Transcriptional responses to glucose at different glycolytic rates in *Saccharomyces cerevisiae*

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The addition of glucose to *Saccharomyces cerevisiae* cells causes reprogramming of gene expression. Glucose is sensed by membrane receptors as well as (so far elusive) intracellular sensing mechanisms. The availability of four yeast strains that display different hexose uptake capacities allowed us to study glucose-induced effects at different glycolytic rates. Rapid glucose responses were observed in all strains able to take up glucose, consistent with intracellular sensing. The degree of long-term responses, however, clearly correlated with the glycolytic rate: glucose-stimulated expression of genes encoding enzymes of the lower part of glycolysis showed an almost linear correlation with the glycolytic rate, while expression levels of genes encoding gluconeogenic enzymes and invertase (*SUC2*) showed an inverse correlation. Glucose control of *SUC2* expression is mediated by the Snf1-Mig1 pathway. Mig1 dephosphorylation upon glucose addition is known to lead to repression of target genes. Mig1 was initially dephosphorylated upon glucose addition in all strains able to take up glucose, but remained dephosphorylated only at high glycolytic rates. Remarkably, transient Mig1-dephosphorylation was accompanied by the repression of *SUC2* expression at high glycolytic rates, but stimulated *SUC2* expression at low glycolytic rates. This suggests that Mig1-mediated repression can be overruled by factors mediating induction via a low glucose signal. At low and moderate glycolytic rates, Mig1 was partly dephosphorylated both in the presence of phosphorylated, active Snf1, and unphosphorylated, inactive Snf1, indicating that Mig1 was actively phosphorylated and dephosphorylated simultaneously, suggesting independent control of both processes. Taken together, it appears that glucose addition affects the expression of *SUC2* as well as Mig1 activity by both Snf1-dependent and -independent mechanisms that can now be dissected and resolved as early and late/sustained responses.

**Keywords**: *Saccharomyces cerevisiae*; Mig1; Snf1; glucose repression; glucose signal.

Addition of glucose to *Saccharomyces cerevisiae* cells growing in the absence of glucose causes an extensive reprogramming of gene expression and metabolism. These changes affect chromatin structure, transcription, mRNA stability, translation and post-translational modifications [1–4]. A range of different signalling pathways, including, among others, the Snf1–Mig1 pathway, the Snf3–Rgt2 pathway and the Ras-cAMP pathway [5], are responsible for these effects. Glucose sensing appears to occur at seemingly simple responses can be dissected into different components with potentially different underlying mechanisms.

This study focused on the effects on mRNA levels of different sets of genes. One such set are genes encoding enzymes of glycolysis. While expression of genes encoding enzymes operating in both glycolysis and gluconeogenesis usually remain constitutive [6,7], expression of genes for enzymes specific to the lower part of glycolysis is stimulated upon glucose addition [8]. The underlying signalling pathway is not understood. However, it has been reported that stimulated expression requires glucose metabolism through the upper part of glycolysis [9]. On the other hand, expression of genes encoding enzymes specific for gluconeogenesis, respiration, or the uptake and utilization of alternative carbon sources, is efficiently repressed by glucose [4]. Glucose repression is a complex process involving different regulators affecting different subsets of genes. Best studied is the Snf1–Mig1 pathway, which is involved in the (de)repression of genes encoding enzymes needed for the utilization of alternative carbon sources as well as for gluconeogenesis and respiration. The protein kinase Snf1 is activated by phosphorylation at low/no glucose [10]. Recently, three protein kinases – Elm1, Tos3 and Pak1 [11–13] – were identified that seem to mediate Snf1 activation. It is unclear how these kinases are controlled.
but it appears that the hexokinases, Hxk1 and Hxk2, may play some role in this process [14–17]. In addition, a decreased Glc7 phosphatase activity may also contribute to Snf1 activation, as has been shown by deletion studies of REG1 by Treitel et al. and McCartney et al. [10,18]. Also, protein interactions, as well as carbon source-dependent phosphorylation of Reg1, may effect Reg1/Glc7 activity [19,20]. An active Snf1 phosphorylates at least four sites in the transcriptional repressor Mig1. Mig1 phosphorylation causes the majority of the protein to exit the nucleus [21]. Recent data, however, suggests that phosphorylation-mediated altered interaction with the two co-repressors Cyc8 (Ssn6) and Tup1 on target promoters is the primary cause for the switch between repression and derepression [22].

