**Original Review Article****DOI: 10.26479/2019.0503.15****GLUCOSE SIGNALING AND TRANSCRIPTIONAL REGULATION OF  
HEXOSE TRANSPORTERS IN *SACCHAROMYCES CEREVISIAE*****Smriti Sinha, Villayat Ali, Sonu Kumar Gupta, Malkhey Verma\***Department of Biochemistry and Microbial Sciences, Central University of Punjab Bathinda,  
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**ABSTRACT:** *Saccharomyces cerevisiae* as a model eukaryotic organism, various studies have been done for investigating the signal transduction components and pathways in the multicellular and complex eukaryotes. Due to similarities in the mechanism, yeast serves to be a great model for this study. The hexose transporters (HXTs) genes present with different affinities for glucose along with Snf3/Rgt2, Snf1 and Hxk2 are studied in this review. The genes and its regulators work in an overlapping fashion which has been dealt in detail. Although *S. cerevisiae* utilizes a big range of carbon sources but sometimes the presence of glucose subdues various molecular activities necessary for the use of alternative carbon sources and it suppresses other processes such as respiration and gluconeogenesis. This effect of glucose is established by various metabolic, sensing and signaling interactions. Recently, several components of the glucose induction pathway are studied such as Ras-cAMP pathway. This review describes glucose induction and repression various HXTs genes in yeast and their effect on its metabolism.

**KEYWORDS:** *Saccharomyces cerevisiae*, hexose transporters, glucose induction, repression, signalling, metabolism.

**Corresponding Author: Dr. Malkhey Verma\* Ph.D.**Department of Biochemistry and Microbial Sciences, Central University of Punjab Bathinda,  
Punjab, India. Email Address: Malkhey.verma@cup.edu.in**1. INTRODUCTION**

Yeast is a fungus and needs a supply of energy for its growth and maintenance. For yeast *Saccharomyces cerevisiae*, glucose is the favoured carbon source and energy source and it imparts a remarkable effect on gene expression. Previous work has established that virtually all cell types

hold a refined genetic program that responds to this rich source of carbon and energy that is glucose. *Saccharomyces cerevisiae* is known for sharing various components of signal transduction that are involved in the detection of glucose with higher eukaryotes. It does not only detect glucose rather it transduces the signals to the interior parts of the cell and then do the required adjustments to the metabolism of cells and gene expression outline. This review will focus on the mechanisms involved in the transmission of glucose signals from the plasma membrane of yeast cell to its nucleus and the transcriptomic response mediated by nuclear organization to glucose induction and repression. *S. cerevisiae* is able to distinguish between varying extracellular glucose concentrations and regulate the expression of the appropriate hexose transporters (HXTs) through sensor molecules and signal transduction pathways. Glucose is involved in the repression of genes that are not essential for its uptake, while it induces the transcription of those genes that mediate its transport and metabolism. In *S. cerevisiae*, there are large numbers of hexose transporter genes which are present such as HXT1-17, Snf3, and Rgt2 and together with a galactose permease gene (GAL2). All these genes belong to a superfamily of monosaccharide facilitator genes. The HXT gene family (HXT1-17, Gal2, Snf3 and Rgt2) helps to compose the multi-factorial uptake system that enables *S. cerevisiae* to utilize a broad range of extracellular glucose concentration [1]. This suggests that these hexose transporters genes of yeast are needed only for transport and do not get involved in signalling pathways. It appears that of the 17 HXTs in yeast HXT1-4 and HXT6 & 7 are physiologically most important. Glucose persuades the expression of 17 HXT genes that encode hexose transporters of varying affinities for glucose [2]. These transporters can be classified on the basis of their regulation and affinity for glucose: (i) expression at low glucose concentration, and repression at high glucose, thus high affinity; (ii) expression is independent of glucose concentration; (iii) induction by high glucose concentration, therefore low affinity; (iv) expressed at starvation conditions, very high affinity [3]. It has been observed in previous research that HXT1 is a low-affinity transporter, with its expression only induced at high glucose concentrations. Maximal induction is achieved at high glucose concentrations when the transcription factor Rgt1 is inactive and a transcriptional activator is active. HXT 2 & 4 are involved in glucose uptake at low concentrations and therefore recognized as high-affinity transporters. The induction of HXT3 does not depend on glucose concentrations, expresses at both high and low glucose concentrations. Snf3 and Rgt2 are putative glucose sensors and are much similar to each other. They are not involved in signalling pathways rather they sense the presence of glucose and signal it and therefore play a vital role in the initial detection of glucose [4]. In the case of no extracellular glucose, the DNA-binding protein, Rgt1, forms a complex with the co-repressors Mth1 and Std1, in the nucleus. Mth1 and Std1 cycle between the cytoplasm and nucleus and at no glucose they are primarily localized in the nucleus. The binding with these two co-repressors enables Rgt1 to bind the HXT promoters [5]. Once bound to the HXT promoters, Rgt1 recruit the co-repressors Tup1 and Ssn6, which allow Rgt1 to function as a repressor [6]. The glucose

regulatory system in yeast represents a parallel network of signaling pathways [7].

