthesis. Under nutrient deprivation, the translational repressor 4E-BP1 binds eIF4E in a manner that displaces eIF4G and blocks ribosome recruitment. Under nutrient-replete conditions, 4E-BP1 is phosphorylated making it incompetent to interact with eIF-4E (Lin et al., 1994; Pause et al., 1994). mTOR activity is required for this phosphorylation and inactivation of 4E-BP1 in vivo (Graves et al., 1995; Lin et al., 1995; Beretta et al., 1996; Brunn et al., 1997b), and it phosphorylates several important sites on the protein in vitro (Fadden et al., 1997; Brunn et al., 1997a; Gringras et al., 1999).

S6K1 controls the translation of a subset of mRNAs known as the 5' -tract of oligopyrimidine (TOP) mRNAs. All of the protein subunits of the ribosome and a number of other translation factors are encoded by TOP mRNAs (Meyuhas, 2000). Thus, TOR not only controls the initiation of translation for the mRNA pool at large, but also controls, via S6K1, the synthesis of the translation apparatus itself. Under nutrient-replete conditions, S6K1 phosphorylates the ribosomal S6 protein, leading to preferential translation of the 5' -TOP mRNAs. Rapamycin inhibits the activation of S6K1 and prevents the phosphorylation of the S6 protein (Price et al., 1992; Chung et al., 1992). Upon activation of the TOR pathway, S6K1 becomes phosphorylated on many sites, and at least two of the sites that must be phosphorylated for activation can be phosphorylated by mTOR in vitro (Burnett et al., 1998; Saitoh et al., 2002; Pearson et al., 1995). The importance of S6K1 in the TOR signaling pathway is well illustrated in fruit flies where overexpression of S6K (the *Drosophila* homolog of S6K1) rescues the TOR-knockout phenotype (Zhang et al., 2000). Additionally, S6K-deficient fruit flies and mice have significantly smaller cells and are smaller overall than their wild-type siblings (Montagne et al., 1999; Shima et al., 1998), reminiscent of the phenotype seen when TOR is inhibited.

Another likely substrate of S6K1 is eEF2 kinase (Wang et al., 2001). As opposed to the phosphorylation mediated by AMPK, phosphorylation of eEF2K by S6K1 inactivates it. Since the elongation factor is active in its non-phosphorylated form, mTOR activation promotes translational elongation (by inactivating the inactivating kinase, eEF2K), in addition to initiation, via S6K1.

As detailed above, mTOR increases protein synthesis at least partially through its targets 4E-BP1 and S6K1. It also inhibits autophagy, the process by which cells nonspecifically engulf cytoplasmic components and degrade them into utilizable biosynthetic precursors in lysosomes. While the proteasome selectively degrades short-lived proteins, most long-lived proteins, which constitute the majority of cellular material, are digested in lysosomes (Yoshimori, 2004). In most cells, autophagy is repressed to a basal level; starvation and hormonal signaling can dramatically stimulate the process. Under nutrient-replete conditions, rapamycin treatment induces autophagy in both yeast and mammalian cells (Noda and Ohsumi, 1998; Shigemitsu et al., 1999; Blommaert et al., 1995). While there is substantial evidence indicating that TOR regulates autophagy, its mechanism is unknown. The control of autophagy by TOR is typical of its role as a homeostatic regulator of nutrient status: under nutrient abundance, TOR inactivates this nutrient-scavenging pathway, but under starvation allows it to proceed.

As mentioned above, mTOR is involved in a myriad of cellular processes including cell-cycle progression, transcription and protein kinase C signaling, and the direct mTOR targets involved in these processes remain elusive.

Shamji et al. (2000) have proposed that mTOR might function as a multichannel processor, with different inputs eliciting different outputs. While the mechanism of how this could happen is not known, the finding that the raptor–mTOR interaction qualitatively changes in response to nutrient conditions provides one possibility (Kim et al., 2002). Stimulus A might cause the raptor–mTOR complex to adopt conformation 1, which creates an interaction surface on the raptor protein that is now accessible to bind the mTOR target X, specifically initiating the concomitant signaling pathway. By contrast, stimulus B might cause the raptor–mTOR complex to adopt conformation 2, which exposes an alternative interaction surface that binds specifically to the mTOR target Y, leading to the activation of this signaling pathway. The absence of any relevant stimulus might create yet another conformation that is not competent to bind and activate any of the mTOR output mediators. Thus, input could be directly tied to output in a high-fidelity coupled system. If mTOR in fact functions in this type of fashion, then it would have significant implications for how we study its activity. This would, for example, make the results of standard *in vitro* or *ex vivo* kinase assays irrelevant to the *in vivo* situation.

