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Yeast as a model system to study glucose-mediated signalling and response

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1. ABSTRACT

Glucose is the principal carbon and energy source for a wide variety of cells, ranging from unicellular microorganisms to higher eukaryotic cells. It is taken up by these cells and metabolized to obtain the energy necessary for cell viability. In addition, the presence of this sugar is able to adjust cellular metabolism, regulate gene expression and even influence cell growth. For this reason, glucose is considered as a "hormone". Specifically, it can trigger different signalling pathways that allow cells to adjust their gene expression programmes in response to glucose availability. Elucidating the molecular mechanisms of glucose response in eukaryotes has been greatly aided by studies conducted in the yeast *Saccharomyces cerevisiae*. This yeast shares with complex multicellular eukaryotes many of the signal transduction components that detect glucose, transmit the corresponding signals to the interior of the cell and make the needed adjustments to cellular metabolism and gene expression. In this manuscript, I will review the current knowledge of some aspects of glucose-mediated signalling in yeast and discuss how these results have contributed to the understanding of similar processes in mammalian cells.

2. INTRODUCTION

Glucose is the most preferred carbon source for the majority of living cells and its metabolism provides the energy necessary for cell viability. In addition, the presence of glucose is able to elicit a complex metabolic response by acting on two levels: i) allosteric modification of different enzymes, and ii) regulation of gene expression. In the first case, in yeast, glucose is able to induce a rapid loss of activity of the enzymes of the gluconeogenic pathway (e.g. fructose 1,6-bisphosphatase) and several sugar transporters (e.g. high affinity glucose transporters, the galactose permease and the maltose permease), and stimulate an increase in the rate of degradation of these proteins by a process known as catabolite inactivation [(1), (2), (3), (4), (5)]. In the second case, glucose is able to induce the expression of several genes, such as those encoding glycolytic enzymes, ribosomal proteins and some glucose transporters, by a process known as glucose induction. At the same time, glucose is also able to repress the expression of a large number of genes, including those involved in the utilization of alternative carbon sources, gluconeogenesis and respiration, by a process known as glucose repression [see (6) for review].

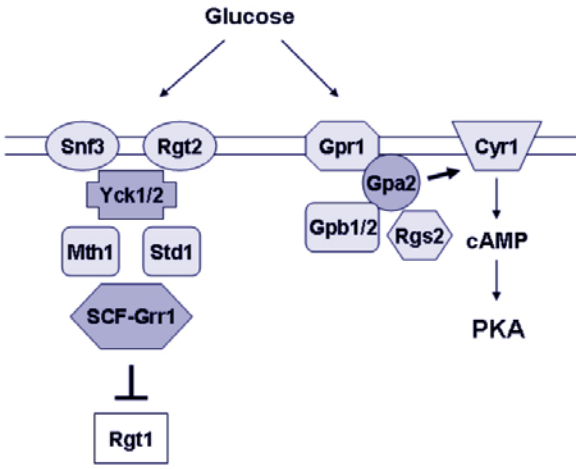

Figure 1. Yeast plasma membrane glucose sensors and their signalling cascades. The yeast plasma membrane glucose sensors Snf3/Rgt2 and Gpr1 and the identified components of their signalling cascades are described. See text for details.

In mammalian cells, glucose is also able to stimulate similar metabolic responses. Moreover, these organisms have developed the extraordinary ability to maintain a constant level of glucose in the bloodstream (around 5 mM). This stability is achieved by the coordinated action of different hormones such as insulin and glucagon, which affect glucose responsive tissues in order to maintain glucose homeostasis. How mammalian cells respond to fluctuations in glucose levels is a major issue, since alterations in this response might contribute to pathological conditions such as obesity and type 2 diabetes. In the study of this process of glucose sensing and response, the use of the yeast *Saccharomyces cerevisiae* has proved to be very useful, since this yeast shares with complex multicellular eukaryotes many of the signal transduction components that detect glucose, transmit the corresponding signals to the interior of the cell and make the needed adjustments to cellular metabolism and gene expression. In addition, *S. cerevisiae* has the advantage of a rapid growth, the ease of using different genetic techniques, a well-defined genetic system and a highly versatile DNA transformation system (7). The genome of this yeast was the first to be completely sequenced. Subsequently, this yeast became one of the key organisms for genomic research, including extensive studies on transcriptome profiling (8), global analysis of gene function by gene disruption (mutants for each ORF have been produced) (9), of protein localization (10), of 2-D protein maps (11), of protein-protein interactions by two-hybrid [(12), (13)] and tandem affinity purification methods [(14), (15), (16), (17)] and of protein phosphorylation [(18), (19)]. All these studies have generated a tremendous amount of information that makes *S. cerevisiae* the organism of choice for the study of complex regulatory networks.

3. EARLY STEPS IN GLUCOSE SIGNAL TRANSDUCTION: GLUCOSE SENSING

In nature, the yeast *Saccharomyces cerevisiae* has to deal with an extremely broad range of sugar concentrations, from higher than 1.5 M (as in drying fruits) down to micromolar concentrations. To be able to adapt to fluctuations in the sugar concentration of the environment, this yeast has developed an unusual diversity of hexose transporter proteins (17 different Hxt's and Gal2), with specific individual properties and kinetics. *S. cerevisiae* has from low affinity hexose transporters, such as Hxt1 and Hxt3 (Km from 50 to 100 mM), that function when there is a good supply of sugar, to intermediate affinity transporters, such as Hxt2 and Hxt4 (Km around 10 mM), and high affinity transporters, such as Hxt6 and Hxt7 (Km around 1 mM), that function when the amount of sugar is scarce. The expression of these transporters is tightly regulated at the level of transcription by glucose signalling pathways that are triggered by the amount of substrate present in the environment. In this way, yeast express only the sugar transporters that are required for each environmental condition. For example, the expression of *HXT1*, a gene encoding a low affinity glucose transporter, is induced in the presence of glucose, whereas the gene expression of the intermediate and high affinity glucose transporters is repressed by the presence of the sugar [reviewed in (6), (20), (21)].

