**The early steps of glucose signalling in yeast**

Juana M. Gancedo

Department of Metabolism and Cell Signalling, Instituto de Investigaciones Biomédicas Alberto Sols, CSIC-UAM, Madrid, Spain

**Abstract**

In the presence of glucose, yeast undergoes an important remodelling of its metabolism. There are changes in the concentration of intracellular metabolites and in the stability of proteins and mRNAs; modifications occur in the activity of enzymes as well as in the rate of transcription of a large number of genes, some of the genes being induced while others are repressed. Diverse combinations of input signals are required for glucose regulation of gene expression and of other cellular processes. This review focuses on the early elements in glucose signalling and discusses their relevance for the regulation of specific processes. Glucose sensing involves the plasma membrane proteins Snf3, Rgt2 and Gpr1 and the glucose-phosphorylating enzyme Hxk2, as well as other regulatory elements whose functions are still incompletely understood. The similarities and differences in the way in which yeasts and mammalian cells respond to glucose are also examined. It is shown that in *Saccharomyces cerevisiae*, sensing systems for other nutrients share some of the characteristics of the glucose-sensing pathways.

**Introduction**

Adaptation to changes in the environment is a key process for the successful survival of organisms. The response of the cells to these changes is mediated by a large variety of signalling pathways that may be sorted into a few basic types, among which two are prominent. In one of them the first element in the pathway is a protein in the plasma membrane, able to bind a nutrient or a hormone and to adopt a new conformation that activates a cascade of reactions, affecting metabolic or regulatory enzymes, transcription factors, etc. (Forsberg & Ljungdahl, 2001a; Kroese *et al*., 2003; Holsbeeks *et al*., 2004; Mascher *et al*., 2006; Monge *et al*., 2006; Hao *et al*., 2007). The second type of pathway depends on the uptake of the nutrient and, in most cases, on its metabolism, which causes changes in the concentration of intracellular metabolites with a regulatory function. The metabolites may, in turn, interact with different kinds of proteins and modify their activity, their stability and/or their localization. The regulated proteins can have a variety of functions: they may have enzymatic activity, they may bind other proteins and they are often able to bind specific DNA regions and to modulate the transcription rate of the corresponding genes (Sellick & Reece, 2005).

An interesting example of the response of a cell to a change in the environment is the response of *Saccharomyces cerevisiae* to the presence of glucose. The mechanisms involved are particularly complex, as this sugar is not only a preferential carbon source for the organism but also a molecule that affects yeast physiology at many levels. In the presence of glucose, there are changes in the concentration of intracellular metabolites (Kresnowati *et al*., 2006), modifications and eventual degradation of some enzymes (Serrano, 1983; Gancedo & Gancedo, 1997; Portela & Moreno, 2006), and alterations in the stability of a number of mRNAs (Mercado *et al*., 1994; Cereghino & Scheffler, 1996; Scheffler *et al*., 1998; Yin *et al*., 2003; Kresnowati *et al*., 2006). In addition, the transcription of different genes is either induced or repressed (Gancedo, 1998; Johnston, 1999; Wang *et al*., 2004). This large remodelling of metabolism results in an increase in the rate of growth of the yeast (Johnston *et al*., 1979).

This review focuses on the early elements involved in glucose signalling in yeasts and on their relevance for the regulation of specific processes. There is a special emphasis on the role of the plasma membrane glucose-sensing proteins Snf3, Rgt2 and Gpr1, and of Hxk2 in *S. cerevisiae*, but reference is made to what is known for other species at the end of each section. Signalling involving intracellular metabolites, and other regulatory elements, such as the TOR (target of rapamycin) pathway or phospholipase C, are also discussed. The main targets for glucose signals considered are the genes repressed or induced by glucose, and the proteins activated, inactivated or degraded in the presence...
of glucose. Most data correspond to *S. cerevisiae* but reference to other yeast species is made, as far as information is available. The review also addresses briefly the question of how yeast senses the presence of other nutrients, and discusses elements shared between the sensing pathways used by different nutrients. Finally, similarities and differences between the glucose-sensing systems of yeast and mammalian cells will be highlighted.

**Sensing elements**

**Plasma membrane glucose-sensing proteins**

Any yeast protein able to bind glucose may, in principle, play a role in a glucose-signalling pathway. In the plasma membrane of *S. cerevisiae*, there is a large family of at least 20 glucose transporters (Wieczorke *et al.*, 1999), the most relevant being Hxt1 and Hxt3, with a low affinity for glucose and high transport capacity, and Hxt2, Hxt4 and Hxt7, with a high affinity and low transport capacity (Boles & Hollenberg, 1997). When glucose repression of the genes MAL2, SUC2 and GAL1 was measured in mutants expressing different sets of glucose transporters, it was found that the rate of glucose transport determined the strength of repression, but that no specific transporter was required for repression to take place (Reifenberger *et al.*, 1997). It has also been shown that one of the pathways of glucose signalling, involving cAMP, can be restored in an hxt1 to hxt6 null mutant by constitutive expression of GAL2, which encodes a galactose permease also able to transport glucose (Rolland *et al.*, 2000). It can be concluded therefore that the glucose transporters do not play a direct role in glucose signalling.

**Snf3 and Rgt2**

Snf3 and Rgt2 are plasma membrane proteins, with 12 transmembrane domains, highly similar to the Hxt glucose transporters (Neigeborn *et al.*, 1986; Özcan *et al.*, 1996), but unable to transport glucose themselves (Özcan *et al.*, 1998). Because they are required for the induction by glucose of the *HXT* genes (Özcan *et al.*, 1996), Snf3 and Rgt2 act likely as receptors that sense external glucose, but their capacity to bind glucose has not been demonstrated directly. Snf3 appears to be a sensor for low levels of glucose, as it is needed for the induction of *HXT* genes by low glucose, while Rgt2 would be a sensor for high levels of glucose, as it is required for maximal induction of *HXT1* by high glucose (Özcan *et al.*, 1996). Snf3 and Rgt2, unlike the yeast glucose transporters, have long C-terminal tails in the cytoplasm, which play an important role in glucose signalling (Özcan *et al.*, 1998; Dlugai *et al.*, 2001), but are not an absolute requirement, as shown by the fact that an overexpressed, tail-less Rgt2 can be functional (Moriya & Johnston, 2004). The replacement by a lysine of an arginine residue in Snf3 or Rgt2, conserved in all glucose transporters and situated in a cytoplasmic loop preceding the fifth transmembrane domain, allows total induction of *HXT2* and partial induction of *HXT1* by glucose (Özcan *et al.*, 1996). This suggests that the mutated receptors adopt a conformation similar to that of the glucose-bound Snf3 and Rgt2, independent of the carbon source present in the medium.

The schematic diagram of the regulation of *HXT* genes by Snf3/Rgt2, shown in Fig. 1, is based on the following observations.

![Snf3/Rgt2-signalling pathway](image-url)
There is strong experimental evidence that Rgt2 interacts with the membrane-bound, type I casein kinases Yck1 and Yck2 and, upon binding glucose, activates them (Moriya & Johnston, 2004); it is assumed that Snf3 acts in the same way. The activated casein kinases phosphorylate the regulatory proteins Mth1 and Std1 (Moriya & Johnston, 2004), and this reaction is facilitated by the recruitment of these proteins to the C tails of Snf3 and Rgt2 (Schmidt et al., 1999; Lafuente et al., 2000).

Mth1 and Std1 are paralogous proteins (Hubbard et al., 1994), which interact with Rgt1 (Tomás-Cobos & Sanz, 2002; Lakshmanan et al., 2003), a transcriptional repressor of genes induced by glucose (Flick et al., 2003; Kim et al., 2003; Palomino et al., 2005; Belinchón & Gancedo, 2007a). This interaction prevents the dissociation from the corresponding promoters of a repressing complex formed by Rgt1, Mth1/Std1 and the proteins Cyc8 and Tup1 (Polish et al., 2005). Serine-rich sequences have been identified in both Mth1 and Std1, which are possible targets for Yck1/Yck2 (consensus target sequence SXXS). When the serine residues are replaced by another amino acid, Mth1 and Std1 are converted into constitutive repressors, which are no longer degraded in the presence of glucose (Moriya & Johnston, 2004). This indicates that the phosphorylation of Mth1 and Std1 by Yck1/Yck2 targets them for degradation. In fact, Grr1, an F-box protein, component of an SCF ubiquitin ligase complex, and required for the induction of the HXT genes (Özcan & Johnston, 1995), binds phosphorylated Mth1 or Std1, which are then ubiquitinated by the SCF\textsuperscript{Grr1} complex, and degraded via the proteasome (Spielewoy et al., 2004; Kim et al., 2006). Although both Mth1 and Std1 are degraded in the presence of high glucose, cellular levels of Mth1 decrease much more than those of Std1. This is due to the fact that expression of MTH1 and STD1 is regulated in a different way: while under these conditions MTH1 is repressed, the repression of STD1 by Rgt1 is relieved (Kim et al., 2006).

The DNA-binding capacity of Rgt1 is lost when Rgt1 is hyperphosphorylated in the presence of glucose (Kim et al., 2003). This phosphorylation allows an intramolecular interaction between a central region of Rgt1 and its DNA-binding domain, thus preventing the binding of Rgt1 to DNA (Polish et al., 2005). Because, in the absence of Mth1 and Std1, Rgt1 no longer acts as a repressor (Flick et al., 2003; Lakshmanan et al., 2003), it has been proposed that binding of Mth1 to Rgt1 prevents its phosphorylation (Polish et al., 2005). Although Std1 is, to some degree, functionally redundant with Mth1, it appears to play a specific role that has not yet been clarified (Polish et al., 2005).

It should be stressed that HXT1 expression at high glucose concentrations is strongly reduced in an rgt1 mutant, and therefore Rgt1 acts formally as a transcriptional activator (Özcan & Johnston, 1995). Moreover, a fusion protein lexA-Rgt1 acts as an activator of the transcription of a lexO-lacZ reporter gene in a glucose-grown yeast (Kim et al., 2006). This contrasts with the observation that the binding of Rgt1 to the HXT1 promoter takes place only in the absence of glucose (Mosley et al., 2003). A possible interpretation for these, apparently contradictory, findings is that in yeast grown in a high-glucose medium, phosphorylated Rgt1 activates the transcription of a putative gene encoding a transcriptional activator of HXT1 or, less likely, inhibits the expression of a gene encoding an HXT1 repressor.

Glucose sensing through receptors similar to Snf3/Rgt2 is not restricted to \textit{Saccharomyces}. In \textit{Kluyveromyces lactis}, a single membrane protein, Rag4, appears to play the same role as Snf3/Rgt2 (Betina et al., 2001), and there is an Rgt1 orthologue, K\textit{IRgt1}, that represses \textit{RAG1}, a gene encoding a high-capacity, low-affinity glucose transporter (Rolland et al., 2006). A casein kinase 1, Rag8, has been found to bind Rag4, and both Rag4 and Rag8 are required to relieve repression by Rgt1, in the presence of high glucose, although, in contrast with the situation in \textit{S. cerevisiae}, Rgt1 remains bound to the \textit{RAG1} promoter under derepressing conditions (Rolland et al., 2006). Another difference between \textit{S. cerevisiae} and \textit{K. lactis} is that K\textit{IRgt1} is not required for maximal expression of \textit{RAG1} (Rolland et al., 2006). The gene \textit{KLLA0F15928g} encodes a protein similar to Mth1/Std1 (Génolevures (Sherman et al., 2006)), but its role in regulating \textit{RAG1} has not yet been ascertained. In \textit{Pichia angusta} (\textit{Hansenula polymorpha}), a hexose transport homologue, Gcr1, has been identified as being involved in glucose repression (Stasyk et al., 2004); there is no information, however, on other elements of the putative glucose-signaling pathway. In \textit{Candida albicans}, Hgt4 is the orthologue of Snf3/Rgt2 and there are also proteins similar to Std1 (orf19.6173) and to Rgt1, which act like the corresponding proteins of \textit{S. cerevisiae} (Brown et al., 2006). On the other hand, no obvious orthologues of the different regulatory proteins appear to be present in \textit{Schizosaccharomyces pombe}, while in \textit{Yarrowia lipolytica}, although gene \textit{YAL10B04796g} encodes a protein with some similarity to Mth1/Std1 [Génolevures (Sherman et al., 2006)], there are no clear relatives of Snf3/Rgt2 or Rgt1.