Time-course analyses suggested that the process of glucose repression consists of a short- and a long-term response (minutes and hours, respectively) [23,24]. Those could be distinguished on the basis of their different requirements for sugar kinases, suggesting different signalling pathways. While long-term glucose repression required Hxk2, for short-term repression any of the three sugar kinases, Hxk1, Hxk2 or Glk1, was sufficient [23,24]. It should be noted that Hxk2 does not have a unique role in glucose repression, as often claimed in the literature, but that Hxk1 also contributes to glucose and, in particular, to fructose repression [24].

Earlier studies showed a correlation between glucose consumption rate and glucose repression [25–27]. Our previously reported series of strains, in which sugar uptake is mediated by the individual expression of different native and chimeric hexose transporters [28,29], display a wide spectrum of glucose uptake rates. These strains are therefore useful for investigating the effects of different glycolytic rates on glucose-induced signalling pathways. For this study we have chosen four strains, which represents the full range of glycolytic rates: a wild-type, with a high glycolytic rate; a HXT-null strain, which does not take up glucose owing to the deletion of all known hexose transporter (HXT) genes; a strain expressing Hxt7 as the sole sugar transporter, which displays relatively high sugar uptake rates; and a strain that expresses Hxt-Tm6*, a chimera of Hxt1 and Hxt7. Hxt-Tm6* mediates low uptake rates and, for that reason, the strain does not produce ethanol also in the presence of high external sugar levels [28,29].

Materials and methods

Strains

The strains used are listed in Table 1 and all derive from CEN.PK2-1C MATa leu2-3 122 ura3-52 trpl-289 his3-D MAL2-8· SUC2 hxt12Δ [30]. KOY.PK2-1C83 (wild-type) is the prototrophic version of the CEN.PK2-1C strain [28]. In KOY.VW100P (HXT-null), all known hexose transporters have been deleted and an expression cassette has been introduced in the HXT3-6-7 locus [28]. KOY.HXT7P (HXT7) and KOY.TM6*P (HXT-Tm6*) have HXT7 and the chimera HXT-Tm6*, respectively, cloned into this expression cassette [28,29].

Plasmid pRS316 carrying either SNF1 [10] or MIG1 [31] tagged with the haemagglutinin (HA) epitope at the C-termini was transformed into KOY.PK2-1C82, KOY.HXT7, KOY.TM6* and KOY.VW100P strains, which are isogenic to the strains listed above except that they contain the ura3-52 marker. The resulting transformants are hence prototrophic. For Mig1-GFP localization, plasmid BM3315 [21] was transformed into the same strains.

Cultures

Cells were precultured at 30 °C for 48 h in 50 mL of complete minimal medium [32], supplemented with 1% (v/v) ethanol. Fermentors containing 1.5 L of minimal medium (5x concentrated) were inoculated to an attenuation (D), at 610 nm, of 0.05. Conditions were maintained constant at 30 °C, 1500 r.p.m. and pH 5.0. Off gas was maintained at 0.75 L min⁻¹ by using a mass flow regulator. Gas was passed through a condenser to avoid evaporation. Carbon dioxide production and oxygen consumption were measured on-line (type 1308; Bruel and Kjaer, Nærum, Denmark). At a D610 of 1 to 1.5, glucose was added to a final concentration of 5% and samples were taken at 1, 5, 10, 15, 20, 30 and 60 min as well as at residual glucose concentrations of 1.5–2.5%. For the HXT-null strain, samples were taken in the ethanol consumption phase following glucose addition.

