Nutrient Sensing through the Plasma Membrane of Eukaryotic Cells

Glucose as a hormone: receptor-mediated glucose sensing in the yeast *Saccharomyces cerevisiae*

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Abstract
Because glucose is the principal carbon and energy source for most cells, most organisms have evolved numerous and sophisticated mechanisms for sensing glucose and responding to it appropriately. This is especially apparent in the yeast *Saccharomyces cerevisiae*, where these regulatory mechanisms determine the distinctive fermentative metabolism of yeast, a lifestyle it shares with many kinds of tumour cells. Because energy generation by fermentation of glucose is inefficient, yeast cells must vigorously metabolize glucose. They do this, in part, by carefully regulating the first, rate-limiting step of glucose utilization: its transport. Yeast cells have learned how to sense the amount of glucose that is available and respond by expressing the most appropriate of its 17 glucose transporters. They do this through a signal transduction pathway that begins at the cell surface with the Snf3 and Rgt2 glucose sensors and ends in the nucleus with the Rgt1 transcription factor that regulates expression of genes encoding glucose transporters. We explain this glucose signal transduction pathway, and describe how it fits into a highly interconnected regulatory network of glucose sensing pathways that probably evolved to ensure rapid and sensitive response of the cell to changing levels of glucose.

Glucose fuels life
Glucose is the principal carbon and energy source for most cells (perhaps because it is the most abundant monosaccharide on the earth), and many (all?) organisms have evolved sophisticated means for sensing glucose and utilizing it efficiently. Microorganisms living in an animal’s gut must be able to detect the frequent fluctuations in glucose availability they encounter. Plants must have mechanisms for communicating between cells in the photosynthetic ‘source’ tissue that produce sugar and cells in ‘sink’ tissues that store sugar. Cancer cells must realize that their glucose supply is diminishing as the pace of tumour growth outstrips the capacity for vascularization, and adjust their lifestyle accordingly. The importance of glucose sensing to mammals is apparent in their remarkable ability to maintain an almost constant level of glucose in the bloodstream (approx. 5 mM) both during the brief periods when they feast and during the long periods when they fast.

Learning how cells sense and respond to glucose is thus of great interest and major significance.

Glucose is particularly important to the yeast *Saccharomyces cerevisiae*. It is by far the preferred carbon source of this yeast. Yeast cells can sense glucose and utilize it efficiently over a broad range of concentrations (from a few micromolar to a few molar!). Accordingly, yeasts have evolved sophisticated mechanisms for sensing the amount of glucose available and responding appropriately (reviewed in [1,2]).

Yeast’s unusual glucose metabolism determines its unique lifestyle
*S. cerevisiae* has an unusual lifestyle: it prefers to ferment rather than oxidize glucose, even when oxygen is abundant [3,4]. Glucose is metabolized through glycolysis to pyruvate, which has two fates (Figure 1). In the presence of oxygen, most organisms convert pyruvate to carbon dioxide and water (via the tricarboxylic acid cycle), generating many ATPs (up to 36 per molecule of glucose used, according to the textbooks, but fewer are actually produced) via oxidative phosphorylation (the thin black arrow in Figure 1). Only when...
oxygen becomes limiting do most cells resort to fermentation (denoted by the thick downwards-pointing arrow in Figure 1), because it yields only 2 ATPs per molecule of glucose via ‘substrate-level phosphorylation’ of ADP. S. cerevisiae is one of the few organisms that prefers to ferment glucose, even when oxygen is abundant. The resulting low yield of ATP production demands that yeasts aggressively utilize the available carbon at the expense of their more efficient competitors [5]. Because this unusual metabolism results in the production of ethanol and copious amounts of carbon dioxide, yeasts have been exploited for thousands of years for the production of ethanol and copious amounts of carbon dioxide, yeasts have been exploited for thousands of years for food and beverage production [6].