**Gpr1**

Gpr1 is a plasma membrane protein, with seven transmembrane domains, coupled to the G protein Gpa2 (Yun et al., 1997; Xue et al., 1998) and involved in the increase in cAMP levels triggered by glucose (Yun et al., 1998; Kraakman et al., 1999a; Rolland et al., 2002). The Gpr1-Gpa2 couple is responsive to glucose and to sucrose but not to other sugars such as fructose, 2-deoxyglucose or xylose; mannos acts as an antagonist (Rolland et al., 2000). Unexpectedly, the
system has a better affinity for sucrose than for glucose, as the effector concentration for half-maximum response (EC_{50}) is about 0.5 mM for sucrose and 20 mM for glucose (Lemaire et al., 2004). There is no direct evidence for the binding of glucose to Gpr1. However, the fact that mutants of Gpr1 have been obtained, deficient in glucose-induced, but not in sucrose-induced, cAMP increase, strongly suggests that there is a binding site for the sugars in Gpr1 and that some mutations in this site, such as A640C, Q644C or E648C, affect specifically the interaction with glucose (Lemaire et al., 2004). G proteins are usually heterotrimeric proteins consisting of α, β and γ subunits, and ligand binding to the G protein-coupled receptor stimulates a GDP to GTP exchange in the Gα subunit and the release of the GpGγ dimer. Gpa2 behaves as a Gα subunit but no canonical Gβ and Gγ subunits associate with it; instead Gpa2 forms a complex with proteins with a different structure, the kelch-repeat proteins Krh1/Gpb2 and Krh2/Gpb1 (Harashima & Heitman, 2002; Batlle et al., 2003). Gpa2 also interacts with the protein Rgs2 that stimulates the intrinsic GTPase activity of Gpa2, facilitating the formation of the inactive GDP-bound Gpa2 (Versele et al., 1999), but there is no information on a possible regulation by glucose of Rgs2 activity. It is thought that the binding of glucose to Gpr1 directs the formation of the GTP-bound, active form of Gpa2 (Fig. 2), and it has been shown that adenylate cyclase, Cyr1, binds to GTP-Gpa2 but not to GDP-Gpa2 (Peeters et al., 2006). This mechanism explains the rapid increase in cAMP levels observed when glucose is added to glucose-deprived cells. Increased levels of cAMP cause the activation of protein kinase A (PKA), as cAMP binds to the regulatory subunit Bcy1 and dissociates it from the alternative catalytic subunits Tpk1, Tpk2 or Tpk3, which then become active (Toda et al., 1987a, b). Because PKA phosphorylates a large number of proteins, it can mediate many of the effects produced by glucose, as it will be discussed below.

The regulatory role of the Krh1/Krh2 proteins is still subject to debate. They act as negative regulators of PKA (Lu & Hirsch, 2005) but two different mechanisms have been proposed to explain their effect. In the absence of Krh1/2 the interaction between Tpk1 and Bcy1 is reduced, as shown in a two-hybrid assay (Peeters et al., 2006). Therefore, Krh1/2 could facilitate the association between the regulatory and catalytic subunits of PKA. On the other hand, it has been found that Krh1/2 bind to a conserved C-terminal domain of Ira1 and Ira2, which function as GTPase-activating proteins on GTP-bound Ras (Tanaka et al., 1990), and stabilize them (Harashima et al., 2006). In the absence of Krh1/2, Ira1 and Ira2 are more readily degraded and elevated levels of Ras2-GTP lead to increased cAMP production. Both mechanisms are not mutually exclusive and it should be noted that the Gpr1-Gpa2 system is able to regulate the activity of PKA in an adenylate cyclase mutant where the system cannot control cAMP levels (Peeters et al., 2006). In this mutant, both a constitutively active GPA2 allele and a deletion of KRH1/2 lower the concentration of external cAMP required to allow yeast growth.

A particularly relevant substrate of PKA is the transcriptional repressor Rgt1, as the hyperphosphorylation of Rgt1 triggered by glucose integrates glucose signals relayed through Snf3/Rgt2 and Gpr1. It has been shown that after Rgt1 is phosphorylated by the Tpk3 isoenzyme (or by a Tpk3-dependent protein kinase), it dissociates from the HXK2 promoter, thereby relieving repression of the gene (Palomino et al., 2006). There is also direct evidence that the serine residues from Rgt1 located in consensus sequences for phosphorylation by PKA are required for the intramolecular

**Fig. 2.** Gpr1-signalling pathway. In the absence of glucose, Gpa2 and the Ras proteins are mainly in their GDP-bound, inactive form, their GTPase activity being stimulated by Rgs2 and Ira1/2, respectively. Protein kinase A (Tpk) has a low activity, as Krh1/2 favors its binding to the regulatory subunit Bcy1, and cAMP levels are low due to a reduced activity of adenylate cyclase (Cyr1). When glucose is present, it binds Gpr1, which then interacts with Gpa2, facilitating the formation of the GTP-bound, active form of the protein. Glucose metabolism also causes an activation of the Ras proteins, possibly due to inhibition of Ira1/2, and the activated Ras and Gpa2 stimulate together Cyr1 activity. In the presence of glucose, there is also a decrease in the activity of Krh1/2, which destabilizes Ira1/2 and facilitates the dissociation of the Bcy1-Tpk complex.
interaction that regulates the capacity of Rgt1 to bind DNA (Kim & Johnston, 2006).

In S. pombe, the proteins git3 and Spgpa2 have been identified as being involved in the activation of adenylate cyclase triggered by glucose, thus being functionally similar to Gpr1 and Gpa2 (Welton & Hoffman, 2000). However, the putative glucose-binding protein git3 has only a partial sequence homology with Gpr1 (Welton & Hoffman, 2000, and Spgpa2, able to bind to an N-terminal domain of adenylate cyclase (Ivey & Hoffman, 2005), is associated with canonic Gq and Gy subunits (git5 and git11), which are required for a normal response to glucose in S. pombe (Landry & Hoffman, 2001).

In C. albicans, there are conflicting reports on the role of the homologues of Gpr1 and Gpa2: in one case, they were found to be required for a glucose-dependent increase in cellular cAMP (Miwa et al., 2004), while in another it was concluded that Gpr1 and Gpa2 did not mediate glucose-induced cAMP signalling (Maidan et al., 2005). While there is a functional Gpa2 protein in K. lactis, involved in cAMP signalling (Saviñón-Tejeda et al., 1996), the role of a protein with homology to Gpr1, encoded by ORF KLLA0F24750g [Génolevures (Sherman et al., 2006)], has not been investigated. No information on a cAMP-signalling pathway in Y. lipolytica is available, although a gene search shows the existence of ORF YALI0A09592g encoding a protein similar to Gpa2 and of ORFs YALI0D13552g and YALI0F19426g encoding proteins with some similarities to Gpr1 [Génolevures (Sherman et al., 2006)].

**Hxk2**

Relevant intracellular proteins from S. cerevisiae able to bind glucose are the hexose kinases Hxk1, Hxk2 and Glk1 (Lobo & Maitra, 1977). It has long been known that in hxxk2 mutant strains, glucose repression of some genes such as SUC2 or GAL1 is very much reduced (Zimmermann & Scheel, 1977; Entian & Zimmermann, 1980). While it was initially proposed that Hxk2 has a specific regulatory domain required for glucose repression (Entian & Frohlich, 1984), later experiments with strains expressing mutated forms of Hxk2 showed a parallelism between glucose repression and the capacity of the strains to phosphorylate glucose, and suggested that Hxk2 plays only a metabolic role (Ma et al., 1989b; Rose et al., 1991). Although there have been more recent reports on mutant forms of Hxk2 with reduced catalytic activity but still functional in glucose signalling (Kraakman et al., 1999b; Mayordomo & Sanz, 2001), there is not yet evidence for a mutant form of Hxk2 devoid of catalytic activity but maintaining its regulatory capacity. New perspectives were provided when the intracellular location of Hxk2 was investigated, using isolated nuclei and specific antibodies or expressing an Hxk2-green fluorescent protein (GFP) fusion. It was found that a small proportion of Hxk2 is located within the nucleus (Herrero et al., 1998; Rández-Gil et al., 1998a) and that under conditions where Hxk2 does not enter the nucleus glucose repression of the genes SUC2, HXK1 or GLK1 does not take place (Herrero et al., 1998; Rodríguez et al., 2001). These results indicate a nonmetabolic role for Hxk2 that requires a nuclear localization.

Another question to be solved is the identification of the molecular target(s) for Hxk2. A first candidate was the protein kinase Snf1, required for the expression of glucose-repressed genes. When a high concentration of glucose is available to yeast cells, Snf1 is inactivated (Carlson, 1999) and it was assumed that this inactivation does not take place in the absence of Hxk2 (Treitel et al., 1998), an assumption consistent with the following observations. In an hxxk2 mutant, glucose repression of some genes that require Snf1 to be transcribed is weak (Zimmermann & Scheel, 1977; Entian & Zimmermann, 1980), and Snf1 phosphorylates the repressor Mig1, even in the presence of high glucose (Treitel et al., 1998; Ahuatzi et al., 2007). The interaction between Snf1 and the regulatory protein Snf4, low at high glucose and required to lift Snf1 autoinhibition, is considerably increased in the absence of Hxk2 (Jiang & Carlson, 1996; Sanz et al., 2000). REG1 overexpression partially suppresses the effect of an hxxk2 mutation on glucose repression (Sanz et al., 2000), an observation suggesting that the presence of Hxk2 facilitates the dephosphorylation, and subsequent inactivation, of Snf1 by the protein phosphatase complex Glc7-Reg1. However, while an interaction of Snf1 with Hxk2 was observed in extracts from cells grown in either high or low glucose (Ahuatzi et al., 2007), no interaction was observed, in a two-hybrid assay, between Hxk2 and Reg1 or Glc7 (Sanz et al., 2000). Moreover, actual measurements of the capacity of Snf1 to phosphorylate a peptide substrate in vitro did not show an increased activity of Snf1 in extracts from an hxxk2 mutant grown on glucose (K. Hedbacker & M. Carlson, pers. commun.). Therefore, it remains unclear whether Hxk2 controls the intrinsic activity of Snf1, or only its capacity to phosphorylate Mig1.

Other possible targets for Hxk2 have been uncovered during the last years, suggesting a model for the Hxk2 role in the control of transcription of some glucose-repressed genes (Fig. 3). An interaction of Hxk2 with the protein Med8, a subunit of the Srb/mediator complex (Myers et al., 1998; Myers & Kornberg, 2000), has been observed, using two-hybrid assays, coprecipitation experiments and gel mobility analysis with purified proteins (de la Cera et al., 2002). This interaction may be physiologically relevant, as Med8 binds to regulatory elements in genes controlled by glucose, such as those found in the promoter of the SUC2 gene and also in the coding region of the HXX2 gene (Chaves et al., 1999; Moreno-Herrero et al., 1999; Palomino et al., 2005). In the
case of glucose-repressed genes, the function of the binding of Hxk2 to Med8, which takes place in glucose growing cells, could be to interfere with the recruitment of RNA polymerase II by the mediator complex. However, for a glucose-activated gene such as HXX2, it is harder to visualize how the binding of Hxk2 to Med8 could facilitate transcription. A possible explanation for the positive role played by Hxk2 in HXX2 transcription is that, in the presence of glucose, Hxk2 sequesters the transcriptional repressor Rgt1, thus helping to relieve its block on HXX2 transcription (Palomino et al., 2006).

It has also been demonstrated, using different techniques, that Hxk2 interacts directly with the transcriptional repressor Mig1 and that the Lys6-Met15 decapeptide from Hxk2 is needed for the interaction (Ahuatzi et al., 2004). The interaction between Mig1 and Hxk2 is required for Hxk2 to be retained within the nucleus and there is a correlation between the level of Mig1 in the cell and the amount of Hxk2 located in the nucleus (Ahuatzi et al., 2004). The fact that a nuclear localization of Mig1 requires the presence of high glucose in the culture medium (DeVit et al., 1997) explains the observation that the partial nuclear localization of Hxk2 is also glucose-dependent (Ahuatzi et al., 2004). It should be noted that Mig1 does not need to form a complex with Hxk2 to enter into the nucleus, as it is found in the nucleus in an snf1 hxk2 double mutant (Tomás-Cobos & Sanz, 2002; Ahuatzi et al., 2007). The binding of Hxk2 and Mig1 in the nucleus suggests that the main role of Hxk2 in glucose repression is to hinder the contact between Mig1 and Snf1, blocking phosphorylation of Mig1 and thus maintaining its repressing capacity (Ahuatzi et al., 2004). The interaction between Hxk2 and Mig1 would be especially important in avoiding cross-talk between different signalling pathways, as it has been observed that in sodium-stressed cells, growing in the presence of high glucose, Snf1 is activated but does not phosphorylate Mig1 (McCartney & Schmidt, 2001).

It is likely, however, that Hxk2 plays some additional role, as lack of Hxk2 has a stronger effect than lack of Mig1 on relieving glucose repression of SUC2 (Entian, 1981; Neigeborn & Carlson, 1987; Nehlin & Ronne, 1990; Walsh et al., 1991; Lutfiya & Johnston, 1996; Salgado et al., 2002). This could involve the protein Hxk2 itself or products of glucose metabolism. Because, in the absence of Hxk2, Hxk1 is able to support repression of invertase by fructose (De Winde et al., 1996) and, when overexpressed, allows partial repression of invertase by glucose (Ma & Botstein, 1986), a metabolic role for the glucose-phosphorylating enzymes appears likely.

