REVIEW

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Nutrient–sensing mechanisms and pathways

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The ability to sense and respond to fluctuations in environmental nutrient levels is a requisite for life. Nutrient scarcity is a selective pressure that has shaped the evolution of most cellular processes. Different pathways that detect intracellular and extracellular levels of sugars, amino acids, lipids and surrogate metabolites are integrated and coordinated at the organismal level through hormonal signals. During food abundance, nutrient–sensing pathways engage anabolism and storage, whereas scarcity triggers homeostatic mechanisms, such as the mobilization of internal stores through autophagy. Nutrient–sensing pathways are commonly deregulated in human metabolic diseases.

Nutrients (also referred to as macronutrients) are simple organic compounds that are involved in biochemical reactions that produce energy or are constituents of cellular biomass. Glucose and related sugars, amino acids and lipids are important cellular nutrients, and distinct mechanisms to sense their abundances operate in mammalian cells. Essentiality is not necessarily a hallmark of nutrients; for certain amino acids, such as arginine, cysteine, glutamine, glycine, proline and tyrosine, essentiality is context dependent. In healthy people, the *de novo* synthesis of these amino acids from other molecules meets organismal requirements, but under particular metabolic needs, such as during the rapid growth of infants[1,2], they must also be obtained from the environment. Nutrient scarcity has operated as a strong pressure for selecting efficient mechanisms of nutrient sensing in all species. Considering the importance of nutrient homeostasis for all living organisms, and for human health in particular, it is surprising that we know relatively little about direct nutrient-sensing mechanisms.

The sensing of a particular nutrient may involve the direct binding of the molecule to its sensor, or occur by an indirect mechanism relying on the detection of a surrogate molecule that reflects nutrient abundance. Regardless of the manner in which nutrient sensing occurs, for a protein to be considered a sensor, its affinity must be within the range of physiological fluctuations of the concentration of the nutrient or its surrogate.

Unicellular organisms are directly exposed to environmental fluctuations of nutrients, and sense both intracellular and environmental nutrient levels. By contrast, most cells in multicellular eukaryotes are not directly exposed to changes in environmental nutrients, and homeostatic responses aimed at maintaining circulating nutrient levels within a narrow range exist. Nevertheless, internal nutrient levels do fluctuate, and hence intracellular and extracellular nutrient-sensing mechanisms are also present in mammals. In multicellular organisms, nutrients also trigger the release of hormones, which act as long-range signals with non-cell-autonomous effects, to facilitate the coordination of coherent responses in the organism as a whole.

In this Review, we discuss intracellular and extracellular glucose-, amino-acid- and lipid-sensing mechanisms and signalling events in mammals; discuss how these sensing mechanisms become deregulated in human disease; and describe how internal nutrient stores are mobilized during nutrient scarcity.

Lipid sensing

Lipids are a large and diverse set of nutrients (for example, fatty acids or cholesterol) characterized by hydrophobic carbon backbones that are used for energy storage and membrane biosynthesis, among other cellular processes. Owing to their non-polar nature, lipids are normally either packaged into lipoproteins and chylomicrons or bound by albumin in the serum[3]; they are rarely found free in a soluble form in the organism. Despite the morbidity caused by high levels of lipid intake and deregulated lipid storage, which occurs in obese states, our knowledge of lipid-sensing mechanisms, with some exceptions, is quite limited.

Fatty–acid signalling

A family of G-protein-coupled receptors, best characterized by GPR40 and GPR120, detects long-chain unsaturated fatty acids. In mechanisms that are not fully understood, free fatty-acid stimulation of GPR40 at the plasma membrane of pancreatic β-cells augments glucose-stimulated insulin release[4] (Fig. 1a). GPR120 also mediates insulinotropic activity, albeit by an indirect mechanism, involving production of GLP1 in the gut and the release into circulation. GLP1 belongs to a group of gastrointestinal hormones called incretins that promote insulin release in β-cells[5]. These examples demonstrate how an increase in one particular nutrient (fatty acids) anticipates a response to the imminent increase in another nutrient (glucose), as food intake rarely provides solely one nutrient species. In addition, activation of GPR120 at the plasma membrane of white adipocytes leads to a signal transduction cascade that promotes phosphatidylinositol-3-OH kinase (PI(3)K) and AKT activation, leading to the cell-autonomous induction of glucose uptake[6] (Fig. 1a). Genetic mutations that disrupt GPR120 function occur in people who are obese, and ablation of Gpr120 in mice contributes to diet-induced obesity, suggesting that this signal transduction pathway has a key role in the systemic control of nutrient homeostasis[7]. Naturally, these findings have spurred interest in the development of GPR120 agonists to control the onset of obesity[8].

In addition to GPR120, the CD36 (also known as FAT) receptor has been implicated in direct binding and uptake of intestinal lumen fatty acids[9], and, interestingly, GPR40, GPR120 and CD36 have fatty-acid-sensing properties in cells within the oral epithelium that are involved in gustatory perception[10–13] (Fig. 1a).

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Cholesterol sensing

Our limited knowledge about the sensing of other lipid species is in contrast with our profound understanding of the cholesterol-sensing mechanism, which was deciphered by Brown and Goldstein[14]. Sterols, including cholesterol, are fundamental constituents of mammalian membranes that provide membrane fluidity and are needed for the synthesis of steroid hormones. Cholesterol can be obtained from the diet, as well as synthesized *de novo*. Hence, adequate sensing of internal cholesterol levels allows the energetically demanding cholesterol biosynthetic pathway to be controlled, so that it is only active when external supply and internal levels of sterols are low. Cholesterol sensing occurs in close proximity to the regulation of the cholesterol biosynthetic pathway: the cholesterol-sensing protein (cholesterol-sensing protein SREBP1 cleavage activating protein, SCAP), and the transcription factor that induces the expression of enzymes involved in the cholesterol biosynthetic pathway, form a constitutively bound complex on the endoplasmic reticulum (ER). SCAP directly binds cholesterol by a region originally found to span its five transmembrane sterol sensing domains (SSDs)[15,16]. The initial mapping observations were later refined to a loop in the ER lumen side of the membrane, probably embedded in the lipid bilayer[17] (Fig. 1b). SCAP is constitutively bound to sterol regulatory element-binding proteins (SREBPs), which transactivate genes that are crucial for cholesterol synthesis. When cholesterol levels are high, cholesterol binding to SCAP triggers a conformational change that increases its affinity for the INSIG proteins[18], an anchor for SCAP and SREBP within ER membranes. Conversely, when cholesterol levels are low and SCAP is not bound to cholesterol, the SCAP–SREBP tandem dissociates from INSIG and shuttles to the Golgi apparatus[19] (Fig. 1b). This step is essential because the presence of the SCAP–SREBP complex at the Golgi allows the cleavage and release of the cytoplasmic amino-terminus of SREBP by proteases that are resident at the Golgi[20,21]. In turn, the cleaved cytoplasmic fragment of SREBP translocates to the nucleus and induces genes involved in lipid anabolism. Replete cholesterol levels then initiate a slow negative feedback by interacting with SCAP and inhibiting further cleavage of SREBP[22].

