Minireview

Multi-level response of the yeast genome to glucose
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Abstract

The yeast *Saccharomyces cerevisiae* shows a great variety of cellular responses to glucose via several glucose-sensing and signaling pathways. Recent microarray analysis has revealed multiple levels of genomic sensitivity to glucose and highlighted the power of genome-wide analysis to detect cellular responses to minute environmental changes.

Over the years, the yeast *Saccharomyces cerevisiae* has consolidated its position as an excellent model for the analysis of signal transduction pathways and their control of metabolic pathways, stress responses, growth and differentiation. In particular, the molecular mechanisms underlying the metabolic responses to different nutrient conditions have been studied extensively. With the elucidation of the complete yeast genome and the introduction of genome-wide expression analysis, the impact of distinct nutrient conditions on transcriptional responses can be explored on a genome-wide scale. Because of the powerful set of genetic and genomic tools that are available, yeast is the perfect organism for combining genome-wide studies of gene expression with the experimental elucidation of signaling networks; such studies have, among other findings, detected changes in expression of many genes of unknown function. Recent work has extended genome-wide studies of nutrient regulation to include the yeast response to very low concentrations of glucose [1].

Glucose signaling pathways regulating transcription

S. cerevisiae has a strong preference for glucose as its carbon source. This is reflected in the preferential conversion of glucose into ethanol, even under aerobic conditions, and in the preferential utilization of glucose when cells are grown in mixtures of glucose and other sugars. In addition, growth on

glucose is faster than on other carbon sources and a range of glucose-dependent effects can be observed on properties correlated with growth and stationary phase. During its evolution, *S. cerevisiae* has gained sophisticated regulatory mechanisms to sense fluctuating levels of glucose, and as a result has many different glucose-sensing and signaling pathways [2]. Three of these pathways are particularly well understood. The first is the main glucose repression pathway which controls, for example, respiration and the preferential utilization of glucose [3]; the second is the Snf3/Rgt2 pathway, which controls the expression of glucose carriers [4]; and the third is the Gpr1/Gpa2 pathway, which controls the synthesis of cAMP and activity of the protein kinase A (PKA) pathway [5] (Figure 1). In addition, many other glucose-induced regulatory effects have been described for which the glucose-sensing mechanism(s) and signaling pathway(s) are unclear. Glucose sensing via these pathways affects specific targets at the post-translational and/or transcriptional level, and a variety of transcription factors have been identified as targets of the glucose-signaling pathways (summarized in Table 1).