It has been reported recently (Sarma et al., 2007) that, at high glucose and in the absence of Hxk2, about 50% of Mig1 remains in the nucleus, but excluded from the perinuclear compartment. This observation contrasts with earlier experiments showing that, in an hxk2 mutant, a Mig1-GFP fusion protein remains in the cytoplasm in the presence of glucose (DeVit et al., 1997; Ahuatzi et al., 2007), although it was already indicated that, under these conditions, a small amount of fluorescence was visible in the nuclei of some cells (DeVit et al., 1997). In any case, it should be noted that the localization of Mig1 in the nucleus is not sufficient to cause repression, as shown by the fact that, in a mutant lacking the nuclear exportin Msn5, GALI is derepressed normally, although Mig1 always remains in the nucleus (DeVit & Johnston, 1999). It appears that phosphorylation of Mig1 by Snf1 is all that is needed to block its capacity to repress gene transcription.
A disputed point has been the role of the serine 14 of Hxk2 in the capacity of the enzyme to mediate catabolite repression of genes such as SUC2. This serine was called Ser15 (corresponding to codon 15) until Hxk2 was sequenced and it was found that the N-terminal amino acid is valine (corresponding to codon 2) and not methionine (Behlke et al., 1998). Although serine 14 can be phosphorylated by PKA in vitro (Kriegel et al., 1994), PKA is not likely to catalyze the reaction in vivo, because phosphorylation of serine 14 is reduced under conditions where the cAMP-dependent protein kinases are activated (Vojtek & Fraenkel, 1990). Different groups have looked at the consequences of replacing serine 14 by an alanine residue and have reported contradictory results. While some laboratories found that the modified enzyme was still functional in glucose signalling (Herrero et al., 1998; Mayordomo & Sanz, 2001), another group reported that the mutation impaired the functionality of Hxk2 and concluded that it is the phosphorylated Hxk2 that transmits the glucose signal (Rández-Gil et al., 1998b). This, however, does not appear to agree with the observation that the proportion of phosphorylated Hxk2 is higher under derepressing than under repressing conditions (Vojtek & Fraenkel, 1990; Rández-Gil et al., 1998b). It has been suggested that the discrepancy between the results of different groups investigating the effects of Hxk2S14A could be due to variations in the amount of mutated hexokinase synthesized in the diverse strains used (Moreno & Herrero, 2002) and, in fact, the number of copies of the mutated HXK2 gene was not the same in the different experiments, as the gene was carried either in an integrative plasmid (Rández-Gil et al., 1998b) or in centromeric (Mayordomo & Sanz, 2001) or multicopy (Herrero et al., 1998) episomal plasmids. Taking into account that a modified Hxk2 lacking the 14 N-terminal amino acids can still mediate repression of SUC2 or induction of HXT1 by glucose (Ma et al., 1989a; Mayordomo & Sanz, 2001), it can be concluded that serine 14 is dispensable for glucose signalling, and that a more relevant feature is the hexokinase activity in the cell. The functionality of an Hxk2 lacking its 14 N-terminal amino acids would also indicate that, at least under some circumstances, full glucose repression may take place in a strain with an Hxk2 lacking the Lys6-Met15 decapetide, although this decapetide is required for interaction with Mig1 (Ahuatzi et al., 2004).

For most other yeasts, there is little evidence for hexokinases playing a nonmetabolic role in glucose repression. In K. lactis there is a single hexokinase, Rag5, required for glucose repression (Bar et al., 2003) and a glucokinase that allows growth on glucose at a very slow rate (35 h doubling time) in the absence of Rag5 (Kettner et al., 2007). Although this makes it hard to distinguish between a metabolic and a regulatory role for Rag5, it has been found that Rag5 is not efficient for restoring glucose repression of invertase in hxl2 S. cerevisiae mutants (Prior et al., 1993; Petit & Gancedo, 1999). In P. angusta (H. polymorpha), a hexokinase, HpHxk1, and a glucokinase, HpglK1, have been identified and any of them supports repression by glucose of maltase, alcohol oxidase or catalase (Kramarenko et al., 2000). However, although HpglK1 allows growth on glucose of an S. cerevisiae triple kinase mutant, in the S. cerevisiae transformants synthesis of invertase and maltase remains insensitive to glucose repression (Laht et al., 2002). Whereas HpHxk1 has been cloned (Karp et al., 2003), its effect in S. cerevisiae has not been reported. In S. pombe, two hexokinases have been identified: SpHxk1 with low affinity for glucose and much higher activity with fructose than with glucose as a substrate, and SpHxk2, a kinetically conventional hexokinase (Petit et al., 1996). As occurred with the kinases from K. lactis and P. angusta, SpHxk1 could not restore glucose repression of invertase in an S. cerevisiae triple mutant (Petit et al., 1998) and repression in the presence of SpHxk2 was only partial (Petit & Gancedo, 1999). In contrast, in a similar experiment, Hxk1 from Y. lipolytica was fully effective (Petit & Gancedo, 1999) and there is preliminary evidence that YIHxk1 is involved in glucose repression of the LIP2 gene encoding an extracellular lipase in Y. lipolytica (Fickers et al., 2005). It has also been reported that in mutants of Schwanniomyces occidentalis or Pachysolen tannophilus, which lack a specific isoenzyme of hexokinase, glucose repression is defective (McCann et al., 1987; Wedlock & Thornton, 1989).

**Signalling by intracellular metabolites**

Because most glucose effects require glucose metabolism (Belinchón & Gancedo, 2007a, b), it may be concluded that binding of glucose to a receptor is usually not sufficient to signal the presence of glucose in yeast. Although there have been some reports on glucose signalling, independent of glucose uptake (Liang & Gaber, 1996; Özcan, 2002), they should be considered with caution, because the hxt strain used showed a residual growth on glucose, indicating that glucose could still be transported and metabolized. While growth could be completely blocked by adding antimycin to the medium, the experiments on the signalling capacity of glucose were performed in the absence of antimycin.

An intracellular metabolite that plays an important role in glucose signalling is cAMP, as shown by the fact that many of the transcriptional changes elicited by glucose can be reproduced by activation of the GTP-binding proteins Ras2 or Gpa2. This can be done, in the absence of glucose, using genes encoding constitutively active forms of Ras2 or Gpa2, placed under the control of a conditional promoter (Wang et al., 2004). Activation of either Ras2 or Gpa2 causes an increase in cAMP levels (Toda et al., 1985; Nakafuku et al., 1988), which stimulates the cAMP-dependent protein
kinases Tpk1, Tpk2 and Tpk3 (Toda et al., 1987b). Surprisingly, from the many potential substrates already identified for the three isoenzymes Tpk1, Tpk2 and Tpk3 (thereafter PKA) present in S. cerevisiae (Budovskaya et al., 2005; Ptacek et al., 2005), there is none clearly related to the regulation of important elements mediating glucose control, such as the Snf1 kinase and the Glc7 phosphatase complexes (Santangelo, 2006).

Glucose signalling through the Ras-cAMP system requires the phosphorylation of the sugar (Rolland et al., 2000, 2001). It has been suggested that the metabolism of glucose causes an inhibition of Ira1/Ira2, stimulators of the GTPase activity of the Ras proteins, and therefore, increases the GTP-loading of Ras1 and Ras2 (Colombo et al., 2004). GTP-loaded Ras proteins in turn activate adenylate cyclase, increasing the intracellular concentration of cAMP [see a recent review (Santangelo, 2006) for details].

As indicated above, it is also possible to mimic the glucose response, in the absence of the sugar. Although the changes in transcript levels resulting from Ras2 or Gpa2 activation are dependent on PKA, it has also been shown that most glucose-responsive genes can also be regulated through a PKA-independent pathway (Wang et al., 2004). Information is not yet available on the elements of this regulatory pathway, but it may start with changes in the concentration of some intracellular metabolite(s) other than cAMP (Boles & Zimmermann, 1993; Gonçalves et al., 1997). Glucose-6-phosphate and fructose-1,6-bisphosphate may appear as good candidates, as their concentrations are strongly responsive to the presence of glucose (Bañuelos et al., 1977); however, there is no clear correlation between the intracellular concentration of these hexose phosphates and the degree of repression by different carbon sources of the gluconeogenic enzyme fructose-1,6-bisphosphatase (FbPase) (Rodríguez & Gancedo, 1999; Belinchón & Gancedo, 2003). Although studies on the induction of glycolytic enzymes in a variety of mutants suggest that different metabolites control the expression of different genes (Boles & Zimmermann, 1993; Müller et al., 1995; Boles et al., 1996), the identity of the regulatory metabolites has not been established and their mechanism of action is not known.

In mammalian cells, the AMP-dependent protein kinase, a homologue of the protein kinase Snf1 from yeast, is activated under stress conditions due to an increase in the concentration of intracellular AMP (Hardie, 1999). In S. cerevisiae, however, there is no evidence that AMP controls Snf1, or even that the AMP concentration decreases in the presence of glucose (Kresnovati et al., 2006). It has been proposed that yeast could, in some unknown way, sense the ‘glucose flux’ or the rate of glucose consumption and adjust in this way the degree of repression of genes involved in the utilization of alternative carbon sources (Bisson & Kunathigan, 2003). However, there is evidence that glucose repression of a gene such as SUC2 is not directly related to the glucose flux (Meijer et al., 1998). The identification of regulatory elements in pathways alternative to that controlled by PKA therefore remains an important point that requires further investigation.

An interesting regulatory metabolite that accumulates in yeast cells metabolizing glucose is fructose-2,6-bisphosphate (Lederer et al., 1981). It is worth noting that this compound has been found to activate the phosphorylation of FbPase by PKA in vitro (Gancedo et al., 1983), and is therefore likely to control the phosphorylation and partial inactivation of FbPase, which takes place on addition of glucose to derepressed yeast cells (Mazón et al., 1982).

**Other early elements in glucose signalling in S. cerevisiae**

**The TOR pathway**

Although the TOR-signalling pathway has been investigated mainly in relation to the nitrogen source in the medium (Cooper, 2002), it may also mediate some of the yeast responses to glucose (Hardwick et al., 1999). In the presence of rapamycin, an inhibitor of Tor1 and Tor2, the transcript levels of several genes induced by glucose decrease, while a number of glucose-repressed genes are expressed at higher levels (Hardwick et al., 1999). In the case of the low-affinity glucose transporter Hxt1, rapamycin not only decreases the levels of HXT1 mRNA (Hardwick et al., 1999; Tomás-Cobos et al., 2005) but also causes instability and mislocalization of Hxt1 (Schmelzle et al., 2004). It therefore appears that the TOR pathway acts both on transcription and on posttranscriptional processes. It has been proposed that glucose signalling through TOR takes place independently of the TOR effectors Tap2 and Sit4, and is mediated by the Ras/cAMP pathway (Schmelzle et al., 2004). It is not yet known, however, whether the Tor proteins sense intracellular glucose or some other indicator of carbon source availability.

The lack of Tor1 also impairs the downregulation by glucose of different amino acid permeases (Peter et al., 2006). In addition, in the absence of Tor1, mitochondrial respiration is increased during growth on glucose. This increase, which is not observed in glycerol-growing cells, is due to an enhanced translation of mRNAs corresponding to subunits of the oxidative phosphorylation complex encoded by mit-DNA (Bonawitz et al., 2007).

**14-3-3 proteins**

The 14-3-3 proteins Bmh1/Bmh2, positive regulators of the TOR kinase pathway (Bertram et al., 1998), affect some processes regulated by glucose. In a double mutant bmh1bmh2 catabolite inactivation of maltose permease is
impaired (Mayordomo et al., 2003), glucose repression of ADH2 is partially relieved (Dombek et al., 2004) and induction of HXT1 by high glucose is prevented (Tomás-Cobos et al., 2005). It has been shown that Bmh1 and Bmh2 interact with Reg1, a regulatory subunit of the type 1 protein phosphatase complex (Dombek et al., 2004) and that the complex Bmh1/2-Reg1 interacts with Grr1, a component of the SCF\Grr1 ubiquitination complex (Tomás-Cobos et al., 2005). This would suggest that the Bmh proteins mediate HXT1 induction by facilitating, in a Reg1-dependent process, the interaction of Grr1 with Std1. The Bmh proteins may also play a Reg1-independent role, possibly related to the activation of the TOR pathway, as the bmh1bmh2 and reg1 mutations release synergistically glucose repression of ADH2 (Dombek et al., 2004). It has also been reported that, in the presence of glucose, Bmh1/2 interacts with the protein kinase Yak1 (Moriya et al., 2001); under these conditions, Yak1 is phosphorylated by PKA (Zappacosta et al., 2002) and is exported from the nucleus in a Bmh-dependent process (Moriya et al., 2001). Upon glucose depletion Yak1 is activated, enters the nucleus and phosphorylates Pop2, a subunit of the Ccr4-Not complex that mediates mRNA deadenylation, causing cell-cycle arrest (Moriya et al., 2001). Activated Yak1 also phosphorlates the transcription inhibitor Crf1, causing a decrease in the transcription of the ribosomal genes (Martin et al., 2004).