Substantial evidence supports an additional sterol-sensing event that occurs within the ER, involving the enzyme HMG-CoA reductase. HMG-CoA reductase, a transcriptional target of SREBP, catalyses the rate-limiting step in *de novo* cholesterol synthesis in response to low cholesterol levels. The carboxy-terminus of HMG-CoA reductase, containing its catalytic activity, is exposed to the cytoplasm, whereas several transmembrane domains, including the sterol-sensing domain reminiscent of that of SCAP, are embedded in the ER membrane[23]. High levels of intermediate lipid species in cholesterol synthesis, such as lanosterol, trigger the binding of HMG-CoA reductase to INSIG, which is also bound constitutively to an ubiquitination complex formed by VCP, GP78 and UBC7. This interaction promotes the ubiquitin-mediated degradation of HMG-CoA reductase[24] (Fig. 1c). As mentioned, HMG-CoA reductase catalyses an early (and rate limiting) step in cholesterol synthesis, but the levels of HMG-CoA reductase are regulated by a slow, transcriptional mechanism that is shut off only after cholesterol levels have been replenished. Hence, the interaction of HMG-CoA reductase with INSIG, leading to its turnover by the proteasome, constitutes a faster regulatory loop that aims to put a brake on cholesterol synthesis when the presence of precursor molecules guarantees its imminent increase.

Sensors upstream of adipokines

Adipokines, hormones secreted by adipocytes, exert systemic effects that include the regulation of appetite, energy expenditure and other processes that contribute to nutrient homeostasis. Their levels do not necessarily reflect circulating lipid levels, but relate to organismal lipid storage[25], and some adipokines, such as leptin, can be considered as surrogate indicators of lipid-storage abundance. Surprisingly, the identity of the sensor that connects high levels of stored lipids with leptin production remains a mystery, despite the identification of regulatory elements in the promoter region of the *LEP* gene[26]. We know considerably more regarding the systemic effects downstream

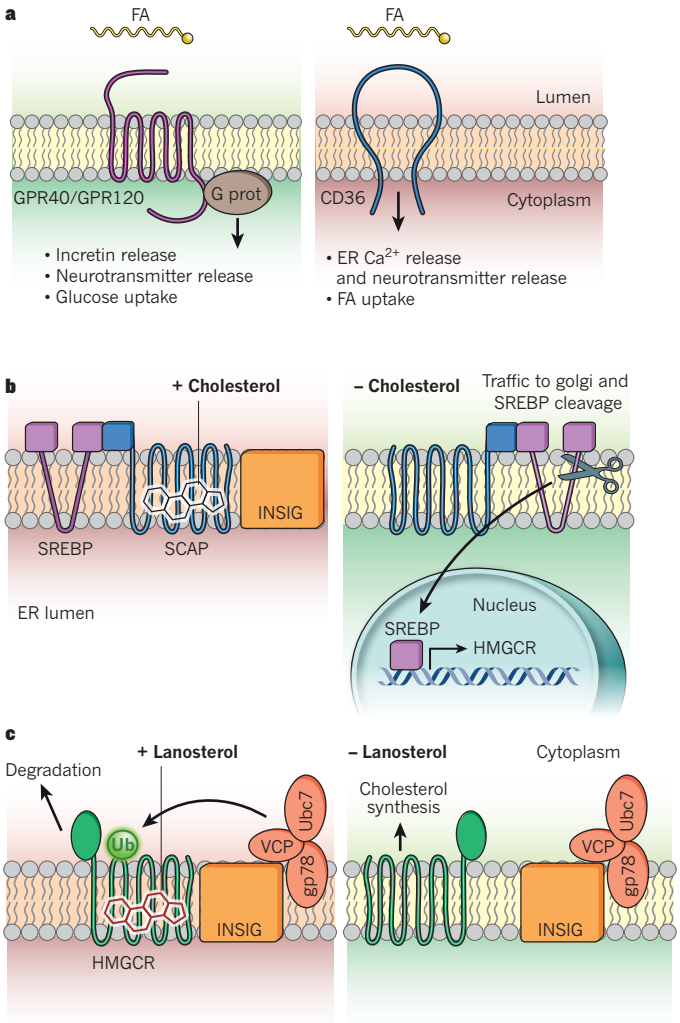

Figure 1 | Lipid-sensing mechanism. **a**, Fatty-acid (FA) detection mechanisms by GPR40 and GPR120 (left) and CD36 (right). GPR family members are expressed in several cell types including enteroendocrine cells, taste buds and white adipocytes; in the enteroendocrine cells, binding to fatty acids occurs on the luminal side, and the signal is transduced through a G protein, leading to the release of incretins into the circulation; in taste buds, they trigger the release of neurotransmitters; and in white adipocytes, activation of GPR120 indirectly promotes glucose uptake. Binding of CD36 to free fatty acids in oral taste buds triggers calcium release from the endoplasmic reticulum (ER) and neurotransmission; in enterocytes, it directly promotes fatty-acid uptake. **b**, Cholesterol sensing by SREBP1 cleavage-activating protein (SCAP). In the presence of cholesterol, the SCAP–SREBP complex binds the INSIG proteins at the ER membrane and remains anchored in the ER. When cholesterol is absent and SCAP–SREBP does not bind INSIG, the complex traffics to the Golgi where the cytoplasmic tail of SREBP gets released by proteolytic cleavage, and triggers a cholesterol synthesis transcriptional program at the nucleus, including the synthesis of HMG-CoA reductase (HMGCR). **c**, The enzyme HMGCR catalyses a rate-limiting step in cholesterol synthesis, and is synthesized when cholesterol levels are low. HMGCR is embedded in the ER membrane and has cytoplasmic domains, which include its catalytic activity (right). In the presence of abundant intermediate species in the cholesterol biosynthetic pathway (such as lanosterol), HMGCR interacts with the INSIG proteins, constitutively bound to an ubiquitination complex. This leads to HMGCR ubiquitination and degradation and halts the synthesis of cholesterol in a rapid regulatory mechanism, which is key to the anticipation of an imminent increase in cholesterol levels.