The tendency of most types of cells to resort to fermentation only when oxygen becomes limiting is known as the ‘Pasteur effect’, named after its discoverer [7]. The contrasting lifestyle of yeast cells – their propensity to carry out aerobic fermentation – is called the ‘Crabtree effect’ after the oncologist who discovered this phenomenon in mammalian tumour cells in the 1920s [8]. (Essentially the same phenomenon was discovered independently, also in tumour cells, and is also called the ‘Warburg effect’ after its discoverer [9]). In fact, aerobic fermentation has been used as a diagnostic criterion for tumour cells, and is the basis of modern tumour imaging techniques, because its demand for a high rate of glucose uptake renders such cells visible to the appropriate imaging methods when they are fed radioactive glucose derivatives [10]. The mechanistic basis of the Crabtree/Warburg effect in tumour cells, though not well understood, shows some remarkable similarities to the mechanism responsible for this phenomenon in yeasts (more on this below).

**Yeast has many glucose transporters**

The importance of glucose to yeast is underscored by the large number of hexose transporters it possesses – 18. At least six of these (Hxt1, 2, 3, 4, 6 and 7) are known to be glucose transporters [14,15], one (Gal2) is a galactose transporter [16] and the others (Hxt5 and Hxt8-17) are probably transporters of other hexoses such as fructose and mannose, which are closely related to glucose [15]. Each of the glucose transporters has a different affinity and capacity for glucose [17]. Hxt1 is a low-affinity, high-capacity transporter that is most useful when glucose is abundant (>~1%); Hxt2 is a high-affinity, low-capacity glucose transporter that is necessary when glucose is scarce (~0.2%). The other hexose transporters have evolved for dealing with different concentrations of glucose and under different conditions. Thus, yeast cells have many different glucose transporters to deal with many different conditions.

Several of the glucose transporters are expressed only when glucose is available, and only under the appropriate conditions [14]. The low-affinity, high-capacity Hxt1 glucose transporter is only expressed when glucose is abundant; the high-affinity, low-capacity Hxt2 glucose transporter is only expressed when glucose is scarce. HXT3, which encodes a glucose transporter of intermediate affinity, is expressed in cells exposed to both low (0.2%) and high (2%) levels of glucose [18].

**Mechanism of glucose induction of HXT gene expression**

The signal transduction pathway responsible for induction of expression of the HXT genes by glucose is shown in Figure 2. It culminates in the Rgt1 transcription factor, which binds to the promoters of HXT genes and represses their transcription in cells grown in the absence of glucose (bottom right in Figure 2). The Rgt1 repressor does this in conjunction with Mth1 and Std1, paralogous proteins that bind to Rgt1 and...
Figure 2 | Diagram of the Rgt2/Snf3-Rgt1 glucose signal transduction pathway responsible for glucose induction of HXT gene expression

Glucose binding to the Snf3 and Rgt2 glucose sensors activated YckI, which phosphorylates Mth1 and Std1 that are bound to the C-terminal cytoplasmic tails of the sensors. Phosphorylated Mth1 and Std1 are substrates of the SCF<sup>Grr1</sup> ubiquitin-protein ligase, which targets them for degradation in the proteasome. Rgt1 lacking Mth1 and Std1 can no longer bind to DNA, resulting in derepression of HXT gene expression.

The glucose signal transduction pathway that leads to Mth1 and Std1 degradation begins at the cell surface with two glucose sensors – Snf3 and Rgt2 – that are probably glucose receptors, since they are clearly related to glucose transporters [23]. The signal generated by the glucose sensors in response to glucose activates casein kinase I (YckI), a protein kinase that is tethered to the membrane through a C-terminal palmitate moiety and that also interacts with the glucose sensors [20]. Mth1 and Std1 are probably the substrates of YckI, because they bind to the long C-terminal cytoplasmic tails of the glucose sensors [24, 25]. The proximity of activated YckI to its substrates causes it to phosphorylate them, which targets them to the SCF<sup>Grr1</sup> ubiquitin-protein ligase. The ensuing ubiquitination of Mth1 and Std1 targets them to the proteasome for degradation (V. Brachet, unpublished work).

Below are brief summaries of what is known about each component of this glucose sensing and signalling pathway beginning from the bottom of the pathway.

Rgt1 contains a C<sub>6</sub>-Zn<sub>2</sub> ‘zinc cluster’ DNA-binding domain that recognizes the sequence <sub>5</sub>CGGANN<sub>3</sub> [22]. It is unclear how Rgt1 can regulate specific genes through such a simple sequence. One possibility is that it binds cooperatively (or synergistically) to multiple sites. Indeed, most of the HXT genes regulated by Rgt1 have many copies of this sequence in their upstream regions that operate synergistically, with multiple sites contributing more than additively to repression.