**Phospholipase C**

Another regulatory pathway controlled by glucose involves phospholipase C (Plc1). Addition of glucose to starved yeast cells activates Plc1 and thereby increases phosphatidylinositol turnover and the intracellular level of different species of diacylglycerols (Coccetti et al., 1998). The fact that Plc1 interacts with the glucose sensor Gpr1 and its coupled G protein Gpa2 (Ansari et al., 1999) suggests that activation of Plc1 is mediated by glucose binding to Gpr1. This is further supported by the observation that the Plc1-dependent, glucose-triggered increase in free intracellular Ca\(^{2+}\) requires Gpr1 and Gpa2 (Tisi et al., 2002). It has also been observed that the transient elevation of cytosolic calcium caused by glucose requires the phosphorylation of the sugar (Tökés-Füzesi et al., 2002) and it has been suggested that the intracellular calcium concentration is related to the ratio glucose-1-P/glucose-6-P (Aiello et al., 2002). There is also preliminary evidence for inositol triphosphate acting as a mediator of glucose-induced calcium signalling (Tisi et al., 2004). The primary target of the diacylglycerols formed upon activation of Plc1 is the protein kinase C (PKC), and PKC may be involved in the control of carbon metabolism. This control, however, is complex because plc1 mutants show defects both in the induction of HXT genes by glucose and in the derepression of SUC2, alcohol dehydrogenase or glycerol kinase that takes place on glucose depletion (Brandão et al., 2002; Salgado et al., 2002; Gomes et al., 2005). For this function, PKC does not require the MAP kinase cascade formed by the kinases Bck1, Mkk1/2 and Mpk1, but may act by regulating the intracellular localization of DNA-binding proteins such as the repressor Mig1 or the transcription factor Adr1 (Salgado et al., 2002; Gomes et al., 2005).

**Others**

A different way for glucose to act, when it reaches the 100 mM range, is as a weak osmolyte, able to activate the Sln1-dependent branch of the HOG pathway (Tomás-Cobos et al., 2004). This activation has been shown to be absolutely required for glucose to induce transcription of the HXT1 gene (Tomás-Cobos et al., 2004). Still other elements have been shown to affect processes controlled by glucose, but there have only been suggestions on their mode of action, rather than clear evidence. The increased induction of HXT1, which takes place in the absence of Cdc55, has been attributed to interference of Cdc55 with the activity of the protein kinases Yck1/2 that phosphorylate Std1 and Mth1, negative regulators of HXT1 transcription (Tomás-Cobos et al., 2005). The protein kinase Pho85 is required to control the important remodelling of gene expression that takes place when the glucose of the medium is depleted; in its absence downregulation of glycolytic genes is defective, induction of genes involved in gluconeogenesis and in the glyoxylate cycle occurs before the glucose of the medium is completely exhausted, and the expression of genes required for proper mitochondrial function is impaired (Nishizawa et al., 2004). The molecular mechanisms underlying these effects are not known, but it is likely that the Pho85-associated cyclins Pcl6 and Pcl7 are involved in the formation of functional mitochondria, as mutants lacking Pho85, Pcl6 or Pcl7 are unable to grow on nonfermentable carbon sources (Gilliquet & Berben, 1993; Lee et al., 2000). The observation that in a mutant defective in Tps1, a subunit of the trehalose synthase complex, glucose-induced effects are impaired led Thevelein & Hohmann (1995) to conclude that Tps1 is involved in the transduction of a glucose signal. Although the inability of tps1 mutants to grow on glucose complicated the interpretation of the earlier observations, later work showed that Tps1 is dispensable for glucose signalling (Rodriguez & Gancedo, 1999).

**Targets for glucose signals**

**In S. cerevisiae**

**Genes repressed by glucose**

An important signal for glucose repression is an increase in intracellular cAMP, which allows the activation of PKA. This
is shown by the large number of genes for which the transcription rate decreases on activation of Ras2 (Wang et al., 2004). Under artificial conditions, this downregulation of transcription can take place in the absence of glucose, but is dependent on the presence of a protein kinase subject to activation by cAMP. This shows that, for a number of genes, the activation of PKA is sufficient to trigger transcriptional repression. An analysis of the data of Wang et al. (2004) shows, however, that not every gene repressed by glucose responds in the same way to the activation of Ras2 and PKA, as illustrated by some selected cases shown in Table 1. For some genes, cAMP is the main signal and glucose repression in a tpk<sup>−</sup> bcy1 background is impaired or absent. Representative examples are genes related to the metabolism of trehalose or glycogen (TPS1, TPS2, GH1), glucose utilization (HXX1, GLK1, TKL2, GDP1), or ubiquitination (COQ3, YJR036). In contrast, other genes do not respond to the activation of Ras2, among them SUC2, one of the model genes used to study repression by glucose. It should be noted that genes involved in the same or related metabolic pathways do not always behave in parallel. For instance, considering genes encoding enzymes from the gluconeogenic pathway or the glyoxylate cycle, it can be observed that activation of Ras2 blocks transcription of FBP1, while its effect on the transcription of PCK1, ICL1 or MLS1 is weak or absent. This is consistent with results showing that external cAMP has a stronger inhibitory effect on derepression of Pbp1 than on derepression of Pck1 or Icl1 (Zaragoza et al., 1999). Among genes involved in respiratory metabolism, CYB2 and FOX2 are strongly repressed when Ras2 is activated, while the response of COX6 is weak and CYC1 shows no response. For most glucose-repressed genes signalling through cAMP is redundant with another glucose-signalling pathway, as shown by the fact that glucose repression takes place normally in a tpk<sup>−</sup> bcy1 strain. The nature of the corresponding signal has not been yet elucidated, but it does not involve the plasma membrane glucose sensors Snf3 or Rgt2, and depends on glucose metabolism (Belinchón & Gancedo, 2007b). In fact, in mutants where glycolytic flux is strongly reduced, glucose repression is partially relieved (Gamo et al., 1994; Elbing et al., 2004).

For the repression of some genes such as SUC2, HXK1 or the GAL genes, the glucose-phosphorylating enzyme Hxk2 is specifically required (Zimmermann & Scheel, 1977; Entian, 1980; Rodriguez et al., 2001) (see section on Hxk2). On the other hand, repression of ADH2 or FOX1 is not relieved in an hxx2 mutant (Dombek et al., 1993; Stanway et al., 1995) and relief is only partial for genes such as CYC1, CYB2, GLK1 or GDH2 (Ma & Botstein, 1986; Brown et al., 1995; Rodriguez et al., 2001; Belinchón & Gancedo, 2007b). Some genes such as FBP1, PCK1 or ICL1 are completely repressed by glucose, even in a double mutant hxx1 hxx2 (Yin et al., 1996; Belinchón & Gancedo, 2007b) and, while long-term repression of SUC2 requires Hxk2, SUC2 mRNA levels show a strong, transient, decrease on addition of glucose to an hxx1hxx2 strain (Sanz et al., 1996). A further element modulating repression by glucose is the protein kinase Pho85, because in its absence genes involved in gluconeogenesis and the glyoxylate cycle can be expressed at low concentrations of glucose (Nishizawa et al., 2004).

### Genes induced by glucose

A genome-wide screening has shown that, for most of the genes induced by high glucose, the increase in transcript

### Table 1. Elements affecting different glucose repressed genes

<table>
<thead>
<tr>
<th>Element(s)</th>
<th>Stimulus</th>
<th>Strain used</th>
<th>Strong response</th>
<th>Weak response</th>
<th>Response very weak or absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP</td>
<td>Galactose*</td>
<td>GAL10-RAS2&lt;sup&gt;Gal1&lt;/sup&gt;</td>
<td>FBP1, GDH2, HXK1, COQ3, CYB2, TPS2, FOX2</td>
<td>COX6, ADH2, MSL1</td>
<td>ICL1, PCK1, CYC1, SUC2</td>
</tr>
<tr>
<td>cAMP</td>
<td>External cAMP&lt;sup&gt;1&lt;/sup&gt; (no glucose present)</td>
<td>pde2</td>
<td>FBP1</td>
<td></td>
<td>SUC2</td>
</tr>
<tr>
<td>cAMP-dependent protein kinase</td>
<td>2% Glucose*</td>
<td>tpk&lt;sup&gt;−&lt;/sup&gt; bcy1</td>
<td>FBP1, ICL1, PCK1, FOX2</td>
<td>TPS1, HXK1, GLK1, CK1</td>
<td>–</td>
</tr>
<tr>
<td>Snf3, Rgt2, Gpr1</td>
<td>2% Glucose&lt;sup&gt;1&lt;/sup&gt;</td>
<td>snf3 gpr2 gpr1_hxx2</td>
<td>FBP1, GDH2, SUC2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hxx2</td>
<td>2% Glucose&lt;sup&gt;1, 6&lt;/sup&gt;</td>
<td>hxx2</td>
<td>FBP1, ICL1, ADH2, FOX1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hxx1, Hxx2, Glk1</td>
<td>2% Glucose&lt;sup&gt;1&lt;/sup&gt;</td>
<td>hxx1 hxx2 glk1</td>
<td>FBP1, ICL1, PCK1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hxx1, Hxx2, Glk1</td>
<td>2% Glucose&lt;sup&gt;1&lt;/sup&gt;</td>
<td>hxx1 hxx2 glk1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

To establish the role of different elements related with glucose signalling on transcriptional repression, repression of selected genes has been measured in different mutant strains (see text for details). Strong response is equivalent to the response of a wild-type strain to 2% glucose.

*Wang et al. (2004)
1Zaragoza et al. (1999).
3See text for other references.
levels triggered by glucose is modest, between 1.5- and 4-fold. This increase is usually rapid, < 20 min, but it is sometimes transient, mRNA levels decreasing markedly at 60 min. There are exceptions, as for some genes the degree of induction is higher, about fivefold for RPA12 or RPC53, 20-fold for HXT3, and even 100-fold for HXT1, while for genes such as TRP3, ARG3 or MET14, the kinetics of induction are slower (Wang et al., 2004). The role that different sensing elements play in the induction of different genes is examined in the next paragraphs.

Binding of glucose to the plasma membrane sensors Snf3/Rgt2 triggers the induction of a limited number of genes through a cascade of reactions discussed previously. Most of these genes encode glucose transporters (Ozcan & Jonhston, 1999), but a few others have been identified (Kaniak et al., 2004), and among them MIG2 is of special interest, as increased levels of the DNA-binding protein Mig2 contribute to glucose repression (Lutfiyya et al., 1998). The other plasma membrane sensor, Gpr1 (see the corresponding section), is often required for a maximal response to glucose; in its absence, the degree of induction decreases by a factor of two in many cases (Wang et al., 2004). Gpr1 is not needed, however, for the complete induction of HXT1 or pyruvate decarboxylase (Belinchón & Gancedo, 2007a) (Table 2).

Artificial activation of Ras2, in the absence of glucose (see section on ‘Signalling by intracellular metabolites’), can cause an increase in mRNA levels for many genes; for some genes the extent of induction is similar to that triggered by cAMP Galactose (Belinchón & Gancedo, 2007a) through a cAMP-regulated PKA (Wang et al., 2004). It should be noted, however, that for HXT1 and HXT3 there was at least a 10-fold induction by activated Ras2 in this background (supplementary information in Wang et al., 2004). Although these results have not yet been confirmed by northern analysis or quantitative reverse transcriptase (RT)-PCR, it would be interesting to follow this lead and investigate a potential alternative pathway for Ras signalling.

To investigate whether a cAMP-regulated PKA is not only sufficient, but also necessary, for glucose induction of transcription, glucose was added to a tpk wil bcy1 mutant strain growing on glycerol (Wang et al., 2004), because in such a strain PKA is no longer sensitive to the changes in cAMP intracellular concentration triggered by glucose. In a large number of cases there was a strong induction of transcription by glucose (at least 10-fold), thus showing that metabolism of glucose can provide for most genes an induction mechanism independent of an increase in PKA activity. It may be observed that for many genes, mRNA levels were much lower (5–20-fold) in the mutant strain than in the wild type, during growth in glycerol. This suggests that in a wild-type yeast grown in glycerol, the PKA activity is greater than in the mutant and sufficient to cause a partial induction of the corresponding genes. Surprisingly, while galactose does not act as an inducer in the wild-type strain, for some genes it can cause large increases in mRNA levels in the tpk wil bcy1 background, although the strain lacks Gall and is therefore unable to metabolize galactose (Wang et al., 2004). This is a result that remains difficult to interpret.