Biochemical determinations and consumption rates

Glucose and ethanol were measured in the supernatant (1 min at 16 000 g) using enzymatic combination kits (Roche). Several samples were taken during logarithmic growth on glucose, and the specific glucose consumption rate was determined at a specific time-point.

Quantitative PCR (QPCR)

Samples for RNA extraction were taken into ice-cold water. RNA was extracted, treated with DNase, and checked for purity by agarose-gel electrophoresis. Samples were prepared [28] and normalized against the quotient between the levels of the ACT1 and IPP1 mRNAs. The lowest value for each gene was set to 1. The standard deviation of the QPCR is < 0.25 cycles and at least two independent fermentations were performed. Duplicate samples from each fermentation were analysed.

Protein extracts and Western blot analysis

Cells were harvested and proteins extracted as described in McCartney et al. [10]. For the detection of Mig1-HA, samples were separated by PAGE on 7.5% (w/v) SDS gels and blotted onto nitrocellulose membranes. Membranes were blocked at room temperature for 1 h in TTBS [TBS containing 0.1% (v/v) Tween-20] containing 3% (w/v) BSA, and washed three times (5 min each wash) in TTBS, incubated at 4 °C for 3 h with HA mAb (1 : 1000) (Amersham) in TTBS containing 3% (w/v) BSA, washed three times (5 min each wash) in TTBS, and incubated for 1 h at room temperature with secondary anti-mouse immunoglobulin (1 : 1000 dilution) in TTBS containing 3% (w/v) BSA. The membrane was washed three times (5 min each wash) in
Table 1. *Saccharomyces cerevisiae* strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tr>
<td>KOY.PK2-1C83</td>
<td>MATa <em>MAL2-8′</em> <em>SUC2</em></td>
<td>Prototrophic [28]</td>
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<tr>
<td>KOY.PK2-1C82</td>
<td>MATa <em>MAL2-8′</em> <em>SUC2</em> <em>ura</em> 3-52</td>
<td>Auxotrophic: this study</td>
</tr>
<tr>
<td>KOY.VW100P</td>
<td>KOY.VW100P Integration into the cassette: <em>HXT7</em> [46], the KlURA3 open reading frame containing the truncated, constitutive promoter of <em>HXT7</em>, the KlURA3 open reading frame for counter selection, and the <em>HXT7</em> terminator.</td>
<td>Prototrophic [28]</td>
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<tr>
<td>KOY.HXT7P</td>
<td>KOY.VW100P Integration into the cassette: <em>HXT7</em> [46]</td>
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<tr>
<td>KOY.TM6*P</td>
<td>KOY.VW100P Integration into the cassette: <em>HXT-TM6</em> [46]</td>
<td>Prototrophic [28]</td>
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<tr>
<td>KOY.HXT7</td>
<td>KOY.VW100P Integration into the cassette: <em>HXT7</em> [46]</td>
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<td>KOY.TM6*</td>
<td>KOY.VW100P Integration into the cassette: <em>HXT-TM6</em> [46]</td>
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TTBS prior to detection by chemiluminescence using ELC plus (Amersham). Snf1-HA samples were dialysed against buffer overnight [150 mM NaCl, 1% (v/v) Triton X-100, 0.5% (w/v) deoxycholate, 50 mM Tris/HCl, pH 8.00, supplemented with 50 mM sodium fluoride and 5 mM sodium pyrophosphate], and 400 mg of total protein was used for immunoprecipitation of Snf1-HA [10]. The precipitate was dissolved in SDS sample buffer, separated by PAGE on a 7.5% (w/v) SDS gel, blotted onto nitrocellulose membrane and phospho-Snf1 was detected by using the α-PT210 antibody, as described by McCartney et al. [10]. As a control for equal loading, membranes were stripped and the HA epitope on Snf1 was detected by a monoclonal anti-HA immunoglobulin, as described above.