Two putative glucose sensors, Snf3 and Rgt2, are involved in the glucose-response regulation of the expression of the *HXT* genes (Figure 1). They belong to the family of hexose transporters found in bacteria, plants and mammals and contain the typical 12 predicted transmembrane spanning domains. However, they are not able to transport glucose, but instead they signal the presence of the sugar; Snf3 is a high affinity glucose sensor which is activated by low concentrations of the sugar, whereas Rgt2 is a low affinity glucose sensor, which is active when there is a good supply of the sugar. Snf3 and Rgt2 contain unusually large cytosolic C-terminal tails that are critical for glucose sensing (deletion of these tails results in absence of glucose sensing activity). The fact that Snf3 and Rgt2 do not transport glucose and the fact that they have critical cytosolic C-terminal tails suggest that the mechanism by which they sense glucose is not related to the uptake of the sugar, but instead to the binding of glucose to extracellular domains of these two sensors and to the production of conformational changes in the cytosolic tail. This suggestion is confirmed by the isolation of constitutively active *SNF3-1* and *RGT2-1* mutants, containing similar substitutions (R229K and R213K, respectively) in the cytosolic loop of the fifth transmembrane domain, that are able to trigger the glucose signal in the absence of the sugar [reviewed in (6), (20), (21), (22), (23)]. Snf3 and Rgt2 have non-redundant functions. Snf3 regulates the expression of the high affinity glucose transporters, whereas Rgt2 regulates the expression of the low affinity glucose transporters (24).

Yeast contains another glucose sensing mechanism that employs a G-protein coupled glucose receptor [(25), (26)] (Figure 1). Gpr1 is a member of the G-protein coupled seven-transmembrane spanning receptor superfamily. It interacts with the Galpha subunit Gpa2 and its GTPase-activating protein (GAP), Rgs2 [(27), (28)]. Gpa2 also interacts with Gpb1 and Gpb2, two putative

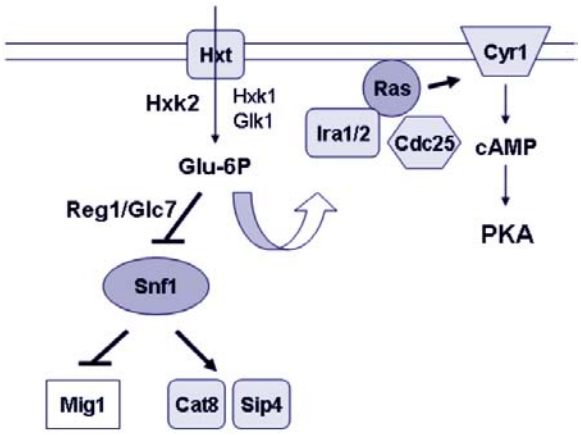

Figure 2. Glucose signalling mechanisms dependent on glucose uptake and phosphorylation. The identified components of the Snf1 protein kinase-dependent glucose repression pathway and the Ras pathway are described. See text for details.

Gbeta subunits (no Ggamma subunit has been identified yet) (29). Triggering of the glucose signal by this system does not require the entry of the sugar inside the cell. It is assumed that Gpr1 detects extracellular glucose and thereby activates Gpa2. This suggestion has been recently confirmed by the isolation of a constitutively activated form of Gpa2 that triggers the glucose signal in the absence of the sugar (30). Activation of Gpa2 stimulates production of cAMP by adenylate cyclase (Cyr1), which in turn leads to the activation of protein kinase A (PKA).

In yeast, PKA can be activated by an alternative pathway, which involves the monomeric G-protein, Ras, its corresponding GTP-exhange factor (GEF), Cdc25, and its GTPase-activating protein (GAP), Ira1/Ira2 (Figure 2). Ras is normally palmitoylated, which anchors it to the cytoplasmic face of the plasma membrane (31). Activation of Ras stimulates the production of cAMP by adenylate cyclase (Cyr1), which subsequently leads to the activation of PKA. This pathway is not dependent on Gpr1, but instead appears to be activated by intracellular phosphorylated glucose, since it does not function in the absence of glucose phosphorylating enzymes (Hxk2, Hxk1 and Glk1) (32). Glucose is only necessary to activate Ras and not for downstream events, since a constitutively active form of Ras can trigger the glucose signal in the absence of the sugar (30).

In mammalian cells, the presence of a putative glucose sensor at the plasma membrane is poorly defined. Several lines of evidence suggest that the glucose transporter GLUT2 may be involved in glucose signalling. For example, in insulinoma cell lines lacking GLUT2, the secretion of insulin is regulated by glucose only if GLUT2, but not GLUT1, is expressed in these cells (33). Other authors observed that a 70-80% reduction in GLUT2 expression in beta-cells of transgenic mice expressing GLUT2 antisense RNA, leads to a decreased glucose-induced insulin secretory response and to diabetes, although transport activity is not limiting for glucose metabolism (34). Additionally, it has been described that GLUT2 has a large intracytoplasmic loop (between the sixth and seventh transmembrane spanning domains) that is involved in glucose signalling. Overexpression of this loop prevents the induction of the expression of several genes (GLUT2, L-puryvate kinase) by glucose (35). Two-hybrid analysis using this loop as bait has identified karyopherin-alpha2 as a binding partner. The interaction is specific since the same loop from GLUT1 is not able to interact with karyopherin-alpha2. Since glucose leads to the exit of karyopherin-alpha2 from the nucleus to the cytosol, the authors suggest that karyopherin-alpha2 would then bind to GLUT2 at the plasma membrane and there load the cargo to be imported to the nucleus under low glucose conditions, to regulate glucose-sensitive gene expression [(36), (37)].