The first of the three well-characterized pathways, the main glucose repression pathway (Figure 1a) has been studied extensively for many years and is responsible for the repression - when glucose is available - of genes involved in respiration, gluconeogenesis and the metabolism of alternative
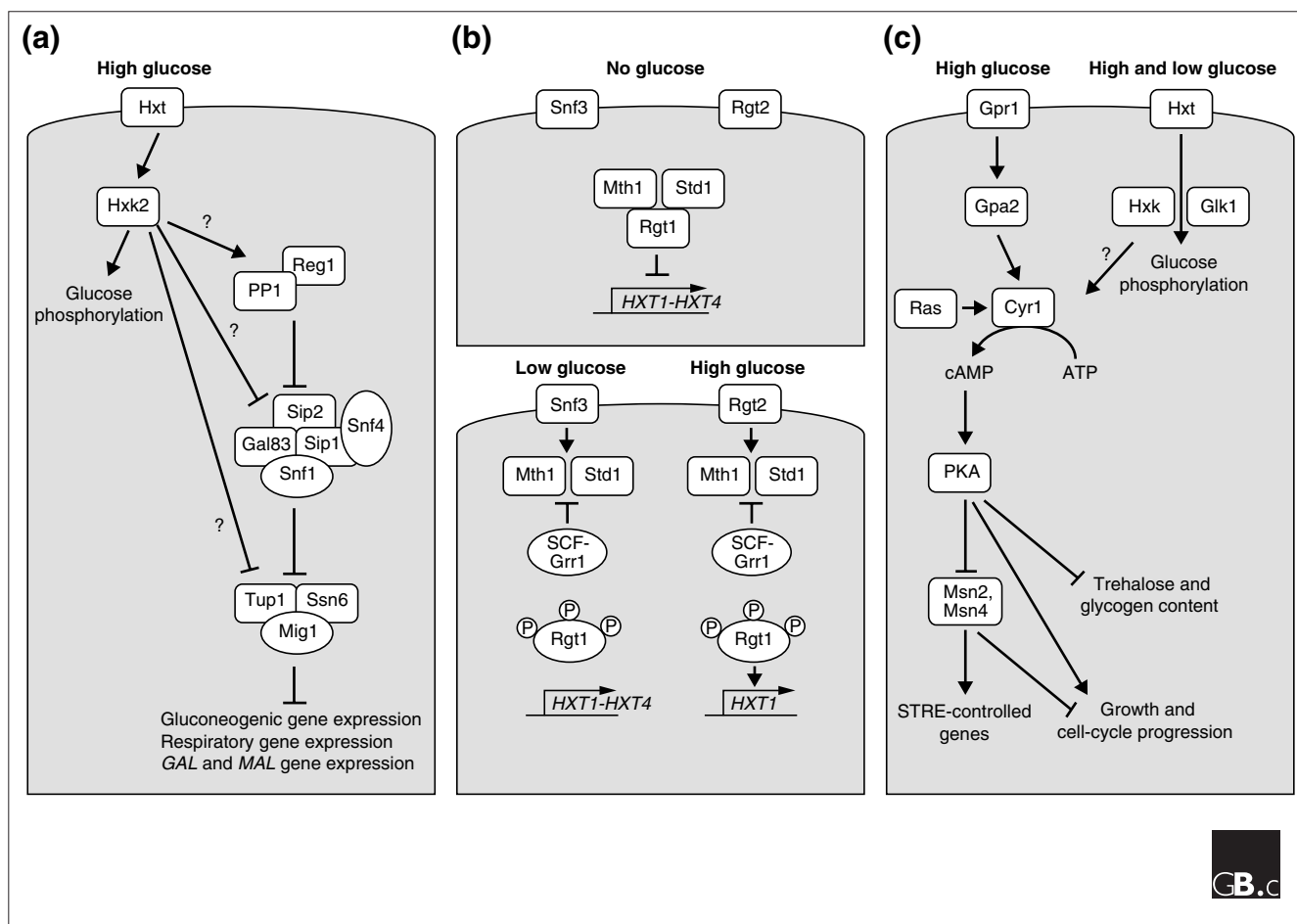
Figure 1
A simplified schematic representation of the three well-characterized glucose-response pathways in *S. cerevisiae*. **(a)** The main glucose repression pathway. In response to high glucose concentrations, the complex containing the Snf1 kinase inhibits the Mig1 repressor-containing complex and thus represses genes involved in respiration, gluconeogenesis and the metabolism of alternative carbon sources, such as galactose (*GAL* genes) and maltose (*MAL* genes). Protein phosphatase type 1 (PP1) acts in a complex with Reg1 to down-regulate Snf1 in low-glucose conditions. Glucose phosphorylation by Hxk2 is required for this pathway, but the step at which it acts is not known. **(b)** The Snf3/Rgt2 glucose-sensing pathway. In the absence of glucose, Rgt1 acts in a complex with Std1 and Mth1 as a transcriptional repressor of the *HXT1-HXT4* genes. When glucose is present, the transcription factor Rgt1 is inactivated through SCF-Grr1-mediated inactivation and degradation of Mth1 and Std1, and hyperphosphorylation by an unknown kinase, resulting in dissociation of Rgt1 from the *HXT* promoters. Snf3 triggers the induction of *HXT1-HXT4* in response to low glucose concentrations. High glucose concentrations further enhance *HXT1* expression through Rgt2 in a process that involves conversion of Rgt1 into a transcriptional activator. **(c)** The Gpr1/Gpa2 glucose-sensing pathway. High glucose concentrations activate cAMP synthesis by the adenylate cyclase Cyr1 (which is dependent on Ras) through the Gpr1/Gpa2 G-protein-coupled receptor system in a glucose-phosphorylation-dependent manner. The resulting activation of protein kinase A (PKA) affects a wide variety of target genes involved in, for example, carbon metabolism and stress resistance. Some of these effects are mediated by the Msn2 and Msn4 transcription factors. STRE, stress-response element. See text for further details.

carbon sources [3,6]. A central glucose-responsive repressor complex, made up of the Mig1, Ssn6 and Tup1 proteins, represses the transcription of gluconeogenic genes, respiratory genes and genes encoding enzymes involved in the utilization of galactose and maltose (Table 1). In the presence of glucose, the repressor complex is inhibited by a complex comprised of the protein kinase Snf1, the associated regulatory β subunits Sip1, Sip2 or Gal83, and the activating subunit Snf4 [7,8]. The Snf1 kinase was recently shown to be activated through phosphorylation of a conserved threonine residue in its activation loop by the Pak1, Tos3 and Elm1 kinases [9,10]. Down-regulation of Snf1 in low-glucose conditions is catalyzed by protein phosphatase type 1 (PP1), encoded by *GLC7*, which acts in a complex with the regulatory subunit Reg1/Hex2 [11]. Although several glucose effects on the Snf1 complex have been identified, the precise nature of the glucose-sensing mechanism involved in glucose repression still remains unclear. Glucose phosphorylation by Hxk2 is required for glucose repression, but whether this links to Snf1 function and/or to direct involvement of Hxk2 in transcriptional regulation in the nucleus remains to be elucidated [12].