4. PAS kinase

PAS kinase (PASK, also called PASKIN, GenBank: NP055963), like AMPK and mTOR, is a nutrient-responsive protein kinase that is conserved from yeast to man. Unlike AMPK, it was only recently discovered (Hofer et al., 2001; Rutter et al., 2001) and its roles in regulating metabolism and cell growth remain largely undefined. Its basic structure, a regulatory PAS domain fused to a canonical kinase catalytic domain, immediately suggested an interesting function. Several prokaryotic energy-sensing systems utilize PAS domains as the molecular sensor of oxygen, redox status, ATP or other indicators of cellular metabolic status (Stephenson and Hoch, 2001; Taylor and Zhulin, 1999). In many of these bacterial systems, the PAS domain allosterically regulates an attached histidine kinase domain. Metazoans appear to encode only one protein with both a protein kinase and a PAS domain (Rutter et al., 2001). By analogy with these bacterial PAS-regulated kinases, it is hypothesized that PASK catalytic activity is regulated by a metabolite interacting with the PAS domain. Indeed, the PAS domain of PASK specifically interacts with and inactivates the kinase catalytic domain (Amezcuca et al., 2002; Hofer et al., 2001; Rutter et al., 2001). NMR-based studies showed that this domain is also capable of binding small organic compounds in its hydrophobic core (Amezcuca et al., 2002). At this point, the naturally occurring molecule that binds to the PAS domain and stimulates PASK activity in response to changes in metabolic status remains a mystery.

4.1. Evidence that PASK is a nutrient-regulated kinase in yeast

Genetic and biochemical experiments in *S. cerevisiae* have implicated yeast PASK in the control of two important cellular processes. First, genetic experiments demonstrated that PASK is important for efficient protein synthesis (Rutter et al., 2002). Additionally, PASK phosphorylates three distinct proteins involved in the control of protein synthesis (Caf20, Tif11, Sro9), providing a possible mechanism for the genetic observation. Second, PASK was shown to regulate glucose metabolism, specifically the cellular decision to either store or use available sugar. PASK blocks the deposition of glycogen, the major storage carbohydrate in yeast, through phosphorylation of UDP-glucose pyrophosphorylase (Ugp1) and glycogen synthase, enzymes that catalyze the final two steps in the glycogen biosynthetic pathway (Rutter et al., 2002). Indeed, preliminary experiments have shown that nutrient availability is an important regulator of Ugp1 phosphorylation by PASK (Rutter, unpublished results).

4.2. Evidence that PASK is nutrient-regulated in mammalian cells

Mice in which PASK has been inactivated by homologous recombination show normal development, growth and reproduction (Katschinski et al., 2003). It is not yet known whether these animals have altered other pathways to adapt to the loss of PASK or how they will respond to conditions of nutrient excess or deprivation. Results from studies on pancreatic β -cells suggest that mammalian PASK is regulated by nutrient availability or metabolic status and may play an important role in the regulation of insulin production (da Silva Xavier et al., 2004). The activity of PASK in immunoprecipitates from cultured pancreatic β -cells was reproducibly stimulated when the cells were grown in 30 mM glucose relative to 3 mM glucose. PASK was also shown to be required for the glucose-induced expression of the preproinsulin (PPI) gene. Since glucose-regulated signaling in β -cells is a major factor in the pathogenesis of both type 1 and type 2 diabetes mellitus, comprehending the regulation and function of PASK in β -cells may prove important for understanding these diseases.

The recent discovery of another evolutionarily conserved nutrient-sensing pathway leaves one wondering how many other sensors and pathways remain to be discovered. As mentioned above, the AMPK and mTOR pathways appear to intersect. Does the PASK pathway also crosstalk with other nutrient-signaling pathways? What is the regulatory molecule that binds the PAS domain and activates the kinase? Much more work is needed to understand both the nature of the stimulus activating PASK as well as the phosphorylation substrates that mediate its function in β -cells and elsewhere.