of leptin. Leptin receptor (LEPR) is expressed both in the central nervous system and in peripheral tissues and its activation coordinates food intake and organismal metabolism. In hypothalamic neurons that suppress appetite (anorexigenic neurons), leptin activity

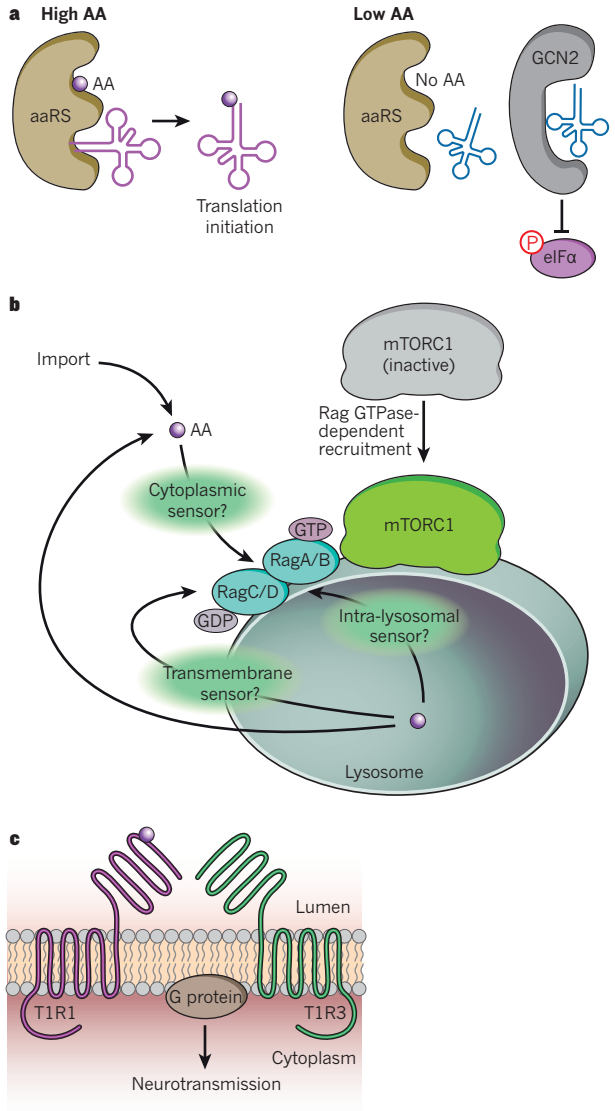

Figure 2 | Amino-acid-sensing mechanisms. **a**, GCN2 detects insufficiencies of cellular amino acids (AAs). During low levels of any amino acid, its cognate aminoacyl transfer RNA synthetase (aaRS) fails to load the tRNA (purple and blue structures). The unloaded tRNA is then detected by GCN2 kinase, which halts translation initiation. **b**, Mechanistic target of rapamycin complex 1 (mTORC1) is activated downstream of elevated intracellular amino acids through its recruitment to the outer lysosomal surface by a Rag GTPase-mediated mechanism. The identity of the sensor for amino acids remains unclear, and several non-mutually exclusive possibilities exist: an intra-lysosomal sensor that transduces the signal through the membrane, a lysosomal transmembrane sensor that both detects and transduces the signal, and a cytoplasmic sensor that operates downstream of amino-acid export from the lysosome. **c**, Sensing of extra-organismal amino acids by oral taste receptors. The heterodimeric receptor T1R1–T1R3 binds amino acids at high concentrations only, and triggers a signal transduction cascade through a G protein. In the intestinal epithelium, it also leads to the localization of GLUT2 to the apical membrane, facilitating glucose import.

antagonizes the effect of appetite-stimulating neuropeptides and neurotransmitters. Lipid mobilization by adipocytes, as occurs in fasting states, results in decreased leptin production, thereby stimulating appetite and promoting nutrient acquisition behaviour. Indeed, mutations in the *LEPR* gene were found in people who are morbidly obese[27], and mice harbouring inactivating mutations in *Lep*[28] or *Lepr*[29] are hyperphagic to the extent that they can be double the mass of normal mice.

In addition to leptin, adipocytes also synthesize the hormone adiponectin (encoded by *ADIPOQ*)[30,31], although we have even less of an understanding of the regulation of its production[32]. In contrast to leptin, circulating adiponectin levels inversely correlate with lipid storage, and this adipokine exerts a multitude of systemic effects that include the promotion of energy expenditure, insulin sensitivity and loss of appetite[33–35]. Mutations and polymorphisms in the human *ADIPOQ* gene strongly correlate with obesity and the development of type 2 diabetes[36–38].

Amino-acid sensing

Amino acids are the building blocks for proteins, the most abundant macromolecules in cells. Protein synthesis is energetically expensive and complex; accordingly, cells sense extracellular and intracellular amino acids to couple their abundance to use. When amino acids are scarce, proteins constitute reservoirs of amino acids that catabolic programs, such as proteasome-mediated degradation and autophagy, mobilize. Amino acids are subsequently recycled and allocated for the synthesis of specific proteins required under nutrient limitation. Furthermore, during periods of prolonged starvation and hypoglycaemia, amino acids are catabolized for the production of other forms of energy, such as glucose and ketone bodies, which are required to fuel the particular needs of certain organs (for example, the brain). Hence, the accurate sensing of amino-acid levels is key for the efficient regulation of protein and amino-acid synthesis and catabolism, as well as for the control of food intake.