Another putative mammalian glucose sensor is SGLT3/SLC5A4, a member of the sodium/glucose co-transporter gene family. SGLT3 is incapable of sugar transport, but glucose causes a specific Na^+-dependent depolarization of the membrane potential, suggesting that SGLT3 may be a glucose sensor in the plasma membrane of cholinergic neurons, skeletal muscle and other tissues (38).

4. GLUCOSE INDUCTION PATHWAY

In the presence of glucose, the yeast *S. cerevisiae* is able to induce the expression of several genes, such as those encoding glycolytic enzymes, ribosomal proteins and some glucose transporters [for review see (6), (21), (39)]. As mentioned above, the yeast *HXT1* gene encodes a low affinity glucose transporter whose expression is induced in the presence of glucose and repressed when the levels of glucose are scarce. In the last decade, the expression of this gene has been used as a model to characterize the process of induction of gene expression by glucose. Genetic and biochemical studies have defined several components that are involved in the regulation of *HXT1* expression (Figure 1). Glucose availability in the surrounding media is assessed by the sensor proteins Snf3 and Rgt2 (see above) and the generated signal is transmitted through the SCF-Grr1 ubiquitin-protein ligase complex [(40), (41)] to Rgt1, a transcription factor belonging to the Cys6-zinc cluster protein family that acts as a repressor of the expression of the *HXT1* when glucose is absent (42). Other components of the glucose signalling pathway are Std1 and Mth1, two proteins that bind to Rgt1 and reinforce its repressor activity (43). Recent studies indicate that Std1 and Mth1 may also interact with the C-terminal tails of the glucose sensors Snf3 and Rgt2 [(44), (45)]. The signal generated by the glucose sensors activates the yeast protein casein kinase I (Yck1/Yck2), a protein kinase that is tethered to the membrane through a C-terminal palmitate moiety and that also interacts with the glucose sensors (46). Yck1/Yck2 phosphorylates Std1 and Mth1, allowing them to be recognized and ubiquitinated by the SCF-Grr1 complex, leading to their degradation. The degradation of Std1 and Mth1 allows for the glucose-induced dissociation of Rgt1 from the *HXT1* promoter, derepressing its expression [(46), (47), (48), (49), (50)].

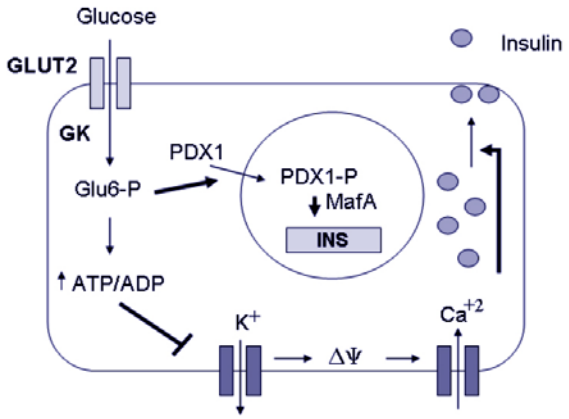

Figure 3. Glucose signalling in pancreatic beta-cells. Glucose is able to promote the secretion of insulin and also to induce the expression of the insulin (INS) gene. See text for details.

Recently, the 14-3-3 proteins Bmh1 and Bmh2 and the TOR kinase pathway have been described as additional components of the glucose induction pathway (51). In combination with the glucose signalling pathway, regulation of *HXT1* expression also requires a functional HOG (high osmolarity glycerol) pathway. Genetic analyses indicate that the HOG pathway modulates *HXT1* expression through regulation of the Sko1-Tup1-SSn6 repressor complex. Thus, the activity of two independent signalling pathways, glucose signalling and the HOG pathway, converge on the regulation of *HXT1* expression by glucose and osmostress (52).

A recent transcriptome profiling report indicates that the glucose signalling pathway defined by Snf3/Rgt2-Rgt1 regulates relatively few genes, suggesting that this pathway is primarily dedicated to regulating the expression of *HXT* genes. This pathway also induces the expression of *STD1* (53), thus providing a feedback regulation since glucose inhibits Std1 function by stimulating its degradation while at the same time induces *STD1* expression through the Snf3/Rgt2-Rgt1 signalling pathway. In this way, the cell is prepared for a rapid reestablishment of repression upon glucose exhaustion [(21), (50)].

In mammalian cells, the coordinated action of different hormones such as insulin and glucagon, maintain glucose homeostasis. The production of these two hormones is also regulated by the levels of glucose in the bloodstream. For example, beta-cells adjust the production and secretion of insulin depending on the levels of glucose. It is generally accepted that no particular glucose sensor exists at the plasma membrane of beta-cells, but instead they respond to fluctuations in the rate of glucose metabolism. These cells have a low affinity glucose transporter (GLUT2) in the membrane and a low affinity glucose phosphorylating enzyme (Glucokinase, GK), which allows them to establish a direct relationship between blood-glucose levels and the formation of Glu-6P inside the beta-cells. Since the rate of glycolysis depends mainly on the kinetic properties of glucokinase, this enzyme has been considered as the "glucose sensor" of these cells. When the levels of blood glucose increase, the intracellular levels of Glu-6P increase, which leads to an improvement in the rate of glycolysis, resulting in an increase in the ATP/ADP ratio. High levels of ATP inhibits the ATP-sensitive K^+ channels, which produces a depolarization of the plasma membrane, the opening of Ca^{++} channels and an increase in the levels of intracellular Ca^{++}. This ionic response triggers the docking of secretory vesicles containing insulin to the plasma membrane and the release of the hormone to the blood stream (Figure 3) [(54), (55), (56)]. In addition to insulin secretion, glucose stimulates insulin gene expression by activation of the PDX1 (pancreatic/duodenal homeobox 1) transcription factor, via the stress-activated protein kinase 2 (SAPK2). In low glucose, PDX1 exists as an inactive protein in the cytosol; high glucose results in the activation of PDX1 by phosphorylation and its translocation inside the nucleus, where it activates the expression of the insulin gene (Figure 3) [(57), (58), (59)]. MafA (a component of the Maf superfamily of transcriptional regulators) is also a crucial regulator of insulin expression; PDX1 and MafA act synergistically and interact with the beta-cell specific factor BETA2, to promote insulin expression (60).