5. Hexosamine biosynthetic pathway flux

The hexosamine biosynthetic pathway (HBP) produces uridine 5'-diphospho-*N*-acetylglucosamine (UDP-GlcNAc). The first and rate-limiting step in this pathway is catalyzed by glutamine:fructose-6-phosphate-amidotransferase (GFAT; GenBank: AAH45641). The hypothesis that flux through this pathway is a form of nutrient sensing came from studying how hyperglycemia induces peripheral insulin resistance. Insulin resistance in cultured adipocytes depends on glucose, glutamine and insulin (Marshall et al., 1991a). Suppression of GFAT levels or activity blocks the hyperglycemia-induced insulin resistance (Marshall et al., 1991a, b), and overexpression of GFAT in skeletal muscle or adipose in mice leads to peripheral insulin resistance (Hebert et al., 1996). Additionally, glucosamine, which can enter the HBP downstream of GFAT, induces insulin resistance in a variety of cells, tissues and organisms (Marshall et al., 1991b; McClain, 2002; Rossetti, 2000). Lastly, both animal models and humans with insulin resistance have elevated UDP-GlcNAc levels (McClain, 2002; Robinson et al., 1995; Yki-Jarvinen et al., 1996; Buse et al., 1997). Together, these data strongly argue that the HBP is a nutrient-sensing system in mammalian cells.

It is not yet known how the signal emanating from increased HBP flux causes insulin resistance. The GLUT4 glucose transporter is primarily responsible for insulin-dependent glucose uptake in adipocytes and skeletal muscle, and the level of GLUT4 at the plasma membrane is decreased by increased flux through the HBP (Robinson et al., 1993; Ross et al., 2000; Chen et al., 1997; Thomson et al., 1997). Insulin signaling through Akt is key for GLUT4 vesicle translocation to the plasma membrane, and the HBP sensing pathways appear to act at or upstream of Akt (Wells et al., 2003).

By what molecular mechanism could increased levels of UDP-GlcNAc impinge on a signal transduction pathway? UDP-GlcNAc is the high-energy donor for *O*-GlcNAc transferase (OGT; GenBank: O15294), an enzyme that attaches *O*-GlcNAc to serine and threonine residues in many cytoplasmic and nuclear proteins (Zachara and Hart, 2002). This modification is dynamic (due to the existence of an *O*-GlcNAcase) and can compete with phosphorylation at certain sites (Comer and Hart, 2000). Importantly, the level of *O*-GlcNAc modification increases when there is increased flux through the HBP (Yki-Jarvinen et al., 1998; Patti et al., 1999; Hanover et al., 1999; Han et al., 2000). Inhibition of *O*-GlcNAcase results in insulin resistance in cultured adipocytes (Vosseller et al., 2002), a result that strengthens the link between nutrient sensing by the HBP and *O*-GlcNAc modification. While many *O*-GlcNAc-modified proteins are known, including transcription factors, components of signaling pathways and metabolic enzymes (Vosseller et al., 2001), demonstrating a clear change in activity based on modification has been challenging. However, recently, glycogen synthase activity was

shown to be impaired with increased *O*-GlcNAc levels (Parker et al., 2003), and the 26S proteasome was shown to be partially inhibited when one or more of the ATPases of the 19S cap was modified with *O*-GlcNAc (Zhang et al., 2003a). Since insulin resistance impairs glycogen synthesis (Ciaraldi et al., 1999), inhibition of glycogen synthase by *O*-GlcNAc modification provides one potential mechanism. One rationale for the regulation of the proteasome by *O*-GlcNAc modification is that in times of excess glucose, muscle protein does not need to be degraded for use as a fuel source.

O-GlcNAc modification has also been implicated in transcriptional regulation. *O*-GlcNAc modification of an Sp1 activation domain inhibits its ability to function as a transcriptional activator (Roos et al., 1997; Yang et al., 2001). The carboxy-terminal domain (CTD) of the RNA polymerase II large subunit is modified with multiple *O*-GlcNAc moieties that have been suggested to play a role in arresting transcriptional elongation (Kelly et al., 1993). More recently, Yang et al. (2002) showed that OGT interacts with a histone deacetylase complex by binding to the co-repressor mSin3A. They found that mSin3A can recruit OGT to promoters for transcriptional repression, potentially by inactivating transcription factors and RNA polymerase II by *O*-GlcNAc modification.