GCN2

In protein synthesis, no amino acid compensates for the absence of another, therefore, the cell must be able to efficiently detect the lack of any amino acid to prevent potential failures in peptide-chain synthesis. The structural unit of protein-synthesis machinery, the ribosome, incorporates amino acids into a nascent peptide by the sequential binding of a specific transfer RNA covalently linked to its cognate amino acid. Amino-acid-specific aminoacyl tRNA synthetases (aaRSs) execute the loading of amino acids to their cognate tRNAs[39], and uncharged tRNAs accumulate during low levels of free amino acids. Failure to finish a peptide chain due to a stalled ribosome under amino-acid scarcity is inefficient and energetically onerous, so cells anticipate this situation by preventing the initiation of translation. The mechanism involves a single protein that is able to detect any uncharged tRNA, regardless of its amino-acid specificity, allowing for the detection of low levels of any amino acid in the context of an abundance of the other 19 amino acids. This protein is general control nonderepressible 2 (GCN2), which has a high affinity to all uncharged tRNAs[40] (Fig. 2a), and represents an elegant example of amino-acid sensing by the detection of a surrogate molecule. Under low intracellular amino acid levels, the binding of GCN2 to a given uncharged tRNA triggers a conformational change that leads to kinase activation and inhibitory phosphorylation of a key early activator of translation initiation: eukaryotic translation initiator factor 2 α (eIF2α)[41]. Mouse models have proven the importance of GCN2 and eIF2α in mammalian responses to transient drops in amino acids[42,43] and, interestingly, this amino-acid-sensing pathway seems to play a key part in the central nervous system for the detection of imbalances in amino-acid composition in food, independent of taste[44–46].

Inhibition of protein synthesis by GCN2 and eIF2α occurs in concert with other cellular responses to amino-acid depletion, such as the inhibition of the mechanistic target of rapamycin (mTOR) pathway (see 'mTORC1'). This restricts translation to those messenger RNAs encoding proteins required for cellular adaptation to nutrient starvation, while impairing synthesis of most other proteins[47]. Minimizing translation also enables amino acids to be used as energy sources.

mTORC1

The mTOR kinase, when part of mTOR complex 1 (mTORC1), controls cellular energetics by inducing numerous anabolic processes, including protein and lipid synthesis[48]. Growth factors activate mTORC1 through

a well-understood signal transduction cascade initiated by the binding of a receptor at the plasma membrane, and culminating in the activation of the Rheb GTPase. Rheb directly binds mTORC1 and activates its kinase in a growth-factor-dependent manner[49–52]. In addition to regulation by hormones, intracellular amino acids also activate mTORC1, so the complex integrates information on both systemic and cellular nutrient levels. In spite of the fact that mTORC1 activity is highly responsive to changes in amino-acid levels, it is not an amino-acid sensor. Indeed, mTORC1 activation is one of the several examples of a key sensing signalling process for which, despite intense interest, the actual nutrient sensors remain unidentified (Fig. 2b). mTORC1 is not equally sensitive to all amino acids — leucine, for example, is particularly important for its activation[53]. We can only speculate about the selective importance of leucine levels for mTORC1 activation; it is one of the most abundant amino acids in proteins, and hence, more likely to be limiting during protein synthesis. Intriguingly, GCN2-knockout mice fed a leucine-deficient diet have a more severe phenotype than the same animals fed diets lacking tryptophan or glycine[43]. Thus, leucine seems to be crucial for the organismal sensing of amino-acid sufficiency and deprivation by different pathways. The molecular characterization of the amino-acid-dependent activation of mTORC1 started only a few years ago with the identification of the Rag family of GTPases[54,55], which regulate mTORC1 through a mechanism distinct to that of growth factors. Whereas growth factors regulate the kinase activity of mTORC1, the Rag GTPases recruit mTORC1 to the outer lysosomal surface, an essential step in its activation[56]. Because mTORC1 kinase activation by Rheb occurs at the outer lysosomal surface, it is only possible following Rag GTPase-dependent recruitment of mTORC1 (Fig. 2b). Hence, amino-acid abundance and the consequent recruitment of mTORC1 is a prerequisite for the activation of mTORC1 by growth factors (Fig. 2). Although the sensors for amino acids have not been identified so far, a few pieces in the puzzle of amino-acid-dependent regulation of mTORC1 have recently been added. Cell-based biochemical studies have identified the proteins responsible for tethering the Rag proteins to the lysosomal surface[56], guanine exchange factors (GEFs) and GTPase-activating proteins (GAPs), as well as other regulatory proteins operating upstream of the Rag GTPases[57–63].

The reason for a lysosomal-centred mechanism of mTORC1 activation may be puzzling, but independent pieces of evidence suggest that the lysosome has a key role in amino-acid homeostasis. The yeast vacuole, an organelle equivalent to the mammalian lysosome, accumulates nutrients such as amino acids[64], and mTORC1 recruitment is conserved in yeast[65]. In addition, high intraluminal concentrations of certain amino acids have also been shown in lysosomes[66]. Protists such as *Dictyostelium discoideum* obtain energy through phagocytosis and lysosomal degradation[67], which is followed by a transient increase in intralysosomal nutrient levels. Finally, both the lysosome and the vacuole are the organelles in which amino acids and other nutrients are scavenged from cellular components, through the catabolic process of autophagy. Hence, high levels of amino acids within the lysosome or vacuole system seem to reflect, to some extent, cellular amino-acid abundance, and so it is reasonable to couple its sensing with recruitment and activation of mTORC1 — a crucial regulator of most anabolic processes, including protein synthesis.

Germline and sporadic mutations in genes involved in the signal transduction of nutrient levels upstream of the Rag GTPases have been found in human syndromes characterized by growth defects, neurological disorders, skin and immunological problems, and tumorigenesis[60,61,68–70].