**Phosphatase treatment**

For phosphatase treatment of Mig1, 50 μg of total protein extract was precipitated with 10% (w/v) trichloroacetic acid and sedimented for 30 min at 4 °C. The sediments were washed twice with ice-cold 100% acetone for 15 min and centrifuged for 15 min between each wash, air-dried, resuspended in 82 μL of H2O containing 10 μL of 10x phosphatase buffer and 8 U calf intestine alkaline phosphatase (Roche), and incubated at 37 °C for 1 h. Samples were again precipitated with trichloroacetic acid, resuspended in SDS sample buffer, boiled for 5 min and electrophoresed. Gels were blotted and proteins detected, as described above (in Western blot analysis), for Mig1-HA detection.

**Determination of invertase activity**

Cells were grown in Erlenmeyer flasks containing 2x minimal medium [32] supplemented with 5% (w/v) glucose to a D₆₁₀ of 1, then harvested by centrifugation. Protein extracts and measurements of invertase activity were performed as described previously [33].

**Microscopy**

Localization of Mig1-GFP was visualized by using a GFP filter on a Leica DMRXA microscope. DNA was stained by 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (1 μg/mL) for 10 min at 30 °C after which the cells were quickly washed three times in growth media.

**Results**

**Four strains displaying different glycolytic rates**

The wild-type, *HXT7* and *HXT-TM6* strains display high (15.8 mmol g⁻¹ h⁻¹), intermediate (10.7 mmol g⁻¹ h⁻¹) and low (3.5 mmol g⁻¹ h⁻¹) glucose consumption rates, respectively [28,29]. The *HXT-null* strain neither takes up glucose nor grows with glucose as the sole carbon source [34] (Fig. 1). In order to follow glucose-induced responses, the yeast strains were grown in the presence of 1% (v/v) ethanol to a D₆₁₀ of 1, pulsed with glucose to a final concentration of 5%, and sampled over a period of 1 h as well as in the subsequent glucose consumption phase (Fig. 1). After the glucose pulse, the wild-type and *HXT7* strains displayed a clear biphasic growth with an initial respiro-fermentative phase where ethanol was produced (Fig. 1) and a subsequent respiratory phase where this ethanol was then consumed (data not shown). In the *HXT-TM6* strain, glucose is only respired, as described previously [28,29]. Following glucose addition the *HXT-TM6* strain initially consumed glucose
and ethanol simultaneously, and once ethanol was depleted it continued to catabolize glucose (Fig. 1). The HXT-null strain continued consuming ethanol, leaving glucose unconsumered.

**Short-term response to glucose addition**

Using QPCR we monitored the response to glucose of four glucose-induced genes encoding enzymes of the lower part of glycolysis (PPII, PGK1, PDC1 and ADH1), of three glucose-repressed genes encoding enzymes in gluconeogenesis and the glyoxylate cycle (FBP1, MDH2, ADH2), as well as of the glucose-repressed SUC2 (invertase) gene. In wild-type cells, expression of all four glycolytic genes was strongly stimulated by glucose, reaching a plateau after about 30 min (Fig. 2). Expression of these genes was not stimulated at all in the HXT-null strain, or rather diminished in the case of PGK1 and TP11. The strains expressing Hxt7 and HXT-TM6* as sole hexose transporter showed intermediate levels of stimulation, which differed in a gene-specific manner (Fig. 2). Generally, it appeared that the degree of induction correlated approximately with the glycolytic rate (measured as the glucose consumption rate) of the strains.

The mRNA level of the gluconeogenic and glyoxylate cycle genes, FBP1, ADH2 and MDH2, was rapidly diminished following glucose addition in all strains able to take up glucose. In the HXT-null strain, the mRNA of all these genes transiently increased and then either plateaued or decreased.

The expression level of SUC2 diminished in the wild-type yeast and in the strain expressing HXT7, while it did not respond to glucose addition in the HXT-null strain. In the HXT-TM6* strain, expression of SUC2 was transiently stimulated.