Glucose is also able to trigger independent signals in tissues other than beta-cells. For example, liver is one of the best studied tissues in which glucose acts as a transcriptional inducer. In this tissue, glucose is able to stimulate the expression of glycolytic and lipogenic genes such as L-pyruvate kinase (L-PK), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), spot 14, among others [(39), (61), (62), (63), (64)]. The promoters of these glucose-induced genes contain a carbohydrate response element (ChoRE), composed of two E boxes, with the consensus sequence of CACGTG, separated by 5bp (65). Recently, a specific transcription factor of the basic helix-loop-helix/leucine zipper (bHLH/LZ) family that binds to the ChoRE of the glucose-induced genes has been described. The DNA-binding properties of this ChoRE-binding protein (ChREBP) are enhanced in the presence of glucose. The activity of this transcription factor is regulated by phosphorylation; AMPK and PKA phosphorylate and inactivate ChREBP, whereas PP2A dephosphorylates and activates ChREBP [(66), (67), (68)]. The activity of the PP2A phosphatase is positively regulated by the levels of xylulose-5P, which provides a direct relationship between glucose metabolism and the activation of ChREBP (69). The cellular localization of ChREBP is also regulated by glucose; in the presence of high glucose, ChREBP is predominantly nuclear, whereas in low glucose conditions, most of the ChREBP is in the cytosol (70). Recently, it has been described that ChREBP interacts with Mlx (Max-like protein X) and forms a heterodimer that regulates the expression of glucose-induced genes (71).

5. GLUCOSE REPRESSION PATHWAY

In the presence of glucose, the yeast *S. cerevisiae* represses the transcription of a large number of genes, including those involved in the utilization of alternative carbon sources, gluconeogenesis and respiration [reviewed in (6), (39), (72), (73)]. Biochemical and genetic studies

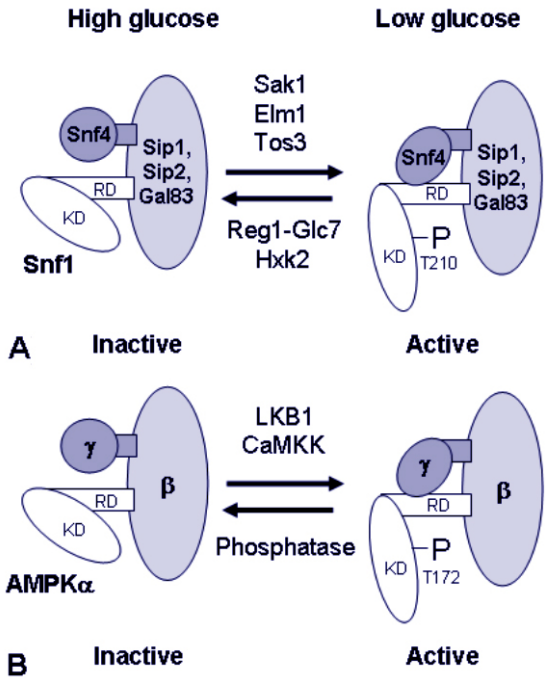

Figure 4. Activity of the Snf1 and AMPK complexes is regulated by phosphorylation and Snf4-dependent rearrangement. A) Snf1 complex; B) AMPK complex. See text for details.

have identified the Snf1 protein kinase as a master player in the glucose repression pathway (Figure 2). Snf1 is a serine/threonine protein kinase that activates transcription by inhibiting transcriptional repressors (e.g. Mig1) or by stimulating transcriptional activators (e.g. Cat8 and Sip4). Snf1 is the yeast homologue of mammalian AMP-activated protein kinase (AMPK) (74), which acts as a sensor of the energy status of the cell (see below). The Snf1 protein kinase (AMPKalpha subunit) is found in complexes containing the activating subunit Snf4 (AMPKgamma subunit) and members of the Sip1/Sip2/Gal83 family (AMPKbeta subunit) (75). Two different domains have been defined in the Snf1 protein kinase: the N-terminal catalytic domain (KD) and the C-terminal regulatory domain (RD). Both, the activating subunit Snf4 and members of the Sip1/Sip2/Gal83 family interact with the C-terminal regulatory domain of Snf1. The interaction with Sip1/Sip2/Gal83 is constitutive, but the interaction with Snf4 is regulated by glucose, increasing under low glucose conditions. Under these conditions, the N-terminal catalytic domain is released from its binding to an autoinhibitory region located in the regulatory domain, adopting an active, open conformation (76) (Figure 4A). The activity of Snf1 is regulated by phosphorylation at residue Thr210 within the catalytic domain (77). This phosphorylation is mediated by three different upstream kinases: Sak1, Elm1 and Tos3 [(78), (79), (80)]. Sak1 has been shown to be the most important upstream kinase in the glucose repression pathway [(81), (82)]. The activity of the Snf1 kinase is regulated by glucose: in low glucose growing cells, the Snf1 kinase is active (phosphorylated), whereas in high glucose containing media, the Snf1 kinase is inactive (dephosphorylated) (76) (Figure 4A). Signalling requires uptake and phosphorylation of the sugar, but unlike mammalian AMPK, Snf1 is not activated directly by AMP (83). Since the activity of the three upstream kinases seems to be constitutive, the regulation of the phosphorylation status of Snf1 must depend on the phosphatase involved in the dephosphorylation of Thr210. In this sense, some reports indicate that the yeast PP1 protein phosphatase, Glc7, and its regulatory subunit, Reg1, are responsible for the dephosphorylation and inactivation of the Snf1 protein kinase, in response to glucose (Figure 4A) [(84), (85)]. In the absence of Reg1, Glc7 can not perform its function, so the Snf1 kinase complex is constitutively in the active, phosphorylated state, even in the presence of glucose [(84), (85)].