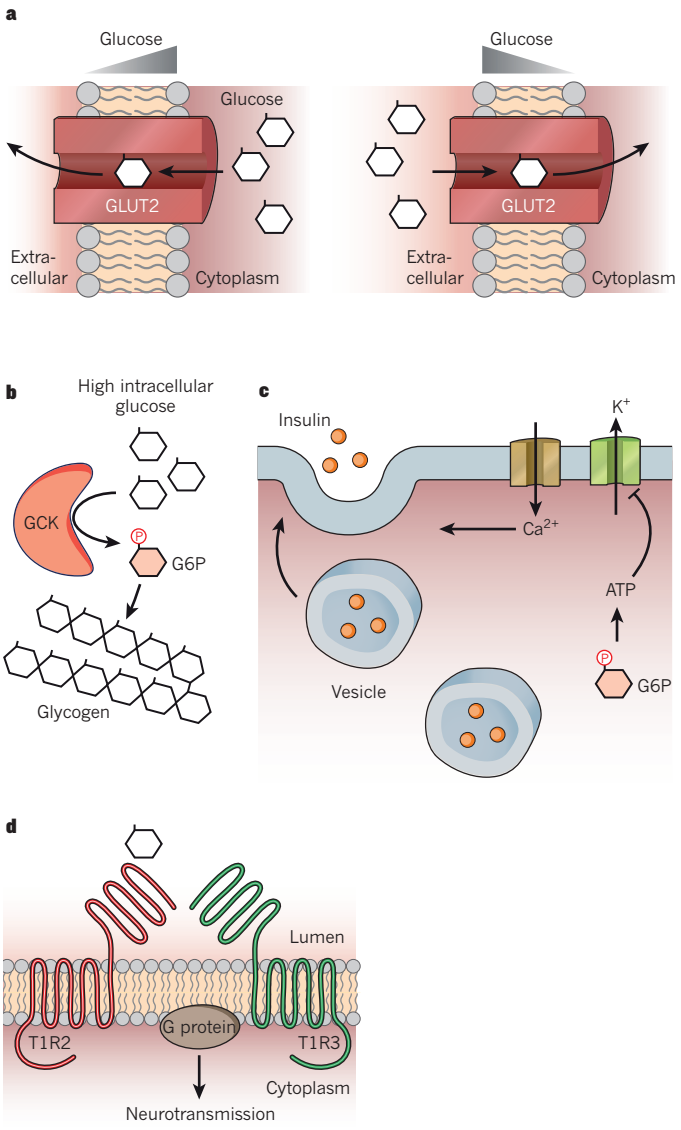

Figure 3 | Glucose-sensing mechanisms. **a**, Glucose sensing by the GLUT2 transporter. Owing to low affinity, this transporter actively imports glucose only during high glycaemic states (right). Its bidirectional properties mean it can also export glucose from hepatocytes into the circulation under hypoglycaemic states if hepatic gluconeogenesis and glycogen breakdown raise the intrahepatic glucose levels (left). **b**, Intracellular glucose sensing by glucokinase (GCK) in hepatic and pancreatic cells. GCK has low affinity for glucose, and shunts glucose-6-phosphate (G6P) into either glycolysis or glycogen synthesis only when glucose is abundant. **c**, The mechanism of insulin release downstream of glucose sensing in pancreatic β-cells. This is a multi-step process that relies on glucose phosphorylation by GCK, subsequent ATP production and ATP-mediated blockade of potassium channels. This leads to a calcium influx that facilitates the release of insulin from vesicles into the bloodstream. **d**, Extra-organismal glucose sensing by oral taste receptors. The dimeric receptors T1R2–T1R3 bind at high concentrations of glucose, sucrose, fructose and artificial sweeteners only, and trigger a signal transduction cascade through a G protein.

Amino-acid-sensing taste receptors

As strict heterotrophs, mammals must obtain energy and nutrients from external organic sources. Predicting the nutritional value of food before digestion allows for the accurate selection of food sources and for the anticipation of increased nutrient abundance. Several mechanisms act synergistically, including experience and social rules in humans, but a fundamental nutrient-sensing event occurs at the level of the oral taste buds. Nutrient sensing by taste receptors is not just a means of sensing extracellular nutrients, it is a mechanism of extra-organismal sensing that allows the interrogation of prospective food sources. In humans, taste is divided into five categories: sweet, umami, bitter, sour and salty, and is generated by signals elicited in taste buds, groups of cells in the tongue, palatal and oesophageal epithelium. Within these cells, the taste receptors are, logically, exposed in the apical membrane oriented towards the environment[71].

Taste receptors belong to the T1R and T2R families of G-protein-coupled receptors, and are characterized by seven transmembrane

domains with an extracellular N-terminus and an intracellular C-terminus. (For molecular and genetic information regarding the different members of the taste receptor genes see ref. 71.) The T2R family is involved in the detection of bitter molecules, a category that includes potentially toxic compounds, and two T1R family members are responsible for sensing the presence of amino acids (the umami taste). Although other taste receptors also exist[71,72], elegant genetic studies using heterologous expression experiments showed that the T1R1–T1R3 heterodimer senses amino acids (Fig. 2c). Human amino-acid taste receptors have a particularly high affinity to glutamate, but other L-amino acids also serve as ligands, whereas D-amino acids do not[73]. Amino-acid binding to a taste receptor triggers signal transduction through the plasma membrane, followed by G-protein activation and neurotransmitter release[74], which is then integrated with other neurotransmission events at the level of the central nervous system.

In addition to the presence of taste buds in the oral epithelium, taste receptors also exist in endocrine cells in certain regions of the gut[75]. Intestinal taste receptors operate through G-protein activation in a similar manner to that of the oral epithelium, but instead of inducing the release of a neurotransmitter that activates an afferent signal to the brain, the cascade elicited by enteral taste receptors culminates in the release of incretins into the blood circulation, serving as an anticipatory signal for the imminent digestion of, and systemic increase in, nutrients.

Interestingly, extracellular amino-acid sensing at the plasma membrane by taste receptors can modulate mTORC1 activation without affecting intracellular amino-acid levels[76], a meaningful cross-talk that engages the anabolic machinery of the cell in anticipation of an elevation in intracellular amino-acid levels, following import.

Glucose sensing

Mammals rely on multiple ways of maintaining glucose levels within a narrow physiological range. Glucose intake, storage, mobilization and breakdown are tightly regulated at different levels, and multiple mechanisms of glucose sensing coexist: extra-organismal, extracellular and intracellular. In addition, a network of hormone signals, exemplified by insulin and glucagon, aim to coordinate coherent responses to systemic glucose levels in distant organs. Deregulated glucose homeostasis mechanisms, from glucose sensing to import, storage and mobilization underlie the pathogenesis of human diseases such as type 2 diabetes.