**Long-term glucose response**

In order to study the long-term glucose response, samples from cells growing exponentially with glucose were taken when 1.5–2.5% of glucose was still present in the culture medium (indicated in Fig. 1). For the HXT-null strain, samples were taken 5–8 h after glucose addition when the strain was still consuming ethanol.

For the glucose-induced glycolytic genes PPII, PGK1, PDC1 and ADH1, the long-term expression level showed an approximately linear correlation with the glycolytic rate, especially for PGK1 and PDC1 genes (Fig. 3). In the HXT-null strain, expression levels of PPII, PDC1 and ADH1 did not differ from those of cells growing in the presence of ethanol only, while the mRNA level of PGK1 was threefold lower. Expression of the gluconeogenic genes FBP1 and MDH2 was strongly repressed by 5% glucose in the wild-type and HXT7 strains and repressed to a lower extent in the HXT-TM6* strain (Table 2). Expression of FBP1 and MDH2 was unaffected by glucose in the HXT-null strain. Expression of ADH2 was strongly repressed immediately after glucose addition and remained repressed in the wild-type and HXT7 strains. In the HXT-TM6* strain, however, ADH2 remained repressed during the phase of glucose/ethanol co-consumption (data not shown), but when ethanol was depleted and the strain only consumed glucose, ADH2 became fully derepressed (Table 2). The reason for this behaviour is unclear. Expression of SUC2 was repressed twofold in the wild-type yeast, slightly increased in the HXT7 strain and stimulated fourfold in the HXT-TM6* strain during growth on glucose. In the HXT-null strain, expression of SUC2 did not seem to respond to glucose (Table 2). In agreement with mRNA levels, invertase activity measurements with glucose-grown cells showed increased activity in the HXT7 and HXT-TM6* strains, while activity remained at a low level in the wild-type yeast. The HXT-null strain, which was grown on ethanol supplemented with 5% glucose, displayed an intermediate level of activity (Fig. 4).
Snf1 and Mig1 phosphorylation in the wild-type yeast, and in HXT7, HXT-TM6* and HXT-Null strains

Because of the interesting expression pattern of SUC2, we investigated the state of the glucose repression signalling pathway by monitoring the phosphorylation patterns of Mig1 and Snf1. Snf1 is activated by phosphorylation at low/no glucose [10,35], and phosphorylation on the critical T210 residue can be monitored by using a specific antibody [10]. Active Snf1 phosphorylates the repressor Mig1 on multiple sites, leading to derepression of target genes, such as SUC2 [18,36,37]. Mig1 phosphorylation can be visualized as a mobility shift by using HA-tagged Mig1 and immunoblotting.

The short-term response was studied by monitoring the electrophoretic migration of Mig1 following the addition of glucose to ethanol-grown cells (the same conditions as in Figs 1 and 2). In ethanol-grown cells, Mig1 appeared as a ladder of bands (Fig. 5A), indicating that the protein was phosphorylated to a different extent and was partially inactive as a repressor. Interestingly, Mig1 from cells growing in the presence of 0.05% glucose migrated as a single slow band, indicating that under these conditions Mig1 is fully phosphorylated and inactive. This fits with the observation that SUC2 expression is much higher in cells growing in the presence of low glucose levels than in ethanol medium ([38], own unpublished data). Mig1 from cells growing with 5% glucose, on the other hand, migrated as a single fast band of fully dephosphorylated and hence actively repressing Mig1 (Fig. 5A, see also Fig. 6).

Interestingly, in all glucose-utilizing strains, the addition of glucose to ethanol-grown cells caused a collapse of the Mig1 ladder to the unphosphorylated (actively repressing) form. Only in the HXT-null strain was the band pattern largely unaltered. While Mig1 remained unphosphorylated in the wild-type yeast throughout the time course of the experiment, it appeared to be rephosphorylated in the HXT7 and HXT-TM6* strain towards the end of the time course.