The role of the glucose phosphorylating enzymes in the induction of transcription by high glucose is not the same for different genes (Table 2). In mutant strains lacking Hxk2, there is a strong decrease in the induction of HXT1 and HXT2, but HXT3 and pyruvate decarboxylase are fully induced (Ozcan & Johnston, 1995; Rodriguez et al., 2001;

---

### Table 2. Elements affecting different genes induced by glucose

<table>
<thead>
<tr>
<th>Element(s)</th>
<th>Stimulus</th>
<th>Strain used</th>
<th>Strong response</th>
<th>Weak response</th>
<th>Response very weak or absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP</td>
<td>Galactose</td>
<td>GAL10-RAS21519 gal1</td>
<td>THR4, RPC53, ROPO31</td>
<td>ARG3, MET14, HXT3</td>
<td>HXT1, HXT2, ENO2</td>
</tr>
<tr>
<td>cAMP-dependent protein kinase</td>
<td>Glucose*</td>
<td>tpk wil bcy1</td>
<td>PDC1</td>
<td>SU2</td>
<td>HXT1, HXT3</td>
</tr>
<tr>
<td>Snf3, Rgt2</td>
<td>Glucose*</td>
<td>snf3 rgt2</td>
<td>PDC1</td>
<td>SU2</td>
<td>HXT1, HXT3</td>
</tr>
<tr>
<td>Gpr1</td>
<td>Glucose*</td>
<td>gpr1</td>
<td>PDC1, HXT1</td>
<td>HXT1, HXT2</td>
<td>HXT3</td>
</tr>
<tr>
<td>HxK2</td>
<td>Glucose*</td>
<td>hxx2</td>
<td>PDC1, HXT3</td>
<td>HXT1, HXT2</td>
<td>HXT3</td>
</tr>
<tr>
<td>HxK1, HxK2, Glk1</td>
<td>Glucose*</td>
<td>hxx1 hxx2</td>
<td>SU2, PDC1</td>
<td>HXT1, PDC1</td>
<td>SU2</td>
</tr>
<tr>
<td>HxK1, HxK2, Glk1, Gpr1</td>
<td>Glucose*</td>
<td>hxx1 hxx2 gpr1</td>
<td>HXT1, PDC1</td>
<td>SU2</td>
<td></td>
</tr>
</tbody>
</table>

To establish the role of different elements related with glucose signalling on transcriptional induction, induction of selected genes has been measured in different mutant strains (see text for details). Strong response is equivalent to the response of a wild-type strain to the same concentration of glucose.

*Induction by 2–4% glucose, unless otherwise indicated.

1Induction by 0.05% glucose.

See text for references.
Belinchón & Gancedo, 2007a). While induction of pyruvate decarboxylase is decreased by 50% in a double mutant hxk1 hxk2 and blocked in the triple hxk1 hxk2 glk1 mutant, in this last mutant there is a partial induction of HXT1 and SUC2 is highly induced. The need for glucose phosphorylation to induce pyruvate decarboxylase is in agreement with the observation that this induction is dependent on products of glucose metabolism such as glucose-6P and three-carbon metabolites (Boles & Zimmermann, 1993). It should be noted that, in the absence of glucose phosphorylation, Gpr1 is absolutely required for the induction of SUC2 but is dispensable for that of HXT1 (Belinchón & Gancedo, 2007a).

A low concentration of glucose, < 0.1%, is able to induce a number of genes, and in this case also, there are different signalling pathways controlling the induction process (Özcan & Johnston, 1995; Belinchón & Gancedo, 2007a). The induction of HXT2, HXT3 and HXT4, encoding glucose transporters, requires the glucose sensor Snf3 and the expression of these genes decreases two- to fivefold in the absence of Hxk2 (Özcan & Johnston, 1995). The induction of SUC2 has only a partial requirement for Snf3/Rgt2 and does not depend on Hxk2, or Hxk1, but it is slightly impaired in the absence of Gpr1 (Belinchón & Gancedo, 2007a) and considerably decreased in mutants lacking PKA (J.M. Gancedo, unpublished results). Induction of pyruvate decarboxylase by low glucose is little affected by the lack of both Hxk1 and Hxk2 (Belinchón & Gancedo, 2007a).

### Proteins activated by glucose

When glucose becomes available to glucose-starved yeast, the activity of a number of enzymes increases. Prominent among them, because it initiates an important signalling pathway, is adenylate cyclase. Activation of adenylate cyclase requires the Ras1/Ras2 proteins, located in the plasma membrane and able to interact directly with the adenylate cyclase complex (Shima et al., 2000). The exact mechanism for activation of the Ras proteins is not known, but it has been established that glucose causes a modest increase in Ras1/2 GTP loading and that this increase requires glucose phosphorylation; the increase is impaired in a strain lacking Cdc25, the GTP/GDP exchange factor for the Ras proteins. Inhibition of the Ira1/Ira2 proteins, which increase the GTPase activity of Ras1/2, contributes to the activation of the Ras proteins triggered by glucose (Colombo et al., 2004). The increased GTP loading of the Ras proteins does not depend on the glucose receptor Gpr1 or on its G-binding protein Gpa2 (Colombo et al., 2004); however, the Gpr1/Gpa2 system is required for full activation of adenylate cyclase (Rolland et al., 2000). Only the GTP-bound, active form of Gpa2 is able to bind adenylate cyclase (Peeters et al., 2006), but this binding is not enough to trigger a full response, because in strains unable to phosphorylate glucose the activation of adenylate cyclase observed, which is dependent on Gpr1 and on a high concentration of glucose, is very weak (Kraakman et al., 1999a). The activation of adenylate cyclase causes an increase in the intracellular concentration of cAMP, which in turn activates PKA. Activated PKA phosphorylates different enzymes such as the neutral trehalase Nh1 (Ortiz et al., 1983; Uno et al., 1983; François & Parrou, 2001) or the 6-phosphofructo-2-kinase Pfk26 (François et al., 1984; Dihazi et al., 2003) and thereby increases their activity.

The activation by glucose of the plasma membrane H⁺-ATPase is also related to phosphorylation of the protein at different positions, but PKA is not involved in the process, at least directly (Portillo et al., 1991). While phosphorylation of Thr⁹¹² from the H⁺-ATPase appears to mediate an increase in the Vₘₐₓ of the enzyme (Portillo et al., 1991), phosphorylation of Ser⁸⁹⁹ would cause a decrease in the Kₘ for ATP (Eraso & Portillo, 1994). Phosphorylation of Ser⁸⁹⁹ is performed by the protein kinase Ptk2 (Eraso et al., 2006), but the protein kinase responsible for Thr⁹¹² phosphorylation has not been identified, in spite of a systematic search using protein kinase mutants (Goossens et al., 2000). The activity of Ptk2 is the same in membranes isolated from cells incubated in the presence or in the absence of glucose and is not affected by glucose itself (Eraso et al., 2006), but it could be modulated by some glucose-derived metabolite as indicated by the fact that activation of the H⁺-ATPase is strongly reduced in an hxl1 hxl2 mutant and extremely low in a mutant unable to phosphorylate glucose (Belinchón & Gancedo, 2007b). On the other hand, the activation of the H⁺-ATPase is independent of the glucose sensors Snf3/Rgt2 and Gpr1 (Belinchón & Gancedo, 2007b).

### Proteins inactivated by glucose

Glucose also triggers the inactivation of different proteins, a phenomenon called catabolite inactivation (Holzer, 1976). Phosphorylation of FbPase by PKA causes a partial inactivation of the enzyme (Gancedo et al., 1983; Rittenhouse et al., 1987); this process is impaired in a mutant unable to phosphorylate glucose and the impairment is stronger when both the glucose phosphorylating enzymes and the glucose sensor Gpr1 are absent (Belinchón & Gancedo, 2007b). This is consistent with the observation that phosphorylation of FbPase by PKA is strongly activated by fructose-2,6-bisphosphate, a regulatory metabolite that is formed when glucose is metabolized (Gancedo et al., 1983). While partial inactivation of isocitrate lyase by glucose is also dependent on phosphorylation by PKA (Ordiz et al., 1996), there is no information on the protein kinase(s) performing the phosphorylation of the malate dehydrogenase isoenzyme Mdh2.
that takes place on glucose addition and causes inactivation of the enzyme (Minard & McAlister-Henn, 1994).

Glucose not only causes the inactivation of metabolic enzymes, but also that of the protein kinase Snf1, which plays a major role in allowing the transcription of glucose-repressed genes. In this case inactivation is due to dephosphorylation of Snf1 by the Glc7-Reg1 complex (Sanz et al., 2000). Glucose may shift the equilibrium between the phosphorylated and nonphosphorylated forms of Snf1, by activating Glc7-Reg1 and/or by inhibiting the protein kinases Sak1, Tos3 and Elm1 that phosphorylate Snf1 (Hong et al., 2003; Nath et al., 2003; Sutherland et al., 2003). However, the mechanism by which glucose modifies Snf1 activity has not yet been unravelled (Hedbacker & Carlson, 2008; Rubenstein et al., 2008).

Proteins degraded by glucose

Glucose also triggers the degradation of a number of enzymes, a process that has been most thoroughly studied for FbPase and Mdh2. Two conflicting views on the mechanism of FbPase degradation have been offered. One of them contemplates a regulated transfer of FbPase to the vacuole and its degradation by vacuolar proteases (Chiang & Schekman, 1991; Shieh et al., 2001); the other one proposes that FbPase is first subject to ubiquitination (Schork et al., 1995) and then degraded by the proteasome (Schork et al., 1994). These views may be reconciled by the observation that, depending on the physiological conditions of the yeast cells, degradation of FbPase, and also of Mdh2, can take place in the vacuole or in the proteasome (Hung et al., 2004). Degradation in the vacuole requires the phosphorylation of glucose but may proceed in the absence of Hxk1 and Hxk2 (Hung et al., 2004; Belinchón & Gancedo, 2007b), while degradation by the proteasome is strictly dependent on Hxk2 (Horak et al., 2002). Reg1 and Grr1 play a role in the degradation of FbPase in the proteasome (Horak et al., 2002), perhaps related to their possible involvement in the ubiquitination process. Reg1 is also required for the transport of FbPase from intermediate vesicles to the vacuoles (Cui et al., 2004). On the other hand, the cAMP-signalling pathway is required for the vacuolar-dependent degradation of FbPase (Hung et al., 2004; Belinchón & Gancedo, 2007b) but not for degradation by the proteasome (Horak et al., 2002; Hung et al., 2004). The transcriptional repressor Rgt1 is required for FbPase degradation in some conditions (Belinchón & Gancedo, 2007b) but not in others (Horak et al., 2002); its specific role, however, has not been elucidated. Degradation of phosphoenoxypruvate carboxykinase appears to proceed by a mechanism similar to that described for FbPase and Mdh2 (Müller et al., 1981; Hämerle et al., 1998).

Mth1, a regulatory protein involved in the transcription control of the HXT genes, is also degraded on glucose addition as described in the section on Snf3 and Rgt2. This degradation is considerably reduced in a reg1 mutant, and this seems to be related to the fact that the Glc7-Reg1 complex is required for glucose activation of the kinases Yck1/2 (Gadura et al., 2006), as activation of Yck1/2 is an early step in the pathway leading to Mth1 ubiquitination and degradation (Moriya & Johnston, 2004). This role of Reg1 could explain the observation that HXT1 induction is strongly impaired in a reg1 mutant but appears in contradiction with the fact that the HXT2, HXT3 or HXT4 genes are normally induced in this mutant (Özcan & Johnston, 1995).

On glucose addition different kinds of transporters are internalized and degraded, among them the maltose/H+ symporter Mal61 (Riballo et al., 1995), the galactose permease Gal2 (Horak & Wolf, 1997), the monocarboxylate/H+ symporter Jen1 (Paiva et al., 2002) and the glycerol/H+ symporter Stl1 (Ferreira et al., 2005). Because the first step in the internalization of the transporters is their ubiquitination (Horak & Wolf, 1997; Lucero & Lagunas, 1997; Paiva et al., 2002), the corresponding signal is likely to be the binding of glucose to Snf3/Rgt2 and the subsequent activation of Yck1/2 as occurs for Mth1 (Gadura et al., 2006). The fact that Gal2 inactivation is not prevented in an snf3 or in an rgt2 mutant (Horak et al., 2002) may be due to the partial functional redundancy of Snf3 and Rgt2. Degradation of the ubiquitinated transporters is independent of the proteasome and takes place within the vacuole. Although Grr1, the F-box protein of one of the SCF ubiquitin ligase complexes, is required for the degradation of the Gal2 transporter, it does not seem to be required for the ubiquitination of Gal2 itself (Horak & Wolf, 2005). Degradation of the maltose permease Mal61 by glucose requires Rgt2 and Grr1 but it is not impaired in an rgt1 mutant (Jiang et al., 1997).