Glucokinase

Glucokinase (GCK) catalyses the first step in the storage and consumption of glucose, glycogen synthesis and glycolysis, and its function constitutes a simple, direct intracellular nutrient-sensing mechanism that controls systemic glucose homeostasis. Like all hexokinases, GCK phosphorylates glucose to make glucose-6-phosphate (G6P), but unlike the other isozymes, only GCK functions as a glucose sensor[77]. This uniqueness occurs because unlike the other hexokinases, which have K_m values (an inverse measure of affinity) for glucose much below the minimum physiological level of glucose, GCK has a significantly lower affinity and is only active when glucose

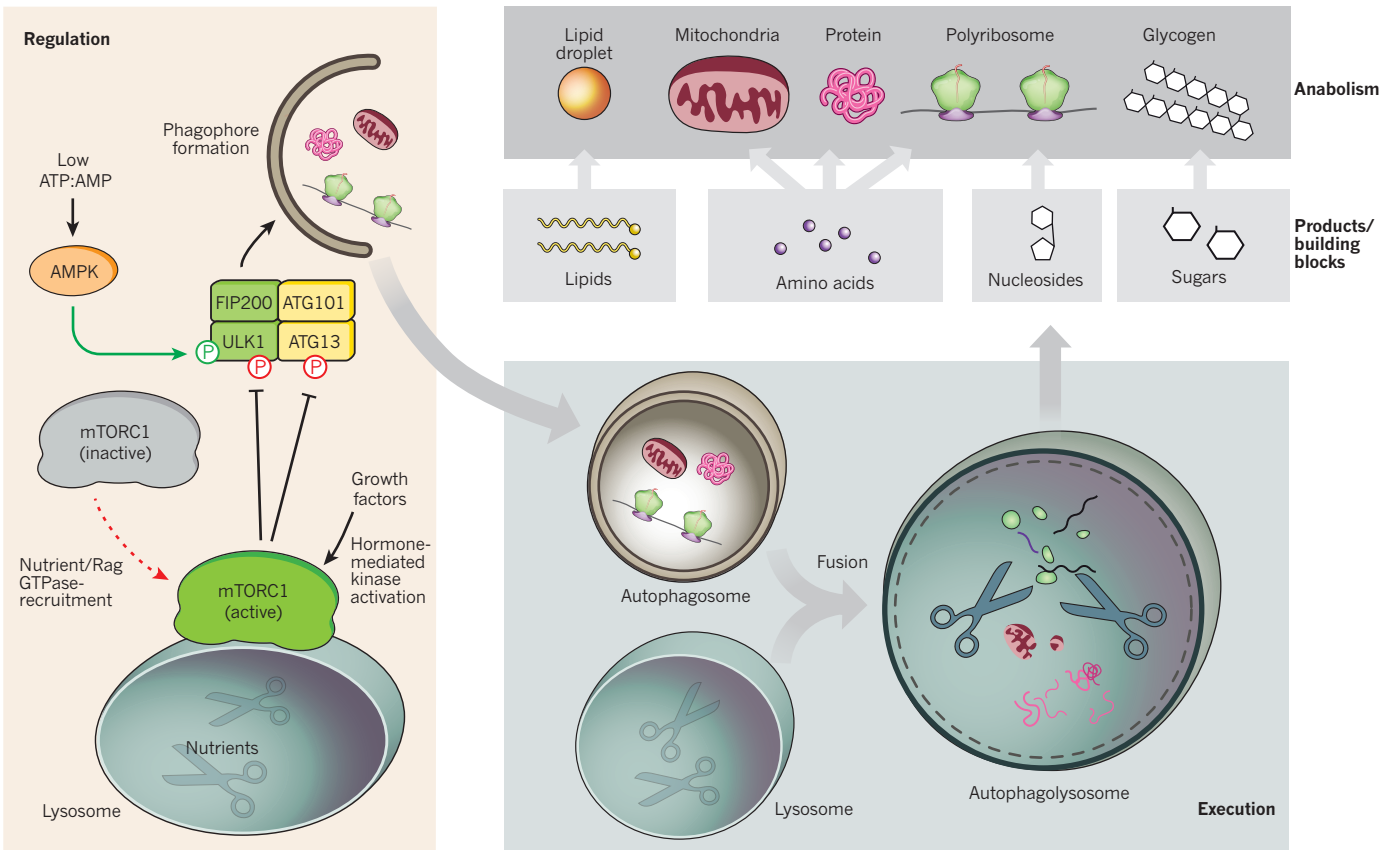

Figure 4 | Nutrients and autophagy. Autophagy serves as an internal source of stored nutrients under conditions of nutrient limitation. Two main regulatory inputs for autophagy are AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin complex 1 (mTORC1). Autophagy initiation can be promoted by the activation of ULK1 through AMPK-dependent phosphorylation during low ATP:AMP ratios. mTORC1 is activated by growth factor-mediated signal transduction at the outer lysosomal surface if cellular amino acids and glucose have recruited mTORC1 through the action of the Rag GTPases. Activated mTORC1 inhibits ULK1 and ATG13 by phosphorylation. Hence, low nutrients promote autophagy by the inhibition of mTORC1. Autophagy starts with the engulfment of the cellular constituents glycogen, lipids from lipid droplets, soluble proteins, ribosomes or organelles in a double-membrane structure that then fuses with lysosomes, in which enzymatic breakdown occurs. The products of autophagy, basic nutrients (sugars, lipids, amino acid and nucleosides), are then exported into the cytoplasm, in which they may be used as a source of energy, or re-used for anabolism.

levels are relatively high (around 120 mg dl^{-1}, or 7 mM, or greater). Hence, although the other hexokinases function as 'phosphorylation machines' regardless of the actual glucose levels, GCK is active only during glucose abundance, and it controls systemic glucose fate through its effects in the liver and pancreas (Fig. 3b). The liver maintains glycaemia through gluconeogenesis and glycogen breakdown during periods of systemic glucose scarcity, or by storing glucose in the form of glycogen when it is in excess[78]. GCK is the most abundant hexokinase in the liver and because it is inactive under conditions of glucose limitation, it permits export of unphosphorylated glucose from the liver in order to supply the energetic demands of the brain and muscles. When hepatic glucose levels are high, GCK-mediated conversion of glucose to the metabolic intermediate G6P allows it to be shunted into glycolysis (for energy production) or glycogen synthesis (for storage).

GCK is also expressed in β-cells (see 'GLUT2'), and in neurons and glial cells in the hypothalamus. Although work remains to be done to understand the role of this glucose sensor in the brain, systemic effects, such as feeding responses and insulin release, are likely to be downstream of hypothalamic GCK activity[79]. Dozens of germline mutations in GCK in people with abnormal glycaemia and diabetes[80], together with conditional deletion of the murine *Gck* gene in the liver and pancreas[81], support the fundamental role of GCK in maintaining organismal glucose homeostasis.