As it appeared that the level of Mig1 increased during the time course of the experiment, we performed QPCR analysis of MIG1 gene expression (Fig. 5B). Indeed,
expression of MIG1 was stimulated upon glucose addition, in accordance with recently published data [39]. Stimulation of expression inversely correlated with the glycolytic rate and, interestingly, was apparent even in the HXT-null strain.

To monitor the long-term glucose response, the four strains were grown in the presence of a high (5%) concentration of glucose to a $D_{610}$ of 1.0. A sample was shifted to a low (0.05%) concentration of glucose as a control, and the phosphorylation state of Snf1 and the mobility pattern of Mig1 were analysed (Fig. 6). Mig1 from wild-type cells migrated as the apparently fully phosphorylated form on the low concentration of glucose and as the dephosphorylated form on the high concentration of glucose (Fig. 6A). Migration of this latter band did not change upon treatment with alkaline phosphatase, confirming that it represents the fully dephosphorylated form (Fig. 6C). Snf1 was largely unphosphorylated in wild-type cells growing in a high concentration of glucose, while the level of phosphorylated Snf1 was increased in cells shifted to a low concentration of glucose. In the HXT-null strain, Mig1 migrated at an intermediate rate (high glucose) or as a diffuse ladder (low glucose), and Snf1 was phosphorylated under both conditions. In the HXT7-expressing strain, Snf1 was (as in the wild-type) unphosphorylated when grown on a high concentration of glucose, whereas Mig1 was partially phosphorylated (Fig. 6A,B), as also illustrated by the fact that the Mig1-band migrated more quickly after phosphatase treatment (Fig. 6C). In the HXT-TM6* strain, Snf1 was strongly phosphorylated in cells growing in conditions of both high and low glucose, consistent with a fully glucose derepressed state of the cell. Interestingly, it appeared that Mig1 assumed an intermediate level of phosphorylation in the HXT-TM6* strain on high glucose (Fig. 6A,B). When comparing the three strains able to take up glucose, it appeared that the phosphorylation of Mig1 correlated well with the glycolytic rate, whereas Snf1 phosphorylation did not (Fig. 6A,6B).

A good correlation was also seen of the glycolytic rate, apparent phosphorylation state of Mig1, and its subcellular localization. Dephosphorylated Mig1, for example in glucose-grown wild-type cells, has been reported to concentrate in the nucleus, and this was also observed in the present study (Fig. 7). Mig1 from HXT7-expressing cells showed increased nuclear localization, although not as strongly as in the wild-type. In HXT-TM6*, as well as in HXT-null cells, Mig1 was localized diffusely throughout the cell after the glucose pulse. In the latter two strains, DAPI staining did not clearly reveal the nucleus owing to a high abundance of mitochondria, which is consistent with the respiratory metabolism of these strains.

**Discussion**

In this study we have used yeast strains with a very broad range of glycolytic rates to study glucose-induced responses while maintaining identical growth conditions as well as high external glucose concentrations.

The results confirm previous reports in that the signalling pathways studied here are triggered inside the cell rather
The phosphorylation level of Mig1 was estimated as a band-shift. Samples were added at 0 h to a final concentration of 5%. (A) The phosphorylation state of Mig1 (see Fig. 6B for phosphatase-treated controls). A total of 60 μg of extract was loaded in each lane. (B) Detection of phosphorylated Snf1 by using an antibody specific for Snf1 phosphorylated at T210. The haemagglutinin (HA) signal was used as a loading control. (C) Treatment of extracts with alkaline phosphatase as a control for the Mig1 phosphorylation state. A total of 50 μg of total protein from the wild-type, HXT7, and HXT-TM6* strains were incubated with and without calf intestine alkaline phosphatase (AP). Untreated wild-type samples were loaded as migration controls.