Another crucial component of the yeast glucose repression pathway is hexokinase PII (Hxk2). In addition to its role as glucose phosphorylating enzyme of the glycolytic pathway, Hxk2 also participates in the regulation of the activity of the Snf1 protein kinase complex (86). Interestingly, the two other glucose phosphorylating enzymes present in yeast (Hxk1 and Glk1) are not involved in the regulation of Snf1, so Hxk2 has particular Snf1-regulating properties not shared by the other glycolytic enzymes. In the absence of Hxk2, the Snf1 kinase complex is maintained in the active phosphorylated state, even in the presence of glucose (85). The function of Hxk2 is mediated by Reg1, since overexpression of Reg1 can complement *hxk2* deficiency. In fact, Hxk2 regulates the phosphorylation status of Reg1 (85). A hypothesis has been formulated on how the phosphorylation status of Snf1 kinase is regulated (85). In low glucose growing cells, the Snf1 kinase is in the open active phosphorylated state, since the Reg1/Glc7 phosphatase is inactive, possibly because under these conditions, the Snf1 kinase interacts with and phosphorylates Reg1, maintaining it in a bound, latent state. Hxk2 is required for maintaining Reg1 in this phosphorylated state; in the absence of Hxk2, Reg1 is dephosphorylated by Glc7 and this dephosphorylated form of Reg1 is not competent to target Glc7 to dephosphorylate Snf1 kinase. In response to a glucose signal, the Reg1/Glc7 complex is activated, allowing the dephosphorylation of the Snf1 kinase, which adopts a closed, inactive conformation (85).

As indicated above, the active Snf1 kinase can phosphorylate and inactivate the Mig1 transcriptional repressor. Mig1 is a C2H2 zinc finger transcriptional factor involved in the glucose-repressed expression of genes involved in the metabolism of alternative carbon sources and gluconeogenic genes, among others. The phosphorylation of Mig1 leads to its exit from the nucleus (87), although this process is not completely necessary to inhibit Mig1 repressor function. In cells lacking the beta-importin Msn5 (involved in the export of Mig1 from the nucleus to the cytosol), Mig1-P is retained in the nucleus, but has no repressor activity [(88), (89)].

The Snf1 protein kinase also regulates a number of transcriptional activators required for the utilization of non-fermentable carbon sources. Of special relevance are

Cat8 and Sip4. Both proteins are C6 zinc cluster transcription factors that bind to carbon source responsive elements (CSRE) present in the promoters of genes related to ethanol utilization, gluconeogenesis, lactate utilization and isocitrate metabolism [(90), (91)]. Phosphorylation of these two factors by Snf1 enhances their transcriptional activator properties [(91), (92)]. Snf1 is also able to activate Adr1, a C2H2 zinc finger transcriptional activator involved in the regulation of *ADH2*, *ACS1* and *ALD6* genes, related to the utilization of ethanol as a non-fermentable carbon source [(93), (94)].

As mentioned above, the mammalian homologue of Snf1 is AMP-activated protein kinase (AMPK). This kinase is activated in an ultrasensitive manner by cellular stresses that deplete ATP (increasing then AMP levels), either by inhibiting ATP production (hypoxia, glucose deprivation, heat shock, inhibition of mitochondrial oxidative phosphorylation, etc.) or by accelerating ATP consumption (exercise, etc.). Once activated, AMPK switches on catabolic pathways and switches off many ATP-consuming processes (anabolic pathways) [reviewed in (95), (96), (97)]. Similarly to Snf1, AMPK is a heterotrimer composed of three different subunits: AMPKalpha (Snf1), AMPKbeta (Sip1/Sip2/Gal83) and AMPKgamma (Snf4). Each subunit has several isoforms (alpha1 and alpha2; beta1 and beta2; gamma1, gamma2 and gamma3) yielding at least 12 heterotrimeric combinations that are present in different amounts depending on the tissue. Studies carried out with the Snf1 yeast homologue have greatly aided the understanding of the regulation of the activity of AMPK and also the structural basis of the interaction between the different subunits. We know that similarly to what happens in the yeast Snf1 complex, the AMPKalpha catalytic subunit also has a highly conserved N-terminal kinase domain (KD), including the catalytic site, and a C-terminal regulatory domain (RD) that also contains an autoinhibitory region. The AMPKgamma subunit contains four tandem repeats of a structure module called CBS, described initially in cystathionine-beta-synthase, that is involved in binding of the allosteric regulator AMP (98); these repeats are also present in yeast Snf4, although the yeast Snf1 complex is not regulated allosterically by AMP (83). The AMPKbeta subunit functions as a scaffold to assemble alpha- and gamma-subunits and also determines the subcellular localization and substrate specificity of the complex [(99), (100)]; the mammalian beta-subunit contains a glycogen binding domain (GBD) [(101), (102)], whereas yeast Sip1/Sip2/Gal83 contain a similar domain originally referred as KIS domain (kinase interacting domain), because it was the region involved in Snf1 binding (75). Similarly to what happens in the Snf1 complex, the interaction between AMPKalpha and AMPKgamma is also regulated by glucose, being increased when the levels of glucose are scarce (99) (Figure 4B).