GLUT2

The glucose transporter GLUT2 (also known as SLC2A2) is a sensor of extracellular glucose levels, and like GCK, GLUT2 has a higher K_m (20 mM) than other glucose transporters of the same family. The K_m for GLUT1 is around 1 mM and that of GLUT4 is about 5 mM (ref. 82), so they are close to saturation even during fasting glycaemia (around 4 mM). The low affinity of GLUT2, by contrast, allows for efficient transport of glucose across the plasma membrane only when glycaemia is high, but not under the low concentrations that still saturate the other transporters. Accordingly, GLUT2 has crucial roles in directing organismal glucose handling after feeding. Hepatic glucose import mediated by GLUT2 is followed by GCK-dependent phosphorylation for storage and energy production, as already described. Importantly, during periods of low glycaemia, hepatic glycogenolysis and gluconeogenesis increase intrahepatic glucose levels. Because GLUT2 can transport glucose in a bidirectional manner, it exports glucose to the circulation (Fig. 3a). Hence, GLUT2-mediated import occurs only during transient hyperglycaemic states, and GLUT2-mediated export only happens when intrahepatic glucose levels are high, thus constituting a key controller of glucose homeostasis. Not surprisingly, inactivating mutations in GLUT2 lead to human metabolic disorders, such as Fanconi–Bickel syndrome characterized by deregulated glycogen accumulation, hepatomegaly and hypoglycaemia, among other symptoms of disrupted glycaemic homeostasis[83].

β-Cells in the pancreas have a specialized role in sensing systemic glucose levels, and are responsible for the synthesis and secretion of insulin. Glucose is imported in β-cells and phosphorylated by the tandem of GLUT2 (or GLUT1) and GCK, and, as it is consumed, leads to an increased ATP:ADP ratio. This closes potassium channels at the plasma membrane, and causes the membrane to depolarize. Dissipation of membrane potential results in a transient increase of intracellular calcium that facilitates the fusion of insulin-containing vesicles with the plasma membrane, releasing its cargo into systemic circulation (Fig. 3c). It is important to mention that whereas the predominant transporter in murine β-cells is GLUT2, the relative abundance of the GLUT2 transporter in human islets seems to be minor compared with that of the high affinity GLUT1 transporter — so the relevance of GLUT2 for glucose transport in human β-cells is not clear[84].

Elevated sugar intake and chronic hyperglycaemia deregulate normal glucose sensing through several mechanisms, including ER stress, increased intracellular Ca^{2+} levels, mitochondrial dysfunction, reactive oxygen species and chronic inflammation, all of which contribute to the corruption of insulin secretion in type 2 diabetes[85].

Finally, although the other glucose transporters (such as GLUT1 and GLUT4) do not behave as sensors, their activities and effects are regulated by different means in order to meet particular requirements of glucose use and storage. GLUT4 is expressed in skeletal muscle and adipose tissue, two organs important for post-prandial glucose uptake and storage[82], and although GLUT4 has a low K_m, glucose uptake in these organs is a regulated process. Insulin triggers a PI(3)K–AKT dependent signal transduction cascade that results in GLUT4 localization to the plasma membrane, allowing glucose uptake in these tissues[86]. Because glucose import and storage are insulin dependent, and thus secondary to direct glucose-sensing mechanisms in the liver and pancreas, they occur only after the organism has reached a threshold of internal glucose abundance. GLUT1 is expressed in fetal tissues and its constant activity provides glucose to all tissues to sustain the rapid growth of the organism.

AMPK and ATP:AMP ratios

AMP-activated protein kinase (AMPK) is a fundamental regulator of cellular metabolism and coordinates several metabolic responses in different cell types. It is exquisitely responsive to cellular energy levels; as a surrogate sensing mechanism for glucose abundance, increased levels of AMP and ADP directly activate the kinase. AMPK has been the subject of a number of excellent reviews addressing its activation, regulation, and downstream consequences[87,88], and will be briefly discussed here in the context of the regulation of autophagy.

mTORC1 and the sensing of glucose

The regulation of mTORC1 through Rag-GTPase-mediated recruitment is not restricted to amino acids; cellular glucose levels also affect the activity of the Rag GTPases[89]. In contrast to the identification of some molecular players involved in the activation of the Rag GTPases by cellular amino acids, less clear is the mechanism by which glucose regulates the Rag GTPases. Some aspects downstream of glucose and amino-acid sensing are shared, such as the involvement of the lysosomal v-ATPase[48,89,90], but additional players remain unidentified. Because the amino-acid- and glucose-sensing mechanisms are generally independent phenomena, as we illustrate, it is very likely that amino-acid and glucose sensing upstream of mTORC1 occur in parallel and converge upstream of the Rag GTPases, but precisely how this integration occurs is unresolved.

Glucose–sensing taste receptors

In a similar manner to amino-acid sensing in taste buds by T1R1–T1R3, the heterodimer composed of T1R2–T1R3 constitutes the glucose taste receptor (Fig. 3d). The extracellular N-terminal domains of both T1R1 and T1R2 are essential for determining their specificity for their natural ligands[91]. Millimolar concentration of the saccharides glucose, fructose or sucrose activate the T1R2–T1R3 receptor[92]; this concentration may seem high, but sucrose concentration in an apple is around 100–200 mM, and so this process is selective and efficient for the detection of highly energetic foods.

T1R2–T1R3 receptors are also expressed in the intestinal epithelium, and although the sensing process is identical to that of the oral epithelium, the signal transduction does not trigger an afferent signal to the brain, but results in the transient localization of the GLUT2 transporter to the apical membrane, leading to increased absorption of glucose from the intestinal lumen after feeding[93,94].

In addition to natural ligands, glucose taste buds also respond to artificial sweeteners such as saccharine, cyclamate and aspartame[92]. Activation of glucose taste receptors by artificial ligands has clinical implications for obesity and type 2 diabetes, as sweeteners may increase nutrient absorption and activate other nutrient-sensing signalling cascades at different levels, regardless of nutritional value. Indeed, some studies have shown that consumers of artificial

sweeteners are at higher risk of developing metabolic disease[95]. The phenomenon of artificial activation of this nutrient-sensing pathway is currently an active field of research.