Other processes controlled by glucose

In addition to the processes already considered, the presence of glucose affects yeast physiology in many other ways. Glucose increases the turnover of a number of mRNA species (Lombardo et al., 1992; Mercado et al., 1994), while it causes a transient stabilization of ribosomal protein mRNAs (Yin et al., 2003). The increased turnover of SDH2 mRNA, encoding the iron–sulfur protein subunit of succinate dehydrogenase, takes place only at high levels of glucose; in contrast, the degradation rates of the FBP1 and PCK1 mRNAs, encoding gluconeogenic enzymes, are already increased by the addition of 0.02% glucose (Yin et al., 2000). Glucose phosphorylation is required to enhance the turnover of SDH2, FBP1, PCK1 or SUC2 mRNAs, but Hxk2 is not specifically needed, except for SDH2 (Cereghino & Scheffler, 1996; Yin et al., 2000). In all cases
the effect of glucose is blocked in an *reg1* mutant, where the activity of the protein kinase Snf1 is less sensitive to glucose (Sanz, 2007). Although external cAMP (in a *pde2* background) can trigger degradation of *PCK1* and *SDH2* mRNAs, the increased degradation of *PCK1* mRNA observed in the presence of 0.02% glucose also occurs in the absence of a Ras-cAMP pathway (Yin et al., 2000). It appears therefore that glucose controls mRNA turnover through alternative, partially redundant signalling pathways. The same applies to the stabilization by glucose of different ribosomal protein encoding mRNAs (Yin et al., 2003). Specifically, the effect of glucose on *RPL3* mRNA stability, but not on that of *RPS6* mRNA, is retained in the absence of glucose phosphorylation, while the Ras-cAMP pathway is required for stabilization of *RPL3* and *RPL24* mRNAs but not for that of *RPS6* mRNA.

Glucose also affects the translation rate of mRNA. On transfer of glucose-growing yeast to a medium lacking glucose, there is a very rapid (1–2 min) inhibition of translation (Ashe et al., 2000). This effect of carbon source removal is specific for glucose (or fructose), as it does not occur in yeast grown in sucrose, maltose or galactose. Inhibition of translation upon glucose withdrawal does not involve the pathways implicated in the inhibition of translation caused by amino acid starvation (Ashe et al., 2000). Translation is restored rapidly (5 min) by addition of glucose (Ashe et al., 2000) and slowly (hours) by incubation of the yeast cells under aerobic conditions in the absence of a carbon source (Uesono et al., 2004). Although several proteins such as Pat1, Ddh1 or Sbp1 have been identified as destabilizing translation initiation complexes upon glucose removal (Coller & Parker, 2005; Segal et al., 2006), there is no information on the mechanism(s) through which glucose may control their activity. Inhibition of translation after glucose removal is prevented if yeast cells are in a metabolic condition that mimics to some extent the absence of glucose. This occurs in mutants with a very low PKA activity (Segal et al., 2006). This effect of carbon source removal (Coller & Parker, 2005; Segal et al., 2006) and slowly (hours) by incubation of the yeast cells under aerobic conditions in the absence of a carbon source (Uesono et al., 2004). Although several proteins such as Pat1, Ddh1 or Sbp1 have been identified as destabilizing translation initiation complexes upon glucose removal (Coller & Parker, 2005; Segal et al., 2006), there is no information on the mechanism(s) through which glucose may control their activity. Inhibition of translation after glucose removal is prevented if yeast cells are in a metabolic condition that mimics to some extent the absence of glucose. This occurs in mutants with a very low PKA activity or in *reg1* or *hsk2* mutants, provided Snf1 is present (Ashe et al., 2000). Translation is also strongly inhibited when yeast cells are transferred from a medium with glucose to a medium with ethanol, and this inhibition requires the Gat1 protein, involved in signalling the quality of the carbon source through the Tor pathway (Kuruvilla et al., 2001).

The yeast vacuolar H⁺-ATPase (V-ATPase) is a multi-subunit complex responsible for the acidification of the organelle. The assembly of this V-ATPase is regulated by glucose, 0.1% glucose increasing the level of assembly from 10 to 25%, while in the presence of 2% glucose, 60% of the subunits are assembled in complexes (Parra & Kane, 1998). Glucose metabolism beyond glucose-6-phosphate is required for triggering V-ATPase assembly but a cAMP signal is neither sufficient nor necessary for the process to take place (Parra & Kane, 1998). Because aldolase is specifically needed for assembly, it has been suggested that aldolase may act in this process as a sensor for the presence of glucose (Lu et al., 2004). V-ATPase assembly is also dependent on the RAVE complex, formed by the Ravl, Rav2 and Skp1 proteins (Seol et al., 2001), but it is not known whether glucose directly controls RAVE.

The growth rate of *S. cerevisiae* and its cell size depend on the carbon source in the medium and both are highest in glucose-grown cells (Johnston et al., 1979). The effect of glucose on cell size is mediated by the Gpr1-Gpa2 system (Tamaki et al., 2005; Vanoni et al., 2005) and requires Cdc25, the Ras GTP-GDP exchange protein (Belotti et al., 2006). Glucose increases the growth rate by inducing a diverse set of genes: glycolytic and ribosomal protein genes, as well as genes related to the cell division cycle. An important signal for this process is the activation of PKA by cAMP, which increases the transcription rate of Rap1 target genes (Klein & Struhl, 1994) and causes an increase in the level of the cyclin Cln3 (Hall et al., 1998). The increase in Cln3 concentration triggered by glucose is due both to a PKA-dependent stimulation of its synthesis (Hall et al., 1998) and to a PKA-independent activation of *CLN3* transcription, involving the transcription factor Azf1 (Newcomb et al., 2002). It has been shown that the induction by glucose of genes such as *CLN3*, *BCK2* and *CDC28*, which promote progress through the start phase of the cell cycle, does not require signalling through Snf3/Rgt2 or Hsk2 (Newcomb et al., 2003). On the other hand, it does not occur in *pjk1* or *pjk2* mutants or in the presence of iodoacetate, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase, thus suggesting that some product of glucose metabolism plays a role in the control of growth rate (Newcomb et al., 2003). The transcriptional activator Gcr1 also affects the response to glucose, as it is required to stimulate cellular metabolism and transcription of *CLN* genes (Willis et al., 2003; Barbara et al., 2007). Although the effects of Gcr1 may be indirect, it appears more likely that Gcr1 acts in concert with the transcription factor Rap1 (Santangelo, 2006).

Glucose blocks the sporulation developmental pathway, initiated in diploid cells by the phosphorylation of the transcriptional activator Ime1. Glucose regulates the process by inhibiting Rim11, a homologue of glycogen synthase kinase 3-β, able to phosphorylate a tyrosine residue, and possibly a serine residue, in Ime1 (Rubin-Bejerano et al., 2004). The glucose signal is transmitted through the cAMP/PKA pathway that mediates, directly or indirectly, the phosphorylation of Ser5, Ser8 and/or Ser12 in Rim11, and the inhibition of its kinase activity (Rubin-Bejerano et al., 2004). An additional element involved in sporulation is the protein kinase Ime2, able to phosphorylate Sic1, an inhibitor of meiotic DNA replication, thus allowing its degradation. Ime2 also stimulates a transcription factor, Ndt80,
involved in the G2-M transition (Benjamin et al., 2003). In the presence of glucose Ime2 is destabilized, a process dependent on ubiquitination by the SCF\(^{Cin1}\) ubiquitin ligase (Purnapatre et al., 2005). The mode of action of glucose has not yet been established, but by analogy with the mechanism of regulation of Mti1 (Moriya & Johnston, 2004) it could involve the binding of glucose to the Snf3/Rgt2 receptors.

In some strains of \textit{S. cerevisiae}, a further developmental pathway may take place, pseudohyphal growth caused by nitrogen starvation (Gimeno et al., 1992; Gancedo, 2001). Under such conditions, glucose (or sucrose) and the Gpr1-Gpa2 system are required to trigger the formation of pseudohyphae (Lorenz et al., 2000). It has been reported recently that pseudohyphal growth can be triggered by maltose or maltotriose through a signalling pathway independent of Gpr1 (Van de Velde & Thevelein, 2008).

**In other yeasts**

In other yeasts, there is only scattered information on the elements controlling repression by glucose. In \textit{S. pombe}, repression of \textit{fbp1}, encoding FbPase, is dependent on the phosphorylation of the transcription factor Rst2 by a cAMP-dependent protein kinase (Higuchi et al., 2002). In mutants lacking this protein kinase, or unable to activate it, \textit{fbp1} is no longer sensitive to repression by glucose (Byrne & Hoffman, 1993; Hoffman, 2005b). In contrast, glucose repression of \textit{inv1}, encoding invertase, is maintained in the absence of the cAMP-signalling pathway (Tanaka et al., 1998). While the lack of Scr1, a repressor protein orthologous of Mig1, relieves repression of \textit{inv1}, it has no effect on the repression of \textit{fbp1} (Tanaka et al., 1998). Nevertheless, in the presence of glucose Scr1 binds to the \textit{UAS2} site of the \textit{fbp1} promoter, and when glucose is removed Scr1 is exported to the cytoplasm, in a process not regulated by the cAMP pathway, and replaced by the activator Rst2 (Hirota et al., 2006). In \textit{K. lactis}, glucose repression of different genes is impaired in strains with a decreased rate of glucose transport (Weirich et al., 1997; Milkowski et al., 2001). The fact that, in \textit{K. lactis}, the strong repression of invertase by glucose is independent of Mig1 (Georris et al., 1999) may be related to the observation that expression of invertase in this yeast does not require Fog2, the orthologue of Snf1 (Goffrini et al., 1996).

In \textit{P. angusta}, glucose fails to repress peroxisomal enzymes in a mutant lacking the orthologue of Snf3, Gcr1 (Stasyk et al., 2004), while repression of alcohol oxidase is only partially relieved in a \textit{mig1 mig2} double mutant (Stasyk et al., 2007).

Regarding the signals controlling induction of transcription by glucose in other yeasts, there are few data available. In \textit{K. lactis}, induction of pyruvate decarboxylase by glucose is abolished in a mutant where the genes \textit{KHT1} and \textit{KHT2}, encoding for glucose transporters, have been deleted (Milkowski et al., 2001). Induction of \textit{RAG1}, a gene encoding a glucose transporter, requires the \textit{RAG5} gene, encoding hexokinase; although the mode of action of Rag5 is not known, it should be noted that induction of \textit{RAG1} by galactose is also dependent on Rag5, suggesting that this protein plays a regulatory role independent of its metabolic function (Prior et al., 1993). Induction by glucose of \textit{RAG1} and of several glycolytic genes is only partial in the absence of the transcriptional activator Sck1 (Lemaire et al., 2002). Because expression of both \textit{RAG1} and \textit{SCK1} is blocked by the repression factor Rgt1 (Rolland et al., 2006; Neil et al., 2007), the Rag4/Rag8 pathway that inactivates Rgt1 (Rolland et al., 2006) is essential for the induction by glucose of \textit{RAG1} and of other glycolytic genes. In \textit{C. albicans}, induction by glucose of genes encoding hexose transporters requires Hgt4, an orthologue of the Snf3 glucose sensor (Brown et al., 2006).

In yeast genera different from \textit{Saccharomyces}, there is little evidence for activation or inactivation of proteins by glucose. An exception is the activation of adenylate cyclase by glucose in \textit{S. pombe} (Hoffman, 2005a, b). As discussed in the section on \textit{Gpr1}, it depends on the binding of glucose to Git3, a plasma membrane protein that plays the same role as ScGpr1, and on the trimeric G-protein formed by the Gpa2, Git5 and Git11 subunits. In contrast with the situation in \textit{S. cerevisiae}, the \textit{SpRas1} protein from \textit{S. pombe} plays no role in glucose/cAMP signalling (Fukui et al., 1986) but the direct activation of adenylate cyclase by Gpa2 requires the Git1 protein (Kao et al., 2006). Git1 lacks sequence homologues in other fungi and its mechanism of action remains to be determined. Regarding catabolite inactivation, only a few systems have been studied and it was found that it does not affect FbPase in \textit{S. pombe} (Vassarotti et al., 1982) or isocitrate lyase in \textit{K. lactis} (López et al., 2004). In methylotrophic yeasts, peroxisomal enzymes can be degraded after peroxisomes have been engulfed within the vacuole (Dunn et al., 2005), but this process of pexophagy is induced both by ethanol and by glucose and is therefore not equivalent to the classical catabolite inactivation. There is, nevertheless, a case where degradation is specifically triggered by glucose; this is the glucose-induced microautophagy in \textit{Pichia pastoris}, which requires the \(\alpha\)-subunit of phosphofructokinase (Yuan et al., 1997). The role of phosphofructokinase has not yet been established, but a catalytically inactive phosphofructokinase is still functional for this process. Interestingly, the glucose-induced degradation of peroxisomal enzymes in \textit{P. pastoris} does not require the Gpr1/Gpa2 proteins, while in \textit{S. cerevisiae} lack of Gpr1 or Gpa2 suppresses the degradation of the peroxisomal thiolase induced by glucose (Nazarko et al., 2007, 2008).