Fig. 5. Mig1 gel mobility pattern in response to glucose addition. Glucose was added at 0 h to a final concentration of 5%. (A) The phosphorylation level of Mig1 was estimated as a band-shift. Samples (30 μg) from wild-type cells grown at high (5%) or low (0.05%) concentrations of glucose were loaded as a comparison. Slow migration indicates fully phosphorylated and fast migration fully dephosphorylated Mig1 (see Fig. 6B for phosphatase-treated controls). A total of 60 μg of extract was loaded for wild-type, HXT7, HXT-TM6* and HXT-null strains. (B) mRNA expression of Mig1 during the first hour after glucose addition, as determined by quantitative PCR (qPCR). Wild-type (■), HXT7 (○), HXT-TM6* (▲), HXT-null strains (□).

Fig. 6. Mig1 and Snf1 phosphorylation in glucose-growing cells. Strains were grown in 5% glucose (H) and shifted to 0.05% glucose (L) for 2 h. The HXT-null strain was grown in 1% ethanol supplemented with 5% glucose (H) and shifted to 0.05% glucose (L) for 2 h. (A) The migration pattern of Mig1. A total of 60 μg of extract was loaded in each lane. (B) Detection of phosphorylated Snf1 by using an antibody specific for Snf1 phosphorylated at T210. The haemagglutinin (HA) signal was used as a loading control. (C) Treatment of extracts with alkaline phosphatase as a control for the Mig1 phosphorylation state. A total of 50 μg of total protein from the wild-type, HXT7 and HXT-TM6* strains were incubated with and without calf intestine alkaline phosphatase (AP). Untreated wild-type samples were loaded as migration controls.

than by plasma membrane-localized receptors. This was first illustrated by the fact that the HXT-null strain, which does not take up glucose, also does not respond to glucose addition. We only observed two potentially relevant deviations: expression levels of gluconeogenic genes transiently increased upon glucose addition to the HXT-null strain, and the expression level of MIG1 was moderately stimulated. These effects could be caused either by minute amounts of glucose diffusing into cells of the HXT-null strain or to signalling pathways sensing external glucose, such as the Gpr1-PKA pathway. That signalling is triggered inside the cells is further indicated by the fact that different glucose consuming, and hence glycolytic rates, caused a different signalling output. The actual signal(s) and sensing mechanisms still remain to be identified, but strains like those used here will certainly be useful in such studies.

We observed an almost perfect correlation between the apparent glycolytic rate and the degree of induction of glycolytic gene expression. This is consistent with previous chemostat studies of the CEN.PK strain cultured at different glycolytic rates within the respiro-fermentative phase, i.e. high dilution rates [40]. Interestingly, all glucose-consuming strains responded equally quickly to glucose addition and the difference was manifested as different amplitudes of expression. This suggests that the – so far elusive – sensing mechanism somehow monitors quantitative differences of the glycolytic rate.

Similarly, expression of gluconeogenic genes was repressed in all three glucose-consuming strains equally quickly. Hence, consistent with previous studies, repression of these genes is very sensitive to glucose [41]. However, gluconeogenic genes were repressed to a much lesser extent in HXT-TM6* cells growing in the presence of high glucose levels, suggesting the interesting scenario that HXT-TM6* cells co-express glycolytic and gluconeogenic enzymes. Potential futile cycling is not likely as a higher biomass is obtained in the HXT-TM6* strain as compared to wild-type yeast [28]. Moreover, the alcohol dehydrogenases seem to be regulated in an interesting way in this strain. Expression of ADH2, which encodes the glucose-repressed alcohol dehydrogenase responsible for ethanol consumption, was strongly repressed in HXT-TM6* cells during glucose/ethanol co-consumption. It is possible, that the enzyme encoded by the glycolytic ADH1, whose expression was stimulated fourfold under these conditions (data not shown) takes over the role of Adh2. Once ethanol was depleted and the strain grew solely on glucose, ADH2 expression was again derepressed to the same level as before glucose addition. The glycolytic rate was identical during glucose consumption and glucose/ethanol co-consumption in the HXT-TM6* cells (data not shown).