DNA-binding seems to be the function of Rgt1 that is regulated by glucose: Rgt1 binds to HXT promoters only in the absence of glucose [22, 26]. This is somewhat surprising, because Rgt1 is known to be a transcriptional activator when glucose is abundant [27]. How can it do this without binding to DNA? Rgt1 DNA-binding is regulated by glucose because Mth1 is required for this function [19, 21]: addition of glucose to cells induces degradation of Mth1, causing Rgt1 to lose its DNA-binding ability. Mth1 is not directly involved in DNA-binding, because <sup>rgt1</sup> mutations that alter its regulation and cause it to bind to DNA constitutively (even in the presence of glucose) relieve the requirement of Mth1 for Rgt1 DNA binding (J. Polish and J.-H. Kim, unpublished results). Mth1 seems to regulate the ability of Rgt1 to be phosphorylated by a yet unknown protein kinase [21], which is correlated with the DNA-binding ability of Rgt1: Rgt1 in glucose-grown cells is hyperphosphorylated and unable to bind to DNA. It is surprising that Std1, which is quite similar to Mth1, does not regulate the DNA-binding activity of Rgt1. Std1 may play a role in regulating the transcriptional activation function of Rgt1.
Degradation of Mth1 (and Std1) is the key event that enables derepression of \(HXT\) gene expression. Mth1 and Std1 are marked for degradation in the proteasome (V. Brachet, unpublished work) by their ubiquitination, catalysed by the Sce\[Glu\] ubiquitin-protein ligase [19]. Proteins must be phosphorylated to be substrates of this enzyme [28], and for Mth1 and Std1 this is achieved by YckI, encoded by \(YCK1\) and \(YCK2\). This conclusion is supported by the observation that removing the serine residues in several consensus YckI phosphorylation sites in Mth1 and Std1 prevents their phosphorylation and subsequent degradation [20]. YckI is located in the cell membrane because of the palmitate it carries on its C-terminus [29,30], and it also interacts with the glucose sensors [20]. This leads to the view that glucose binding to the glucose sensors induces a change in their conformation that activates the associated YckI, which catalyses phosphorylation of Mth1 and Std1. The C-terminal, cytoplasmic tails of the glucose sensors enhance signalling, because they interact with Mth1 and Std1 and thereby bring them to the vicinity of YckI. Mth1 and Std1 must shuttle between the nucleus and the cell membrane, because they bind to Rgt1 in the nucleus and to the glucose sensors at the cell surface. There is no evidence that the subcellular localization of Mth1 and Std1 is regulated.

What is the nature of the glucose signal generated by the glucose sensors, and how does it activate YckI? It seems clear that signal generation is receptor-mediated, because glucose metabolism is not necessary for its generation: there are mutations in the genes encoding the glucose sensors that cause them to generate a signal even in the absence of glucose [23,31]. Thus, the glucose signal cannot be a glucose metabolite. The Snf3 and Rgt2 glucose sensors are probably glucose receptors, because they are clearly derived from glucose transporters and therefore probably have a glucose-binding site [23,32]. The glucose sensors appear to have lost the ability to transport glucose into the cell [23]. We believe that binding of glucose to the glucose sensors induces a conformational change in them that is transmitted to the associated YckI. In this view, the mutations in the glucose sensors that cause them constitutively to generate a glucose signal convert the receptors into their glucose-bound form. To truly understand this signal transduction pathway we will need to learn how this conformational change in the glucose sensors activates the protein kinase activity of YckI.

This is a novel signal transduction pathway that employs a unique type of receptor: a small-molecule transporter that evolution has hijacked for the purpose of nutrient sensing. Soon after the discovery of the glucose sensors [23], a similar receptor for amino acids was discovered [33–36]. In addition, there seems to be another glucose sensing mechanism in \(S.\ cer\)e\(s\)i\(a\)e that employs a G-protein coupled glucose receptor [37]. We submit that discovery of the glucose and amino acid sensors has somewhat changed our view of how cells sense nutrients. The prevailing view was that this occurs mostly through metabolism of the nutrient, as is the case for glucose sensing by insulin-producing cells of the pancreas [38], and is probably the case for the glucose repression pathway that operates through the Snf1 protein kinase and the Mig1 repressor [39]. The discovery of the yeast glucose and amino acid sensors revealed that these nutrients can act like hormones to initiate receptor-mediated signalling. An exciting possibility is that similar nutrient sensors are operating in mammalian cells [40,41]. A better understanding of their function in yeast cells is likely to help answer this important question.