In yeast, regulation of the activity of the Snf1 complex involves two steps, one that requires phosphorylation of the Snf1 catalytic subunit (alpha-subunit) by an upstream protein kinase and one that is mediated by the interaction between Snf4 (gamma-subunit) and Snf1 (alpha-subunit). Phosphorylation of Thr210 can occur independently of the Snf4 step, but to achieve full activity, the complex has to undergo a Snf4-dependent rearrangement (77). After these two steps, the phosphorylated Snf1 kinase (T210-P) opens its active site, making it accessible to substrates. Regulation of AMPK activity follows a similar trend, including phosphorylation of a conserved Thr172 residue of AMPKalpha by an upstream kinase and AMPKgamma-dependent allosteric regulation by AMP. The identification of the putative AMPK upstream protein kinase was a frustrating project until the discovery of yeast Snf1 upstream kinases (see above). Sak1, Elm1 and Tos3 are members of the ELM family of protein kinases (103). While none of them had a clear mammalian homologue, their kinase domains were most closely related to those of the protein kinase LKB1, and the calmodulin-dependent protein kinase kinases CaMKKalpha and CaMKKbeta. Evidence came rapidly indicating that all three, but especially LKB1 and CaMKKbeta, phosphorylated AMPKalpha at Thr172 and thus activated AMPK in vivo [(104), (105), (106), (107)] (Figure 4B). The relationship between the upstream kinases in yeast and mammals was reinforced when it was described that the expression in yeast of mammalian LKB1 and CaMKKalpha phosphorylated and activated Snf1 at its corresponding Thr210 site (108).

Studies on how these upstream kinases are regulated indicate that they are constitutively expressed and that increases in AMP do not stimulate their activity; only a rise in Ca^{++} activates CaMKK's [(105), (109)], thus leaving open the question of how phosphorylation of AMPKalpha is regulated. Perhaps, and as in the case of the yeast Snf1 complex, the regulation depends on the activity of the phosphatase involved in the dephosphorylation of Thr172. Despite several attempts made to characterize this phosphatase, its identity is still unknown. *In vitro* studies have identified PP1, PP2A and PP2C protein phosphatases as possible candidates [(110), (111)] (Figure 4B). In addition, it has also been described that PP2A is involved in the regulation of the interaction of AMPKalpha and AMPKgamma by glucose (99). Also, treatment of primary hepatocytes with different protein phosphatase inhibitors (okadaic acid, microcystine, calyculin A, cantharidin, and tautomycin) lead to the accumulation of active, phosphorylated AMPKalpha, similarly to that observed upon AICAR treatment (an allosteric activator of AMPK). In the presence of naringin, a flavonoid antagonist of okadaic acid action, the phosphorylation of AMPKalpha induced by okadaic acid, microcystin and AICAR was prevented, but not that produced by calyculin A or cantharidine. These data suggest that AMPKalpha may be activated by two independent mechanisms, one being naringin-sensitive (by inhibition of PP2A phosphatases) and the other being naringin-resistant (by inhibition of PP1 phosphatases) (112).

It is worth pointing out that as in the case of the yeast Snf1 complex, the activity of the AMPK complex is also regulated by glucose. High levels of glucose decrease AMPK activity in both beta-cells (113) and skeletal muscle (114). On the other hand, low levels of glucose improve

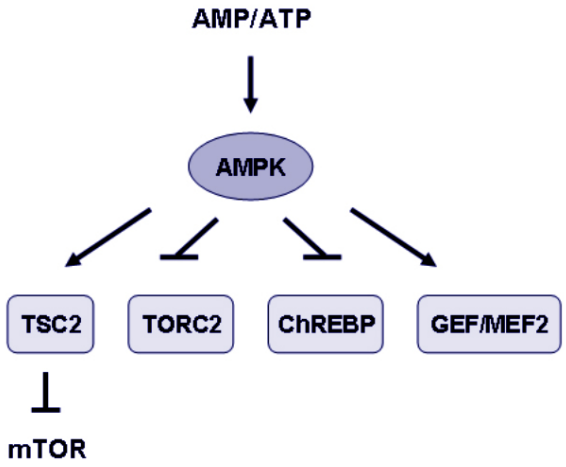

Figure 5. AMPK targets related to glucose signalling. AMPK is able to activate TSC2, the negative regulator of the mTOR kinase; it also activates GEF/MEF2 transcription factors, leading to the expression of GLUT4; it inhibits TORC2, leading to the inhibition of the expression of gluconeogenic genes; it inhibits ChREBP, leading to the inhibition of the expression of glucose-induced genes. See text for details.

AMPK activity. The activation correlates with an increase in the phosphorylation of T172, without change in the AMP/ATP ratio (114) (Figure 4B).

Activation of the AMPK complex leads to both short term (post-translational modification of target proteins) and long term (transcriptional regulation of gene expression) effects. Among the broad variety of AMPK targets, I will focus my discussion on those targets related to glucose regulation of gene expression (Figure 5):

a) AMPK decreases the expression of glucose-induced genes such as ACC1 (acetyl-CoA synthase), FAS (fatty acid synthase), Spot 14, L-PK (L-type pyruvate kinase) and preproinsulin (PPI) [(115), (116), (117)]. However, the overexpression of a dominant negative form of AMPK, which results in inhibition of AMPK activity, does not cause an increase in the expression of glucose-induced genes; therefore, AMPK is involved in the repression of glucose-induced genes, but not in their induction (117). The inhibition of the expression of glucose-induced genes by AMPK is mediated by the inactivation of the carbohydrate-responsive element binding protein (ChREBP). AMPK phosphorylates ChREBP at Ser568 and this modification inactivates its DNA binding activity and consequently downregulates the expression of ChREBP target genes (67).