Thus far, flux through the HBP and *O*-GlcNAc modification as a nutrient-sensing mechanism has only been described in mammals. Yeasts do not have an OGT gene and presumably therefore lack the ability to modify proteins with *O*-GlcNAc. Hence, unlike the AMPK, mTOR and budding PASK fields, researchers studying the HBP have lacked the advantages provided by yeast genetics.

6. Sir2 as a nutrient sensor?

Yeast Sir2 and its metazoan homologs (including the closest mammalian homolog SIRT1; GenBank: AAH12499) are NAD⁺-dependent protein deacetylases. The present intense interest in Sir2 comes from its involvement in longevity; increased SIR2 dose lengthens yeast replicative life span by 40%, while an extra copy of sir-2.1 in *Caenorhabditis elegans* extends the adult life of the worm by 50% (Tissenbaum and Guarente, 2001). Additionally, caloric restriction-induced life span extension requires Sir2 activity, at least in yeast (Lin et al., 2000). Sir2 represses transcription by removing acetyl groups from lysines of histone tails and certain transcription factors (e.g. FOXO and p53) (Hekimi and Guarente, 2003). These findings have led to the intriguing possibility that Sir2 acts as a metabolic sensor, via its NAD⁺ dependence, that links caloric intake to a transcriptional program that modulates life span.

The fact that Sir2 and related deacetylases require the central metabolic cofactor NAD⁺ to catalyze protein deacetylation is surprising; from the chemical perspective, deacetylation does not require the destruction of a high-

energy cofactor. There is also no indication that the breakdown of NAD^+ during the deacetylation reaction is coupled to any form of protein conformational change or other work. Instead, the NAD^+ requirement may serve to link the activity of Sir2 to the metabolic status of the cell. Mutation of the NAD^+ -salvage pathway in yeast lowers the NAD^+ concentration and prevents the life span extension conferred by caloric restriction (Lin et al., 2000); this is similar to what was seen for *sir2* mutants and led to the suggestion that Sir2 activity might depend on the intracellular concentration of some component of the NAD^+ pathway. There is presently an active debate about whether the *in vivo* activity of Sir2 is directly controlled by either the NAD^+/NADH ratio or by inhibition by nicotinamide, one of the products of the deacetylation reaction (Lin and Guarente, 2003; Lin et al., 2004; Anderson et al., 2003a,b).

Support for the idea that Sir2 acts as a sensor of the NAD^+/NADH ratio (or the concentration of some other component that would be influenced by this ratio) comes from a study on mammalian skeletal muscle cell differentiation (Fulco et al., 2003). Prompted by a prior observation that the NAD^+/NADH ratio decreases as muscle cells differentiate (MacDonald and Marshall, 2000), this group hypothesized that Sir2 might regulate muscle gene expression and differentiation. By several different types of experiments, they convincingly show that Sir2 is in fact a negative regulator of muscle cell differentiation. They also found that Sir2 exists in a complex with the histone acetyl transferase PCAF and the muscle-specific transcription factor MyoD and showed that Sir2 could deacetylate PCAF-acetylated MyoD in the presence of NAD^+ . They tested whether the NAD^+/NADH ratio could affect the transcription of muscle-specific genes by adding either pyruvate or lactate to increase or decrease the ratio, respectively. Addition of pyruvate increased the repression of myosin heavy-chain expression, while addition of lactate had the opposite response. The results of control experiments involving catalytically inactive Sir2 and short hairpin RNAs directed against Sir2 suggest that the effect is Sir2-dependent.

Is Sir2 a metabolic or redox sensor? The evidence described above suggests that it may be. However, the difficulty of measuring the NAD^+/NADH ratio and concentrations available to Sir2 *in vivo* causes the question to be presently left unanswered.

7. Involvement of nutrient-sensing pathways in human disease

The nutrient-sensing pathways described above have clear links to diabetes, cancer and potentially the process of aging. The obvious involvement of AMPK, PASK and the HBP with diabetes has driven much of the work in these fields, and this involvement was outlined in the individual sections above. Conversely, the disciplines of cancer

research and metabolism have been deeply divided for many years. Much of the cancer research done in the past two decades has focused on cell-cycle regulation, often ignoring the role of metabolic regulation. However, as tumors grow and invade beyond their homeostatic limits, the cancerous cells are often subjected to insufficient nutrient and oxygen supplies, a problem exacerbated by insufficient vascularization.