Accessing internal nutrient stores through autophagy

Because environmental nutrient availability can be intermittent, cells and organisms have evolved efficient ways of storing nutrients during periods of abundance. This occurs in unicellular organisms and is more obvious and prominent in animals, with the emergence of organs specialized in nutrient storage, such as fat tissue, the liver and skeletal muscle. Mammalian cells accumulate and store glucose in the form of glycogen, lipids within lipid droplets and internal membranes, and amino acids in proteins and organelles; all of which can be mobilized and catabolized to endure periods of nutrient limitation. Cells exploit different means to obtain the basic nutrients from internal stores, including autophagy, the controlled process of recycling of cellular constituents confined within a double membrane structure. Autophagy starts with the *de novo* formation of a membrane structure termed the phagophore, which engulfs its cargo and closes as a cytoplasmic double-membrane autophagosome. An autophagosome then fuses with a lysosome, which leads to the enzymatic breakdown of the autophagosomal cargo into its basic building blocks, which are then exported from the autophagolysosomes and further catabolized to produce energy, or used again in other anabolic reactions (Fig. 4).

The process of autophagy is unique because it can target any cellular component and nutrient storage depot, and, as a key internal source when nutrients are scarce, is highly regulated at multiple levels by nutrients and nutrient signalling[96]. AMPK, directly activated by a low ATP:ADP ratio, phosphorylates and activates ULK1, a kinase that regulates autophagy initiation[97,98]. AMPK also activates the FOXO transcription factors, which transactivate the ATG genes responsible for the initiation and completion of autophagy[99]. Hence, AMPK acutely regulates autophagy, as well as by means of a slower, transcriptional mechanism.

A crucial regulator of autophagy, as shown in all eukaryotes using both cultured cells and model organisms, is mTORC1, through its inhibitory phosphorylation of ULK1 and ATG13 (ref. 100). mTORC1 seems to play a dominant part in the regulation of autophagy, as mTORC1 inhibition is sufficient to induce it[101], whereas its constitutive activation is sufficient to block it[89]. Nutrient depletion is perhaps the most potent inducer of autophagy, and the regulation of mTORC1 by the Rag GTPases downstream of nutrient scarcity seems to be essential for the regulation of autophagy. Mice with constitutive RagA activity, and hence, constitutive activation of mTORC1 regardless of nutrient levels, develop normally but die within the first day of life, similar to mice lacking the essential autophagy genes *Atg5* and *Atg7* (refs 89,102,103). Constitutive RagA activity in neonatal mice leads to a profound glucose and amino-acid homeostasis defect secondary to an impairment in the detection of nutrient shortage after the transplacental supply of nutrients is interrupted at birth. This leads to constitutive mTORC1 activity and the consequent inability to trigger autophagy.

In addition to the regulation of autophagy initiation, mTORC1 activity is required for autophagy termination[104]. Cellular free amino acids, produced by autophagy, result in an increase in mTORC1 activity and the reformation of lysosomes. Systemic levels of nutrients also regulate autophagy through the effects of insulin[105]. The intracellular cascade of insulin activates AKT, a positive input for mTORC1, and a negative regulator of the FOXO transcription factors. Hence, both local and systemic nutrients regulate the process. In addition to nutrients, stresses such as hypoxia, ER stress and DNA damage also regulate autophagy[106].

Several studies that generated autophagy-deficient tissues in a temporal specific manner have determined the importance of autophagy in mammalian physiology. Besides the aforementioned role of autophagy in the early neonatal starvation period[102,103], autophagy is essential for the survival of embryos in the pre-implantation stage[107]. Whole-body acute deletion of autophagy genes in adult mice eventually culminates in neurodegeneration and death, presumably owing to the accumulation of harmful organelles and proteins, which probably cause neuronal toxicity[108,109]. Liver-specific impairment in autophagy results in accumulation of abnormal cellular endomembranes, mitochondria and ubiquitinated proteins[103], and impaired lipid mobilization[110]. Impaired autophagy seems to preferentially affect cells specialized in vesicle trafficking, such as lymphocytes and β-cells[111], but some of these effects may be due to a deranged endomembrane trafficking system, rather than a direct consequence of a nutrient homeostasis defect.

Future directions

Despite intense research, our understanding of nutrient-sensing mechanisms is far from complete. For instance, we have not yet deciphered what links lipid storage levels with leptin synthesis and release. Equally unclear is what the glucose and amino-acid sensors upstream of mTORC1 are. Towards the identification of nutrient sensors upstream of mTORC1, the lysosome seems to be a key organelle in sensing; however, we still need to determine what is sensed, and how, at the lysosome. Besides these and other fundamental unanswered questions of direct nutrient sensing, the mechanisms discussed in this Review are outlined mostly in a modular manner. This reflects our lack of an integrative view of the nutrient-sensing pathways, and connecting the different aspects of nutrient sensing will be one of the challenges of future research. We know that mTORC1 is a node at which hormone and nutrient inputs converge, but we still do not know whether these signalling cascades cross-talk upstream of mTORC1. A complete view of nutrient-sensing mechanisms will address potential cross-regulation between different nutrient-sensing pathways, but also incorporate regulation by other signalling events. For example, we know some of the consequences of chronic inflammation in deregulating nutrient-sensing mechanisms and the signalling cascades downstream, such as those that occur in obese states, but how exercise modulates nutrient inputs, or how ageing affects nutrient-sensing abilities, remain to be determined. From an experimental point of view, advances in genomics will probably provide insight into clinical conditions secondary to deregulated nutrient sensing, such as the identification of novel mutations and polymorphisms in humans. Finally, nutrient abundance not only affects the onset of diabetes, but also influences cancer development and the ageing process. Nutrient sensing and metabolism in cancer cells has received a new wave of attention, partly thanks to advances in next-generation sequencing and metabolomics. On the one hand, cancer cells are exposed to limited nutrients owing to poor vasculature, and deregulated proliferation poses energetic and nutrient demands and liabilities, which act in concert with aberrant activation of growth signals. On the other hand, one of the most successful interventions against the onset of ageing is limitation of nutrient intake, or caloric restriction[112]. Hence, understanding normal nutrient-sensing mechanisms is a prerequisite for designing better interventions against human disease beyond diabetes. ■

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