Activation of AMPK also leads to a decrease in SREBP-1 (sterol-responsive element-binding protein) expression, which in turn leads to a decrease in hepatic gene expression of FAS, L-PK and S14 (118).

b) AMPK decreases expression of gluconeogenic genes encoding PEPCK (phosphoenol pyruvate carboxykinase) and glucose-6P phosphatase, in an insulin-independent manner and thus helps to reverse the hyperglycemia associated with type 2 diabetes (119). A major positive regulator of gluconeogenic genes is peroxisome proliferator-activated receptor-gamma co-activator 1alpha (PGC1alpha) (120). The expression of PGC1alpha is positively regulated by CREB (cAMP-response element binding protein), whose activity is regulated positively by TORC2 (transducer of regulated CREB activity 2). Therefore, the expression of PGC1alpha is directly linked to the activity of TORC2 (121). TORC2 activity is down-regulated by phosphorylation, which allows its binding to cytosolic 14-3-3 proteins, which maintains TORC2 sequestered in the cytoplasm. Among other protein kinases, AMPK phosphorylates TORC2 at Ser171, promoting its sequestration by 14-3-3 proteins in the cytoplasm. AMPK activation leads to lower TORC2 activity, which in turn leads to lower levels of PGC1alpha and a decrease in the expression of gluconeogenic genes. On the contrary, a decrease in AMPK activity leads to more unphosphorylated, nuclear TORC2 that may activate CREB and then PGC1alpha [(122), (123)]. Insulin also increases the phosphorylation of TORC2, whereas glucagon suppresses it. Therefore, TORC2 might serve as the gluconeogenic "molecular switch" that senses hormones and cellular energy status (124).

c) AMPK increases expression of GLUT4 (glucose transporter 4), hexokinase and mitochondrial enzymes [(125), (126), (127), (128)]. In this way, AMPK activation improves glucose uptake, phosphorylation and oxidation of plasma glucose. The increase of the expression of GLUT4 is mediated by phosphorylation of GEF (GLUT4 enhancer factor), that then binds to MEF2 (Myocyte enhancer factor 2) and the complex translocates to the nucleus where it binds to the GLUT4 promoter, activating its expression (129).

6. GLUCOSE INDUCTION AND GLUCOSE REPRESSION ARE INTERCONNECTED

The processes of glucose repression and glucose induction can not be considered as independent pathways because they are clearly interconnected. For example, expression of components of the yeast glucose induction pathway, such as Mth1 and Snf3, are repressed by glucose (45). Moreover, the deletion of Snf3 and Rgt2 glucose sensors produces defects both in glucose induction of *HXT* genes (see above) and also in glucose repression. The absence of the glucose sensors prevents the expression of the hexose transporters, which precludes the entry of glucose inside the cell. The absence of intracellular glucose leads to the derepression of glucose-repressed genes. This interconnection was confirmed by expression of *HXT1* glucose transporter under the control of a constitutive *ADH1* promoter. A double *snf3 rgt2* mutant expressing this construct was then able to grow in glucose and glucose repression was restored, although it was still defective in glucose induction. Therefore, there are two different signals that regulate the induction and repression of gene expression by glucose. Glucose induction requires the Snf3 and Rgt2 glucose sensors, whereas glucose repression requires the internalization and further phosphorylation of

the sugar (130). In addition, it has been described that the Snf3/Rgt2-Rgt1 pathway induces the expression of *MIG2*, encoding a Snf1-independent repressor of the glucose repression pathway, suggesting that the Snf3/Rgt2-Rgt1 pathway also contributes to glucose repression of gene expression (53).

Another example of the interconnection between the glucose induction and repression pathways is the involvement of the Snf1 protein kinase in the regulation of the induction of *HXT1* by glucose. Activation of the Snf1 protein kinase in glucose growing cells (by the use of *hxk2* or *reg1* mutants) prevents *HXT1* induction by glucose. Therefore, Snf1 not only activates the expression of genes involved in the assimilation of alternative carbon sources, but it also represses the expression of genes induced by glucose (131). In this way, Snf1 would mimic the effect of its mammalian counterpart, AMPK, in transcriptional regulation, since, as described above, in mammalian hepatocytes active AMPK inhibits the expression of glucose-induced genes [(116), (117), (132)].

Finally, it has been described that the overexpression of Std1 (a component of the glucose signalling pathway) is able to activate the Snf1 protein kinase by its interaction with the kinase catalytic domain and promotion of an active conformation of the kinase [(131), (133)].

7. RELATIONSHIP BETWEEN GLUCOSE REPRESSION AND THE TOR KINASE PATHWAYS

In yeast, another process that controls growth and metabolism in response to nutrient availability is the TOR (target of rapamycin) kinase pathway. When growth conditions are favorable, the TOR kinase pathway is active and promotes anabolic processes and antagonizes catabolic processes. Inhibition of the TOR pathway results in physiological changes that resemble a starvation state. These changes are both short term effects, including inhibition of protein synthesis, among others, and long term effects, such as a specific reprogramming of gene expression. The key players in this pathway are the TOR protein kinases, Tor1 and Tor2, members of the phosphatidylinositol-3 kinase related kinase family. This pathway is inhibited by nitrogen starvation and by the drug rapamycin, a macrolide antibiotic with antiproliferative and immunosupressive effects [reviewed in (134), (135), (136), (137)].