Table 1

DNA sequences bound by glucose-regulated transcription factors

Transcription factor	Binding site*	Main function	References
Cat8	YCCRTYSRNCCG	Activator of gluconeogenic genes	[27]
Sip4	YCCRTYSRNCCG	Activator of gluconeogenic genes	[28]
Mig1/Mig2	T(C/G)(C/T)GGGG	Repressors of *SUC*, *MAL* and *GAL* genes	[29]
Gcr1	CTTCC	Activator of glycolytic genes	[30]
Rgt1	CGGANNA	Regulator of *HXT* gene expression	[31]
Nrg1/Nrg2	Not determined	Repressors of *SUC*, *GAL* and *FLO11* genes, interact with Snf1	[32-34]
Azf1	AAGAAAAA	Induction of *CLN3* gene expression	[23]
Msn2/Msn4	CCCCT	Induction of STRE-regulated genes	[20]
Gis1	T(T/A)AGGGAT	Activator of post-diauxic shift (PDS) genes	[21]
Pop2-Ccr4-Not	Not determined	Global transcriptional regulator of growth-, metabolism- and stationary phase related proteins	[35]

*Within DNA sequences, Y denotes a pyrimidine and R a purine. Abbreviation: STRE, stress-response element.

The second of the well-studied glucose-response pathways, the Snf3/Rgt2 glucose-sensing pathway (Figure 1b), is involved in controlling the expression of glucose transporter genes [4]. High glucose concentrations induce the *HXT1* gene through Rgt2, while low glucose concentrations induce the *HXT1-HXT4* genes through Snf3. *S. cerevisiae* has seven functional glucose transporter genes (*HXT1-HXT7*) that are well characterized and many more for which the nature and physiological role is rather unclear [4,13]; the expression of many of these genes depends on the concentration of glucose in the medium. The Snf3 and Rgt2 proteins are glucose-carrier homologs but are apparently unable to carry out active glucose transport; instead, Snf3 and Rgt2 specifically mediate regulation of the active glucose carriers by low and high glucose concentrations, respectively [14,15]. In the absence of glucose, Mth1 and Std1 stabilize the Rgt1-Ssn6-Tup1 repressor-corepressor complex. When glucose is present, the transcription factor Rgt1 is inactivated through SCF-Grr1-mediated inactivation and degradation of Mth1 and Std1, and hyperphosphorylation by an unknown kinase, resulting in dissociation of Rgt1 from the *HXT* promoters [16,17]. Remarkably, whereas Rgt1 represses *HXT* gene expression in the absence of glucose, at high glucose concentrations Rgt1 is converted to an activator that is required for maximal expression of the low-affinity glucose transporter *HXT1* (Table 1) [18]. The *HXT2*, *HXT4*, *HXT6* and *SNF3* genes are repressed by Mig1 via the main glucose-repression pathway when glucose is abundant [13].

When cells that have been growing on nonfermentable carbon sources have glucose added to the medium, the third well-studied pathway, the Gpr1/Gpa2 glucose-sensing pathway (Figure 1c), is involved in the rapid increase in cAMP levels that is mediated by the adenylate cyclase Cyr1;

the activity of Cyr1 in yeast depends on the Ras proteins. Activation of the Gpr1/Gpa2 pathway requires both extracellular glucose detection by the Gpr1-Gpa2 G-protein-coupled receptor system and intracellular glucose phosphorylation by one of the sugar kinases Hxk1, Hxk2 or Glk1 [2]. Subsequent activation of PKA affects the transcriptional regulation of genes involved in sugar metabolism, stress resistance and growth [5]. PKA mediates the repression of stress-resistance genes through the inactivation of the transcription factors Msn2 and Msn4. When activated, Msn2 and Msn4 are translocated from the cytoplasm to the nucleus [19], where they act through stress response elements (STREs) in the promoters of responsive genes (Table 1) [20]. The related post-diauxic shift element (Table 1) is affected in a similar way by the Gis1 transcription factor, which acts downstream in the Gpr1/Gpa2-PKA pathway [21].