While the connections between nutrient-sensing pathways and cancer progression are not yet well defined, there are several potential links. The activation of PFK2 by AMPK may contribute to the high glycolytic rate in cancer cells known as the Warburg effect (Hardie et al., 2003). AMPK is important for pancreatic tumor cells to survive nutrient starvation (Kato et al., 2002). The newly discovered AMPK kinase, LKB1, is a tumor suppressor; many different mutations in the gene cause Peutz–Jeghers syndrome, a genetic condition that puts people at risk of developing multiple benign tumors in the intestine, as well as more aggressive forms of cancer. Loss of LKB1 activity is predicted to reduce AMPK activity, leading to altered metabolic regulation (Woods et al., 2003). Additionally, since AMPK inhibits the mTOR pathway via TSC2, loss of LKB1 function also results in aberrantly regulated mTOR (Fig. 3). Not surprisingly, TSC1 and TSC2, which function together as a heterodimer to inhibit mTOR, are also each tumor suppressors. Loss of function mutations in the gene encoding either protein results in the autosomal-dominant disorder tuberous sclerosis characterized by multiple hamartomas (typically benign tumors with very large cells). Therefore, LKB1, TSC1 and TSC2 are each tumor suppressors that function to prevent the overactivation of the mTOR signaling pathway. mTOR integrates nutritional and mitogenic signals to control cell-cycle progression, and it does so at least partially through its regulation of S6K1 and 4E-BP1 (Fingar et al., 2004).

The tumor suppressor PTEN dephosphorylates PIP3, thereby inhibiting the membrane recruitment of Akt and PDK1 (see Fig. 3). In this way, PTEN attenuates growth factor signaling through the mTOR pathway, as well as promoting proapoptotic proteins and inhibiting cell proliferation through separate pathways (Shamji et al., 2003). Somatic PTEN loss is commonly observed in many forms of cancer (Vogt, 2001). Additionally, germline mutations of PTEN can cause Cowden's disease, Bannayan–Riley–Ruvalcaba and Proteus syndromes, diseases characterized by aberrant growth and occasionally massive hamartomas (Shamji et al., 2003; Cantley and Neel, 1999). While loss of PTEN activity leads to tumor progression by a combination of mechanisms, overstimulation of the mTOR pathway is clearly an important component. PTEN-deficient tumors are hypersensitive to inhibition of mTOR by a rapamycin derivative (Mills et al., 2001; Neshat et al., 2001; Podsypanina et al., 2001). These and other studies suggest that rapamycin analogs may be useful for treating a variety of cancers (Shamji et al., 2003), and several of these are

presently in clinical trials (Panwalkar et al., 2004; Dutcher, 2004).

How does hyperactivation of the mTOR pathway lead to cancer? The answer is not yet clear. However, it is apparent that defects in the nutrient-sensing parts of the pathway, distinct from the growth factor components, lead primarily to benign tumors with large cells. Increased phosphorylation of S6K1 and 4E-BP1 promotes cell growth by increasing protein synthesis. Suppressed autophagic proteolysis may also make an important contribution (Ogier-Denis and Codogno, 2003). In order for nutrient signals to regulate cellular proliferation, these signals must in some way impinge on the cell-cycle machinery. Recent results from yeast suggest that an uncharacterized pathway, involving a signal generated by glycolysis, regulates the mRNA levels for a set of cell-cycle genes (Newcomb et al., 2003). Clearly, we do not yet fully understand all the connections between nutrient signaling and cell proliferation; however, the known connections seem too provocative to ignore.

8. Summary

We have tried to give an overview of five different nutrient-sensing pathways that either have or are predicted to have important functions in mammals. For four out of five, yeast genetics is playing a crucial role in the pathway elucidation. There is clearly crosstalk between at least two of the pathways, AMPK and mTOR, and these authors will not be surprised if there are interactions between other pathways as well. In addition to the exciting basic science and medical understanding that has come from discovering these pathways, the resultant convergence of research from disparate fields is also creating a bridge over the chasm that has too often separated the studies of molecular biology and metabolism.

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