The expression of SUC2, a classical model for a glucose-regulated gene, appeared particularly interesting, as it showed very different responses in the four strains.
Employing strains expressing different hexose transporters or a given transporter at different levels, it has previously been observed that there is a good correlation between the apparent glycolytic rate and the degree of long-term glucose repression [42–44]. This is confirmed here, although the picture is complicated by the fact that expression of SUC2 is stimulated by low glucose levels ([38], own data). Stimulated SUC2 expression upon glucose addition in the HXT-TM6* strain illustrates that the glucose repression signalling system perceives a ‘low glucose’ signal, despite the fact that the external glucose level is high. The derepressed state of this strain is confirmed by a high level of phosphorylation of Snf1. In order to achieve complete glucose repression, the wild-type glycolytic rate seems to be required because even the HXT7 strain, which displayed two-thirds of the wild-type rate, did not fully repress SUC2 expression.

Expression of SUC2 and gluconeogenic genes is controlled by the Snf1 kinase and the Mig1 repressor. Gluconeogenic genes are also controlled by the Snf1-dependent Cat8 and Sip4 activators. Monitoring Snf1 and Mig1 phosphorylation revealed some unexpected observations that will require further investigation. Perhaps most perplexing is the observation that Mig1 becomes rapidly dephosphorylated upon glucose addition in the HXT-TM6* strain while, at the same time, the expression level of SUC2 strongly increases. This is in clear contradiction to the current view that dephosphorylation, nuclear Mig1 represses SUC2 expression. This observation suggests that the system which mediates induction of SUC2 at a low glycolytic rate is able to overcome Mig1-mediated repression. Another surprising observation concerns the only partial phosphorylation of Mig1 in the HXT-TM6* strain growing at high glucose levels, despite the fact that Snf1 is strongly phosphorylated. Partial phosphorylation of Mig1 is also seen in the HXT7 strain at high glucose, even though Snf1 is unphosphorylated. This is not caused by the strain being unable to dephosphorylate Mig1, as this species is observed transiently upon glucose addition. This observation suggests that the phosphorylation state of Mig1 is not only controlled by Snf1-dependent phosphorylation but, obviously, also by dephosphorylation, which is mediated by the Glc7-Reg1 system [18]. If indeed the observed Mig1 phosphorylation pattern is caused by simultaneous phosphorylation/dephosphorylation, these two processes might be controlled by different signalling mechanisms. The fact that one distinct Mig1 band is observed under these conditions further suggests that certain phosphorylation sites are used preferentially, which will be tested in the future. The interplay between the two processes apparently allows fine-tuning of the Mig1 phosphorylation level. An almost linear correlation between Mig1 activity and sites phosphorylated by Snf1 has been observed [21]. Future work, for which the strains used here will be instrumental, will address the precise mechanisms controlling Mig1 activity and their interplay with the factor(s) mediating induction by low glucose.

It has previously been proposed that the establishment of glucose repression can be dissected into a short-term and a long-term response. That proposal was based on different roles of the sugar kinases: the hXX2Δ mutant displayed short-term glucose repression but was unable to maintain repression [24]. In a similar way, the HXT7 and HXT-TM6* strains displayed short-term Mig1 dephosphorylation (supposedly activating the repressor, although stimulated SUC2 expression was observed, see above) but subsequently Mig1 became rephosphorylated. Although unlikely, we cannot exclude that in our experiment this biphasic behaviour is caused by properties of the single hexose transporters.
expressed in these cells. Both Hxt7 and Hxt-TM6* are high-affinity glucose transporters, which in wild-type cells are active at low/no glucose and inactivated in medium containing a high concentration of glucose [45]. Hence, it may be that during adaptation to glucose, the levels of active transporters diminish, although quantification of the transporter mRNA of the chimeras shows identical expression during growth on ethanol and glucose (data not shown). Another interpretation for the biphasic behaviour is, like in the hok2A mutant, the initial, acute response and the late sustained response are governed by different regulatory systems. In that scenario, the initial response seems to be more sensitive to glucose, while the sustained response would require higher glucose levels.

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References