**Multiple regulatory mechanisms ensure appropriate \(HXT\) gene expression**

Other regulatory mechanisms are superimposed on Rgt1-mediated regulation to ensure that the cell expresses only those glucose transporters best suited for the amount of glucose available. \(HXT2\), which encodes a high-affinity glucose transporter [42], is induced only by low levels of glucose because its promoter has binding sites for the Mig1 glucose repressor, which represses transcription when glucose levels are high (Figure 3) [43]. By contrast, expression of \(HXT1\), which encodes a low-affinity glucose transporter [42], is induced only by high levels of glucose because of regulation by another mechanism (whose components have not yet been identified) that requires high levels of glucose [43]. In addition, \(HXT1\) expression is induced by high osmolarity through the HOG signal transduction pathway that culminates in the Sko1 transcription factor [44]. It seems likely that contributions of other regulatory mechanisms to regulation of \(HXT\) gene expression will be discovered.

Furthermore, feedback and feedforward regulatory loops govern the expression of genes involved in the Snf3/Rgt2 glucose sensing pathway. We can recognize three such controls (shown in Figure 3) [45]. (1) The glucose induction pathway regulates itself through \(STD1\) expression. This is feedback regulation: glucose inhibits Std1 function by stimulating its degradation while at the same time inducing \(STD1\) expression through the Snf3/Rgt2-Rgt1 signalling pathway. Thus, \(STD1\) expression is turned up at the same time that Std1 levels are decreasing in response to glucose. This regulation should serve to dampen glucose induction of gene expression. It should also provide for rapid reestablishment of repression. (2) Glucose repression regulates the glucose induction pathway through Mig1- and Mig2-mediated repression of \(MTH1\) and \(SNF3\) expression. This regulation of \(MTH1\) expression constitutes feedforward regulation: glucose reduces \(MTH1\) expression at the same time that it stimulates proteasome-mediated degradation of Mth1. This regulation reinforces the inhibitory effect of glucose on Mth1 function and should ensure maximal glucose induction of Rgt1-repressed genes. Repression of \(SNF3\) expression by high levels of glucose probably reflects the likely function of Snf3 as a high-affinity glucose sensor (a sensor of low levels of glucose). (3) The Mig2 repressor is activated by glucose because of induction of expression of \(MIG2\) via the Snf3/Rgt2-Rgt1 glucose induction pathway. Thus, the Snf3/Rgt2-Rgt1 pathway, which we formerly thought was responsible only for induction
of gene expression, also contributes to glucose repression of gene expression, because one of the outputs of the glucose signal that is generated by the Rgt2 and Snf3 glucose sensors is glucose repression of expression of genes that are targets of Mig2.

**Similarities in the basis of aerobic fermentation in yeast and tumour cells**

As mentioned above, many different kinds of tumour cells share yeast’s unusual way of metabolizing glucose (reviewed in [46,47]). As for yeast cells, this lifestyle of tumour cells demands that expression of genes critical for survival under the anoxic, glucose-limited conditions of the tumour be induced. These include the gene encoding vascular endothelial growth factor (VEGF), which stimulates blood vessel growth, and genes encoding proteins necessary for glucose utilization, such as glycolytic enzymes, and, significantly, the low-affinity, high-capacity glucose transporter Glut1, which is essential to provide the cell with the high amount of glucose this lifestyle demands. Expression of these genes is regulated by Hif1 (hypoxia-induced transcription factor) [48–50], which, as its name suggests, is activated when oxygen levels drop, as they do for most cells in a growing tumour. Remarkably, Hif1 function is, like Rgt1, regulated by degradation initiated by its ubiquitination catalysed by an SCF ubiquitin-protein ligase similar to SCF<sup>Upr1</sup> [51,52]. The SCF that operates on Hif1 is SCF<sup>VHL</sup>, one component of which is encoded by the von Hippel–Landau tumour suppresor gene. These similarities in the two cell types bolster our confidence that a deeper understanding of the mechanisms responsible for glucose induction of gene expression in yeasts will inform cancer biology, and may even suggest opportunities for developing therapeutic interventions.

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