As observed in \textit{S. cerevisiae} for pseudohyphal growth, hyphae formation in \textit{C. albicans}, in a glucose-containing medium, is also dependent on both Gpr1 and Gpa2 (Miwa et al., 2004).
Signalling pathways for other nutrients

Amino acids

Saccharomyces cerevisiae may use a large number of compounds as sources of nitrogen, but it does not grow at the same rate on the different nitrogen sources. A regulatory mechanism, called nitrogen catabolite repression, allows the yeast to use preferentially the nitrogen sources that support the faster growth (Cooper, 2002). Superimposed to this control system, other regulatory pathways respond to the presence of amino acids in the growth medium.

An important sensor of external amino acids is the plasma membrane protein Ssy1, with strong structural homology with amino acid permeases (Didion et al., 1998; Poulsen et al., 2005). The targets of this signalling pathway are the transcription factors Stp1 and Stp2, able to activate the expression of a number of genes encoding amino acid permeases (Andréasson & Ljungdahl, 2002). As shown in Fig. 4, Ssy1 forms a complex with the Ptr3 protein and the endoprotease Ssy5 (Forsberg & Ljungdahl, 2001b) and, upon binding an amino acid, activates Ssy5 (Abdel-Sater et al., 2004; Andréasson et al., 2006). Branched amino acids, in particular leucine, are most effective, while charged amino acids have a smaller effect and proline is ineffective (Iraqui et al., 1999; Gaber et al., 2003). Activated Ssy5, in turn, causes the endoproteolytic processing of the transcription factors Stp1 and Stp2 (Andréasson & Ljungdahl, 2002). In the absence of amino acids, Stp1 and Stp2, that are not integral membrane proteins, are associated with the plasma membrane through their N-terminal domains. After the limited endoproteolysis brought about by Ssy5, the released C-terminal domains from Stp1/2 enter the nucleus and activate the expression of different genes encoding amino acid permeases. Although the unprocessed forms of Stp1/Stp2 are not totally excluded from the nucleus, they are prevented to act as inducers of transcription by three inner nuclear membrane proteins, Asi1, Asi2 and Asi3, acting in concert (Zargari et al., 2007).

The processing of Stp1/Stp2 requires the SCF\textsuperscript{Grr1} ubiquitin–ligase complex (Bernard & André, 2001) and the casein kinases Yck1/2 (Abdel-Sater et al., 2004). Yck1/2 phosphorylate Stp1, and possibly Stp2, but this phosphorylation is not dependent on the presence of external amino acids (Abdel-Sater et al., 2004). The substrate of the ubiquitin–ligase complex has not yet been identified, but it has been established that Grr1 acts upstream of the protease Ssy5 (Abdel-Sater et al., 2004), that the protein Ptr3 is modified in the presence of amino acids (Forsberg & Ljungdahl, 2001b) and that the modification of the Ptr3 migration profile is Grr1-dependent (Abdel-Sater et al., 2004). It is therefore tempting to propose that Ptr3 is ubiquitinated by SCF\textsuperscript{Grr1}, and that this modification is required for Ssy5 activation (Fig. 4). It is interesting to note that, while the SCF\textsuperscript{Grr1} complex is required for amino acid signalling, the activity of the proteasome is not necessary (Andréasson & Ljungdahl, 2002; Abdel-Sater et al., 2004), thus suggesting that in this case ubiquitination does not tag a protein for degradation but modifies its capacity to interact with other proteins, a function for ubiquitination that has been discussed previously (Schnell & Hicke, 2003).

An authenticated amino acid permease, Gap1, also plays a role as nutrient sensor, because its interaction with amino acids results in activation of PKA. This activation, however, does not seem to involve an increase in the intracellular.

Fig. 4. Amino acid-signalling pathway. In the absence of amino acids the transcription factors Stp1/2 are phosphorylated by the casein kinases Yck1/2 but are not further processed. Although these forms of Stp1/2 are not fully excluded from the nucleus, they are prevented from activating gene transcription by the inner nuclear membrane proteins Asi1, Asi2, Asi3, acting together. When amino acids are present, they bind the transmembrane protein Ssy1 and activate the protease Ssy5, in a process that requires the protein Ptr3 and the SCF\textsuperscript{Grr1} complex, that may play a role in ubiquitinating Ptr3. Ssy5 performs a limited endoproteolysis of phosphorylated Stp1/2 and the processed Stp1/2 enter the nucleus and activate transcription. See text for details.
concentration of cAMP (Donaton et al., 2003), but depends on Sch9, a protein kinase paralogous to mammalian protein kinase B (Crauvels et al., 1997). It is interesting to note that signals given by different nutrients are integrated to modulate the activity of PKA and control the growth rate of the yeast cells, as well as other processes. For instance, in glucose-grown, nitrogen-starved yeast, the addition of a nitrogen source triggers different effects, such as the activation of trehalase or changes in the rate of transcription of a variety of genes (Pernambuco et al., 1996). Some of these effects depend on the presence of glucose (or fructose) but do not require the phosphorylation of the sugar. Other effects, such as the induction of genes encoding ribosomal proteins, are stronger in the presence of glucose, but only when the sugar can be phosphorylated (Pernambuco et al., 1996).

Intracellular sensors for amino acids have also been described. When the intracellular concentration of amino acids declines, the corresponding tRNAs remain partially uncharged and activate the protein kinase Gcn2 that phosphorylates the translation initiation factor eIF2; phosphorylated eIF2, in turn, stimulates the translation of the GCN4 gene (Hinnebusch, 1997). The subsequent increase in the intracellular concentration of the transcription factor Gcn4 causes large modifications in the pattern of transcription of genes involved in amino acid metabolism (Natarajan et al., 2001). Besides Gcn4, there are other transcription factors that respond to the presence or absence of specific amino acids. Put3, for instance, binds proline and can then activate the transcription of the genes PUT1 and PUT2, which encode proteins involved in proline catabolism (Sellick & Reece, 2005), while the activity of the transcription factor Arg81 depends on its binding to arginine (Amar et al., 2000). Lys14, which activates the transcription of genes involved in lysine biosynthesis, is only operative when bound to α-aminoadipate semialdehyde, an intermediate of the biosynthetic pathway that accumulates in the absence of lysine (Feller et al., 1994). The transcription factor Leu3 responds indirectly to leucine, as it is able to bind isopropylmalate, a product of leucine catabolism. In the absence of isopropylmalate, Leu3 represses the transcription of a number of genes involved in amino acid metabolism, while Leu3 bound to isopropylmalate can act as a transcriptional activator (Sze et al., 1992; Kohlhaw, 2003).

**Sulfur-containing compounds**

The transcription of genes encoding enzymes involved in the assimilation of sulfur-containing compounds and in the biosynthesis of methionine is the subject of a particularly complex regulatory network. This may be explained by the fact that S-adenosylmethionine (SAM) serves as a methyl donor for a very large number of transmethylation reactions involving not only proteins but also nucleic acids or lipids (Menant et al., 2006a). Transcription of many genes is repressed in the presence of methionine or SAM in the medium (Cherest et al., 1969) but also of cysteine or glutathione (Ono et al., 1996). It has been established that under these conditions the expression of most MET genes is turned off, due to degradation or inactivation of the transcription factor Met4, mediated by the ubiquitin ligase SCF<sup>Met30</sup> (Kaiser et al., 2000; Rouillon et al., 2000). Although it had been proposed that the signal for repression of the MET genes is an increase in the intracellular concentration of SAM (Thomas & Surdin-Kerjan, 1997), there is now convincing evidence that it is the intracellular concentration of cysteine that regulates the rate of transcription of these genes (Ono et al., 1996; Hansen & Johannesen, 2000; Srikanth et al., 2005; Menant et al., 2006b). It is not yet known, however, whether cysteine controls directly the activity of SCF<sup>Met30</sup>. Although sulfur amino acid metabolism in S. pombe presents some particularities with respect to <i>S. cerevisiae</i>, it also appears to be regulated by the intracellular concentration of cysteine (Brzywczy et al., 2002).

**Ammonium**

In <i>S. cerevisiae</i>, there are three ammonium transporters, Mep1, Mep2 and Mep3, with similar sequences (Marini et al., 1997). It has been proposed that these proteins include 11 transmembrane domains, an extracytosolic N-terminus and an intracellular C-terminal tail (Marini & André, 2000). Mep2 is specifically required for pseudohyphal differentiation in response to nitrogen starvation, and it has therefore been suggested that it acts as an ammonium sensor (Lorenz & Heitman, 1998). Although Mep2 is N-glycosylated in an asparagine residue at its N-terminus, while Mep1 and Mep3 are not, the glycosylation is not necessary for pseudohyphal growth (Marini & André, 2000). Mep2, and to a lesser extent Mep1, mediate the activation of PKA triggered by ammonium (Van Nuland et al., 2006). As also reported for the activation triggered by amino acids, this process is not associated with an increase in cAMP levels (Thevelein et al., 2005). Because specific mutations in Mep2 did not affect activation of the PKA pathway and pseudohyphal growth in the same way, it has been suggested that the two processes are not regulated through the same mechanism (Van Nuland et al., 2006). This contrasts with the observation that the requirement for Mep2 to trigger pseudohyphal growth when ammonium is limiting is relieved when the PKA pathway is activated by dominant-active forms of Gpa2 or Ras2 or by addition of exogenous cAMP (Lorenz & Heitman, 1998). In <i>C. albicans</i>, when ammonium is absent or present at low concentrations, CaMep2 activates, via its C-terminal cytoplasmic tail, both a cAMP-dependent pathway and a MAP kinase cascade (Biswas & Morschhauser,
Phosphate

*Saccharomyces cerevisiae* responds to phosphate starvation by increasing the transcription rate of genes encoding phosphate transporters and acid and alkaline phosphatases. These genes are activated by the transcription factor Pho4, which is regulated by the proteins Pho85, Pho80 and Pho81. When there is enough phosphate available, the complex Pho80-Pho85, formed by a cyclin and a cyclin-dependent kinase, phosphorylates Pho4, which is then excluded from the nucleus; when phosphate is limiting Pho81, the inhibitor of the cyclin-dependent kinase, inhibits Pho85 activity and Pho4 remains in the nucleus (reviewed in Lenburg & O’Shea, 1996; Oshima, 1997; Persson et al., 2003). It is not yet clear, however, how phosphate availability is sensed and how the corresponding signal is transmitted to Pho81 (Mouillon & Persson, 2006).

As Pho84, a high-affinity phosphate transporter, has some similarity to the glucose sensors Snf3 and Rgt2, it could be a phosphate sensor; however, it is not essential for sensing the external phosphate concentration (Wykoff & O’Shea, 2001). It has been proposed that the low-affinity transporters Pho87, Pho90 and Pho91 are the sensors that monitor external phosphate (Pinson et al., 2004). More recent evidence indicates that, whereas Pho87 and Pho90 are localized in the plasma membrane, Pho91 is found at the vacuolar periphery and is likely to export phosphate from the vacuolar lumen to the cytosol (Hürlimann et al., 2007). While it has been shown that the concentration of intracellular phosphate serves as a signal for the control of the genes regulated by phosphate, the identity of the intracellular phosphate sensor is not known, and a potential candidate, Pho81, is not regarded as likely (Auesukaree et al., 2004).

Addition of phosphate to phosphate-starved yeast cells activates the protein kinase pathway, although it does not trigger a CAMP signal. In this case the phosphate transporters Pho84 and Pho87 may act as phosphate sensors (Giots et al., 2003). The stimulation of processes activated by PKA, triggered by phosphate, requires the presence of glucose in the medium, but may proceed in the absence of glucose phosphorylation, as long as the glucose-signalling pathway depending on Gpr1 is operative (Giots et al., 2003). While the activation of PKA is required for downregulation of Pho84 and its translocation to the vacuole where it is degraded, inhibition of PKA does not affect the down-regulation of acid phosphatase in response to high phosphate (Mouillon & Persson, 2005). This supports the suggestion that the response to phosphate involves two different pathways: there would be a rapid response to external phosphate levels, dependent on Pho84/Pho87, PKA and an abundant carbon source, and a long-term response dependent on the internal concentration of phosphate and a phosphate sensor that remains to be identified (Thomas & O’Shea, 2005; Mouillon & Persson, 2006). Other proteins involved in nucleotide metabolism (adenylate kinase, Adk1, and adenosine kinase, Ado1) or the synthesis of inositol polyphosphate (phosphoinositide-specific phospholipase, Plc1, inositol polyphosphate kinase, Arg82, and inositol hexaphosphate kinase, Kcs1) function upstream of Pho81, but it is not clear whether they play some role in transmitting a phosphate signal (El Alami et al., 2003; Auesukaree et al., 2005; Huang & O’Shea, 2005).

**Final remarks**

**Gene regulation via reverse recruitment**

The effects of glucose on the rate of transcription of different yeast genes have been explained as a consequence of the modification of transcriptional activators and repressors that takes place in the presence of glucose. In the classical recruitment paradigm these modifications affect the capacity of these factors, and also of diverse elements of the polymerase II complex, to assemble on each gene. Recently, it has been proposed that induction of a gene occurs when this gene associates with a preassembled transcription complex situated at the internal nuclear periphery and connected with a nuclear pore; this process has been called gene regulation via reverse recruitment (Santangelo, 2006; Sarma et al., 2007). The mechanism of reverse recruitment is an intriguing possibility, but it is not clear how glucose would act to modify the capacity of the genes to associate with the preformed transcription complex. If the association depends on the presence or on the absence of activators or repressors bound to the gene, the models of direct and reverse recruitment would be equivalent with respect to the way in which early signalling elements control transcription in response to glucose. The difference between the models, however, remains important to establish, from a mechanistic point of view, how the transcription process is regulated.