Apart from these three well-characterized glucose-sensing pathways, other glucose-induced regulatory phenomena have been identified for which the signaling pathway(s) remain unclear. Examples include the glucose-induced upshift of ribosomal protein genes and *CLN3* expression; ribosomal gene expression seems to involve, to some extent, the PKA pathway [22], while induction of the *CLN3* gene requires the Azf1 transcription factor (Table 1) [23], but does not require active growth, is not blocked by mutations in the Ras/cAMP pathway or in the *RGT2, SNF3, HXK2* or *MIG1* genes and apparently depends on active glucose metabolism. The Yak1 kinase is also regulated by an unknown glucose-sensing pathway [24]; when glucose is exhausted, Yak1 accumulates in the nucleus and phosphorylates Pop2, a subunit of the Ccr4-Not complex that regulates the transcription of many genes involved in control of growth, metabolism and proper entry into stationary phase.

In addition to these complex transcriptional regulation pathways, glucose also controls mRNA stability, translation rate, protein turnover and enzyme activity by a range of different mechanisms. Most of these signaling pathways and mechanisms have been studied using classical methods, taking one or a few genes at a time, and glucose signaling in yeast was already recognised as being 'very complex' before the advent of genomic approaches.

Genome-wide analysis of responses to glucose

The introduction of genome-wide gene-expression analysis has extended the scope of glucose regulation to much larger parts of the genome than were previously implicated [25]. The recent work by the group of Al Brown [1] has added another dimension to glucose regulation by revealing different levels of genomic sensitivity to glucose in yeast. They have shown that very low (0.01%), low (0.1%) and high (1.0%) glucose concentrations elicit strikingly different responses in the genome-wide expression pattern. They confirmed the extreme sensitivity of yeast to glucose by showing that genes involved in carbon metabolism are strongly upregulated in the presence of only 0.01% glucose. In addition, the strong differential expression profiles of the different hexose transporter genes (*HXT1-HXT7*) was evident from the transcript-profiling data [1]. Remarkably, the expression of not only gluconeogenic genes, but also genes involved in the glyoxylate and tricarboxylic acid cycles, is very sensitive to these minute amounts of glucose. This shows that even at very low concentrations of glucose, yeast cells start to adapt to fermentative growth, although respiration is still active.

Glucose-controlled mRNA stability

Another novel aspect of the recent work from the Brown group is the influence of different glucose signals on ribosome biogenesis [1]. On the one hand, the microarray data confirmed that ribosomal gene expression is induced in response to high, but not low, glucose signals. On the other hand, mRNA stability assays revealed that glucose, even at concentrations as low as 0.01%, transiently stabilizes the mRNAs for ribosomal proteins. Ribosomal protein biosynthesis requires a major investment of cellular resources because ribosomal protein mRNAs are abundantly present in fast-growing cells and yet have short half-lives. The stabilization of the mRNAs for ribosomal proteins by a low-glucose signal, therefore, appears to be an 'economical' means by which to maintain proliferative capacity in a nutrient-limiting regime without synthesis of new ribosomal proteins. Typically for glucose regulation, this temporary stabilization of ribosomal protein mRNAs is not controlled by just one signaling pathway; rather, the mRNAs for different ribosomal subunits appear to be regulated by distinct glucose-signaling mechanisms.

The genes involved in gluconeogenesis are strongly repressed at glucose concentrations as low as 0.01%. This response to very low glucose concentrations is mediated by two regulatory phenomena: transcriptional repression and accelerated mRNA degradation [26]. Yin *et al.* [26] also showed that the degradation of gluconeogenic mRNA is dependent on the high-affinity glucose sensor Snf3, the phosphorylation of internalized sugars by hexokinases, the protein phosphatase-1 subunit Reg1 and the serine/threonine protein kinase Ume5.

In conclusion, genome-wide transcription analyses will broaden our understanding of the global response of a yeast cell to fluctuating nutrient levels. The glucose response in yeast is an excellent illustration of the complexity of transcriptional regulation by nutrients. Because of this complexity, microarray experiments will be very useful in identifying groups of genes regulated through the different signaling pathways. In addition, this technique will be indispensable for identifying the scope of transcriptional regulation and the extent of overlap between the different signaling pathways, in particular as a function of nutrient concentration. The remarkable sensitivity of the yeast transcriptome to very low glucose concentrations has revealed a new field of application for microarrays: identification of cellular responses to minute changes in the environmental conditions. Microarray analysis appears to provide unprecedented power to identify even the smallest cellular responses.

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