Although the TOR pathway responds especially to amino acid availability, it has been described that it may also control the transcriptional response related to changes in carbon source availability. As an example of the latter case, the TOR kinase pathway affects the subcellular localization of Msn2, a transcriptional activator of genes regulated by stress (STRE regulated genes), by regulating its interaction with Bmh2, a yeast member of the 14-3-3 protein family, that acts as a cytosolic anchor. Inhibition of the TOR kinase pathway results in nuclear accumulation of Msn2 [(138), (139)]. Recently, it has been described that active Snf1 is able to avoid the effects of rapamycin on the regulation of the subcellular localization of Msn2; therefore, active Snf1 and TOR kinases affect similar steps in the regulation of the subcellular localization of this activator of STRE (stress response element)-regulated genes (139).

The mammalian TOR kinase pathway (mTOR) also controls protein synthesis and other growth-related processes in response to growth factors (e.g. insulin), nutrients, energy and stress (137). The mTOR pathway responds to growth factors via the PI3K (phosphatidylinositol-3 kinase) pathway. The Akt/PKB (protein kinase B) kinase of this pathway phosphorylates and inactivates a negative regulator of mTOR named TSC2 (Tuberous sclerosis complex subunit 2) (140). The same inactivation of TSC2 is observed in mouse models containing a heterozygous loss-of-function of PTEN, the specific phosphatase that breaks down phosphatidylinositiol-3-phosphate, resulting in a constitutive activation of Akt/PKB (141). TSC2 is a GAP (GTPase-activating protein) of the small GTPase Rheb (Ras homologue enriched in brain) (142). Rheb binds directly to the kinase domain of mTOR and activates it in a GTP-dependent manner. By inactivating TSC2, the PI3K pathway maintains Rheb in its GTP-bound activated state, promoting in this way TOR kinase activity. Rheb can also be activated by nutrients, especially amino acids, that act either directly or by inhibiting TSC2 activity. On the other hand, mTOR becomes inactivated when the level of energy (ATP/AMP ratio) is low, by the action of AMP-activated protein kinase (AMPK). Activated AMPK directly phosphorylates TSC2 at a site different from the Akt/PKB site and enhances its GAP activity, leading to inhibition of mTOR signalling. Therefore, there is a clear antagonism between the AMPK and mTOR pathways (Figure 5) [(137), (141), (143), (144), (145)].

8. SUMMARY AND PERSPECTIVES

The evidence described in this manuscript suggests that glucose can trigger specific signalling pathways by different mechanisms. In some cases, external glucose binds to sensors at the plasma membrane and produces a conformational change in their structure that is transmitted to the cytosolic part of the sensor, which is then competent to transmit the glucose signal to the next component of the transduction cascade. This is the case of the yeast glucose sensors Snf3/Rgt2 and Gpr1 (Figure 1). In these cases, mutants have been isolated that are able to trigger the glucose signal in the absence of the sugar, which indicates that glucose, per se, is not the intracellular triggering signal. In these sensing mechanisms, the presence of glucose is transduced into the activation of a second protein component (Yck1/Yck2 in the case of the Snf3/Rgt2 system; Gpa2 in the case of the Gpr1 system) which initiates the signalling cascade. In the case of the Snf3/Rgt2-Rgt1 signalling pathway, the presence of glucose does not correlate with increases in any intermediate metabolite, whereas in the case of the Gpr1-Gpa2 sensor, the presence of glucose correlates with an increase in the levels of cAMP, which in turn activates PKA.

In other glucose signalling pathways, the sugar needs to be internalized and phosphorylated to be activated. This is the case of the yeast Snf1-dependent glucose repression pathway and the Ras-dependent activation of PKA (Figure 2). In these cases, the nature of the signal that activates each pathway is not clear yet. Is the signal the level of Glu-6P or the level of some other intermediate metabolite? More work in this area should provide new information about the nature of the signal. In mammalian cells, similar glucose uptake and phosphorylation-dependent mechanisms of glucose signalling exist. For example, in beta-cells, insulin secretion depends on the correct uptake of glucose by the glucose transporter GLUT2 and the correct phosphorylation by glucokinase. In these cells, evidence suggests that the ATP/ADP ratio is the signal that modifies the next step in glucose signalling (inhibition of ATP-sensitive K^+ channel by high ATP levels). However, the signal that activates PDX1 to promote insulin gene expression is still unknown. The AMP/ATP ratio is also the signal that activates mammalian AMPK. A high AMP/ATP ratio promotes the phosphorylation of the AMPKalpha catalytic subunit by making it a better substrate for upstream kinases and a poorer substrate for protein phosphatases and by inhibiting phosphatase activity. The regulation of the activity of AMPK has become an important topic and more work in this area is needed to identify the phosphatase(s) involved in AMPK regulation. In addition to changes in nucleotide levels, glucose also produces other metabolic signals. For example, in hepatocytes the signal of glucose induction of gene expression seems to be mediated by the levels of xylulose-5P, that activate a specific PP2A phosphatase involved in the regulation of the ChREBP transcriptional activator. However, additional work needs to be done to clarify whether xylulose-5P is the only signalling metabolite and whether Glu-6P might have an additional signalling role.

In summary, glucose is able to trigger different signalling pathways by different mechanisms. In some cases, it produces conformational changes in specific plasma membrane glucose sensors. In other cases, it modifies the concentration of some intermediate metabolites, which initiates the glucose signalling cascades. The understanding of how glucose is sensed and how cells respond to this signal will help in the prevention of pathological conditions such as obesity and type 2 diabetes.

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