**Glucose signalling in mammalian systems**

A comparison between glucose signalling in yeast and in mammalian systems shows a number of common themes, but also important variations. In yeast, glucose acts by causing changes in the conformation of the proteins Snf3/Rgt2 and Gpr1 in the plasma membrane, by interacting with the intracellular protein Hxk2 or by modifying the pattern of intracellular metabolites, a process that requires metabolism, except for a small increase in the cAMP concentration, which can be observed in the absence of glucose phosphorylation.
In mammalian cells, glucose operates through two kinds of signals, either by causing a depolarization of the plasma membrane that affects the secretion of the hormones insulin and glucagon, or by modifying the levels of intermediary metabolites, which in turn control the activity of different regulatory proteins (Towle, 2005).

Although two proteins in the plasma membrane of mammalian cells, GLUT2 and SGLT3, act as glucose sensors, their mode of action is unrelated to those of Snf3/Rgt2 or Gpr1. GLUT2, a low-affinity glucose transporter, appears to work mainly through its control of the glycolytic flux in liver cells (Antoine et al., 1997), but not in pancreatic β cells (Efrat et al., 1994). In hepatic cells, GLUT2 also plays a more direct role in the regulation of transcription, through specific binding to karyopherin α2, a protein required for induction of glucose-responsive genes such as l-pyruvate kinase (Guillemain et al., 2002). Glucose induces karyopherin α2 nuclear efflux, but the mechanism(s) regulating this efflux and the precise role of karyopherin in the control of transcription have not yet been elucidated (Cassany et al., 2004). SGLT3, a protein belonging to the sodium/glucose cotransporter family, causes in the presence of glucose a depolarization of the plasma membrane as a result either of Na⁺/glucose cotransport or of the activation of an ion channel sensitive to glucose (Diez-Sampedro et al., 2003). This depolarization triggers an increase in intracellular Ca²⁺ that, in the case of pancreatic β cells, stimulates insulin secretion (Tarasov et al., 2004) and, in the case of glucose-sensing neurons, inhibits glucagon secretion from pancreatic α cells, mediated by the autonomous nervous system (Gromada et al., 2007).

Mammalian glucokinase works as an intracellular glucose sensor and could be seen as the counterpart of yeast hexokinase. However, its mode of action is again different. The low affinity of mammalian glucokinase for glucose makes it able to couple the rate of glucose metabolism to the concentration of glucose available to the cells. Although glucokinase, like yeast hexokinase, is able to shuttle between the nucleus and the cytoplasm, at low concentrations of glucose glucokinase binds the nuclear glucokinase regulatory protein GKR and, has a nuclear localization, while at a high glucose concentration the proteins dissociate and glucokinase is found mostly in the cytoplasm (de la Iglesia et al., 1999). Binding of glucokinase to 6-phosphofructo-2/-kinase/fructose-2,6-bisphosphatase facilitates the retention of glucokinase in the cytoplasm (Payne et al., 2005). Binding is enhanced in the presence of glucose (Baltrusch et al., 2006) and increases 6-phosphofructo-2-kinase activity (Smith et al., 2007), which yields fructose-2,6-bisphosphate, an activator of phosphofructokinase (Hers, 1984). This allows the coordination of two key steps of glucose metabolism; glucose phosphorylation and phosphorylation of fructose-6P.

A high rate of glucose metabolism increases the ATP/ADP ratio and, in mammalian cells, the elevated ATP concentration causes the closure of an ATP-sensitive K⁺ channel and membrane depolarization (Cook & Hales, 1984), with consequences similar to those described above for glucose binding to SGLT3. Other intracellular metabolites, whose concentration changes in relation with the rate of glucose metabolism and that have been shown to act as glucose signals in mammalian cells, are cAMP, AMP, glucose-6P and xylulose-5P, an intermediary in the pentose cycle pathway. It is interesting to note that the relationship between cAMP concentration and nutritional status varies depending of the organism (Gancedo et al., 1985). While the intracellular concentration of cAMP increases in yeast in the presence of glucose (Eraso & Gancedo, 1984), it decreases in mammalian cells due to a lowered glucagon secretion. The decrease in the concentrations of cAMP and AMP diminishes the activity of the cAMP-dependent protein kinase (PKA) and of AMPK, a protein kinase activated by AMP, homologous to the protein kinase Snf1 from yeast (Hardie et al., 1998), while the increase in xylulose-5P activates the protein phosphatase PP2A (Nishimura & Uyeda, 1995). This is important for the activation of the glucose-responding protein ChREBP that, together with Mlx, activates the transcription of different genes, such as those encoding acetylCoA carboxylase, fatty acid synthase or l-pyruvate kinase (Ma et al., 2006). Because ChREBP is phosphorylated by both PKA and AMPK (Kawaguchi et al., 2001, 2002), and dephosphorylated by PP2A (Kabashima et al., 2003), glucose facilitates the formation of the dephosphorylated form of ChREBP, which is the active form able to accumulate in the nucleus and to bind DNA. Finally, glucose-6P and glucose itself are able to bind LXR, a transcription factor found in liver cells that targets genes involved in cholesterol, fatty acid and carbohydrate metabolism (Mitro et al., 2007).

While in yeast Snf1 is required for the induction of gluconeogenic genes (Schöler & Schüller, 1994), in liver active AMPK decreases the expression of genes encoding gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase or glucose-6-phosphatase (Lochhead et al., 2000; Inoue & Yamauchi, 2006).

**Glucose signalling vs. signalling for other nutrients**

The yeast systems sensing the presence of different nutrients share a number of features, regarding both their structure and their regulatory elements. For instance, the first element in a signalling pathway is often a membrane protein, acting as a sensor for external nutrients. This protein can be a transporter, such as Gap1 for amino acids, Mep2 for ammonium, and Pho84, Pho87, Pho90 and Pho91 for phosphate, or a transporter homologue, such as Snf3 and...
Rgt2 for glucose or Ssy1 for amino acids. However, only in the case of glucose (and sucrose) has a G-protein-coupled nutrient receptor, Gpr1, been identified. Once the cell has taken up the nutrient, intracellular sensors may interact directly with it, or with some metabolite whose concentration changes in the presence of the nutrient. Well-characterized examples of such sensors are transcription factors such as Put3, Arg81, Lys14 or Leu3, which bind either an amino acid (proline or arginine) or an intermediary metabolite in a biosynthetic (lysine) or a catabolic (leucine) pathway. A case presenting singular characteristics is the sensing of biotin, as it is not mediated by the biotin transporter or by free intracellular biotin (Pirner & Stolz, 2006). Sensing requires biotin-protein ligase and it has been proposed that biotinyl-AMP formed as intermediary in the reaction catalyzed by biotin-protein ligase acts as a signal for the presence of biotin; however, the downstream molecular target of biotinyl-AMP remains unknown (Pirner & Stolz, 2006).

An important common target for many nutrients is PKA, activated by fermentable carbon sources, amino acids, ammonium or phosphate (Thevelein et al., 2005). In the case of fermentable carbon sources such as glucose, fructose or galactose, activation requires metabolism of the sugar, proceeds through Ras1/Ras2 and involves an increase in intracellular cAMP. Glucose and sucrose produce an additional activation mediated by Gpr1 and its G-coupled protein Gpa2. In contrast, activation of PKA by other nutrients is not accompanied by changes in intracellular cAMP and the activation mechanism is not known (Thevelein et al., 2005).

Other components shared by different nutrient-signalling pathways are the casein kinases Yck1/2 and SCF ubiquitin ligase complexes (Spielewoy et al., 2004). However, while the casein kinases phosphorylate the transcription factor Stp1, independently of the presence of amino acids (Abdel-Sater et al., 2004), their phosphorylation of the regulatory proteins Mth1 and Std1 is dependent on the binding of glucose to Rgt2, or possibly to Snf3 (Moriya & Johnston, 2004). The complex SCF^{Carr1} is required for both glucose and amino acid signalling through the transporter homologues Snf3/ Rgt2 and Ssy1, respectively, but here again the precise regulatory mechanisms differ. In the case of glucose, the ubiquitination of Mth1 and Std1 tags these proteins for degradation in the proteasome (Moriya & Johnston, 2004), while the transmission of the amino acid signals, presumably mediated by ubiquitination of Ptr3 (Abdel-Sater et al., 2004), does not require the activity of the proteasome (Andréasson & Ljungdahl, 2002; Abdel-Sater et al., 2004). SCF^{Carr1} is also involved in the ubiquitination of the protein kinase Ime2, required for sporulation, and there is evidence that ubiquitination destabilizes Ime2, but it is not known whether degradation takes place within the proteasome (Purnapatre et al., 2005). A different SCF complex, SCFMet30, plays a specific role in the control of methionine metabolism. In the presence of high levels of methionine, SCFMet30 ubiquinates the transcriptional activator Met4 and reduces its activity. Although inactivation of Met4 is not necessarily linked to subsequent proteolysis, release of polyubiquitinated Met4 from SCF^{Grr1} results in proteasome-dependent degradation (Chandrasekaran et al., 2006). A number of connections between the changes induced in yeast by different nutrients have become apparent. However, our knowledge is still patchy and we lack a real understanding of how the multiple signalling pathways are integrated to produce an appropriate response (Schneper et al., 2004).

Conclusions

Some aspects of glucose signalling in yeast are now well established. The cascade of reactions triggered by the binding of glucose to the sensors Snf3/Rgt2 in the plasma membrane has been delineated in detail, showing the role of the proteins Yck1/2, Mth1/Std1, Grr1 in the relief of the inhibition by Rgt1 of the transcription of a number of genes. While Hxk2 is required for glucose to affect the transcription of some genes, it is dispensable for the effects of glucose on many other genes. An important consequence of the availability of glucose is an increase in the activity of PKA, mediated both by Gpr1 and by a signalling pathway dependent on metabolism. It has been shown that activation of PKA is sufficient to modify the pattern of gene transcription, with some genes being activated and others repressed. There is also clear evidence that there are no single systems able to cause either repression or induction of transcription by glucose, but that the rate of transcription of different genes responds to different combinations of signals. The effects of glucose are not limited to changes in transcription rates but include modifications in the stability of mRNAs and proteins and in the activity of enzymes, as well as in the concentration of intracellular metabolites.

Transporters have been recruited to act as sensors for many nutrients and in some cases (Snf3/Rgt2, Ssy1) the sensors have lost the capacity to take up the corresponding ligand. Among the intermediary elements in the corresponding signalling pathways, some are shared by different nutrients (Yck1/2, Grr1, PKA), but the role they play may not be the same in all cases.

Although glucose-signalling pathways in yeasts and in mammalian cells share a number of similar elements (Hxk2/ Glucokinase, Snf1/AMPK), these elements are regulated differently and have different functions in the two kinds of cell.

An important aspect of yeast glucose signalling remains to be elucidated: the identification of intracellular metabolites able to play a regulatory role and the determination of their targets.
Note added in proof

It has recently been reported that it is hyperphosphorylation of Ptr3 that mediates activation of SPS signalling, and it has been suggested that Grr1 is required to allow the phosphorylation of Ptr3 by the kinases Yck1/2 (Liu et al., 2008).

Acknowledgements

I acknowledge the work performed by my students in the field of glucose signalling, with special thanks to M.M. Belinchón. I am grateful to C. Gancedo for useful insights during the writing of this review and for carefully reading the manuscript. This work has been supported by grant BFU2004-02855-C02-01 from the Dirección General de Investigación Científica y Técnica (DGICYT).

References


Belinchón MM & Gancedo JM (2007a) Different signalling pathways mediate glucose induction of SUC2, HXT1 and pyruvate decarboxylase in yeast. FEMS Yeast Res 7: 40–47.


Bernard F & André B (2001) Ubiquitin and the SCF (Grr1) ubiquitin ligase complex are involved in the signalling pathway...


Gamo FJ, Lafuente MJ & Gancedo C (1994) The mutation DGT1–1 decreases glucose transport and alleviates carbon catabolite


Kraakman L, Lemaire K, Ma P et al. (1999a) A Saccharomyces cerevisiae G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. Mol Microbiol 32: 1002–1012.


Özcan S & Johnston M (1999) Three different regulatory mechanisms enable yeast hexose transporter (\textit{Hxt}) genes to be induced by different levels of glucose. \textit{Mol Cell Biol} \textbf{15}: 1564–1572.


Wan Y, Pierce M, Schneper L, Guldal CG, Zhang X, Tavazoie S & Broach JR (2004) Ras and Gpa2 mediate one branch of a...