### Signaling of glucose without extracellular sensing, transport and phosphorylation

The Broach University had experimented using microarray and given a clear demonstration of the universal approach of induction and repression overlap in signal transduction of glucose in yeast cells. In the experiment, analysis of yeast transcriptome was done after heterologous induction of active alleles of Gpa2 or Ras2, signal transmitters playing role before time in cAMP-dependent protein kinase pathway (PKA) [8]. The changes that were observed virtually in transcript level after glucose addition was also observed when there was no glucose upon expression of active form of Ras2 [9]. The genes which showed the three-fold change in expression in an hour after glucose addition, 92% of that showed at least two-fold change in a similar manner after Ras activation (see Table 1). The result with Gpa2 was similar but with reduced intensity in comparison to that which was seen in the case of activated Ras [10]. It was also demonstrated that all transcript level changes resulting from Gpa2 and Ras2 activation were conciliated by PKA [11]. The important thing is almost this transcriptional reprogramming can also take place in lack of signaling through cAMP and redundant overlapping pathways (both RAS2/Gpa2/PKA independent and Ras2/Gpa2/PKA dependent) seems to be completely capable in transducing the glucose signal [12]. The perceived transcriptome-wide upregulation and downregulation in response with activated Ras was definitely a bombshell as it had been assumed that yeast primary glucose repression pathways are different from glucose induction and are absolutely Ras-independent [13]. Further, in the absence of either transport or phosphorylation of sugar, stimulation of glucose response is significant. Transport and conversion of glucose (glycolysis) to glucose-6-phosphate by hexokinase is a potentially essential attribute of the response and now seems that most indulging in redundant signaling that operates through or collaterally with cyclic AMP- PKA pathway. So, intracellular or extracellular glucose sensing is compulsory to activate Ras or Gpa2 and is enough for both glucose induction and repression [14].

**Table 1: List of genes that respond in the same manner to glucose addition and Ras activation [9]**

Induced genes			Repressed genes		
Protein biosynthesis <sup>p</sup>	Transcription	transport <sup>q</sup>	Energy reserve metabolism <sup>r</sup>	Protein turnover	Stress response
<i>RPL7B</i>	<i>RPB9</i>	<i>NUP170</i>	<i>PHO85</i>	<i>RPN5</i>	<i>SSA4</i>
<i>TIF3</i>	<i>RPO31</i>	<i>PSE1</i>	<i>MDH3</i>	<i>RPT3</i>	<i>UBC5</i>
<i>HIS1</i>	<i>GCR2</i>	<i>HXT4</i>	<i>PET54</i>	<i>HRD1</i>	<i>MDR1</i>
<i>LYS4</i>	<i>LEU3</i>	<i>ZRT1</i>	<i>COX11</i>	<i>SBA1</i>	<i>PPZ2</i>
<i>HIS3</i>	<i>IFH1</i>	<i>LYP1</i>	<i>COQ1</i>	<i>HRD3</i>	<i>PAU1</i>
<i>CDC60</i>	<i>RRP5</i>	<i>FET3</i>	<i>MRPL23</i>	<i>DOA1</i>	<i>GTS1</i>
<i>ILV1</i>	<i>IMP4</i>	<i>PHO84</i>	<i>COX10</i>	<i>MPD2</i>	<i>PAU3</i>

<sup>p</sup>: Genes involved in translation, processing factors of rRNA and enzymes for protein biosynthesis,

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¶: Genes encoding transport factors and transporters, †: Genes encoding protein or factors responsible for carbon storage and respiration

### **Redundant but combined glucose signaling pathways**

Analysis of downstream regulators in the signaling cascade (mostly functions within the yeast nucleus) gave the indication of overlap between glucose induction and repression. For instance, the repressor Rgt1 blocks the transcription of HXT1 through HXT4 [9]. At high concentrations of glucose, although, the interaction between Rgt1 and HXT1 cannot be detected, Rgt1 gets converted into an activator through the process of hyperphosphorylation. The hexokinase Hxk2 is also needed for glucose induction of HXT1 which is long known to participate in glucose repression [6,15].

Phosphoprotein Gcr1 represents another example of downstream regulatory overlap. It was first identified as an activator of glycolytic genes [16]. Researches and analyses have confirmed that expression of various glucose-repressed genes are derepressed in the absence of Gcr1 at high concentration of glucose and this suggests a new role to this regulator in the expression of transcription [17]. The roles of Hxk2, Rgt1, Gcr1 and snf1/snf4 and various other activator and repressor polypeptides that participate in glucose response are presented in detail in this review. Along with these specific regulators, there are other components of general transcription machinery like Gal11, Sin4, Rgr1, Srb8, Ssn3, Ssn2 etc. that connect with carboxy-terminal domain of RNA polymerase II and play both positive and negative regulatory roles; the working and function of these factors are known to be influenced by glucose signaling. Thus, overlapping of negative and positive circuitry in the glucose response can go from the plasma membrane through to the eventual choice of induction or shut off of initiation of transcription of every glucose-regulated gene by RNA polymerase II [9]. It has become important that the induction/repression duality of these signal recipients like Rgt1, Gcr1, Snf1/Snf4 and Hxk2 and many others should be characterized according to the precise mechanisms involved. Therefore, for the sake of clarity, the discussion of these nuclear regulators are divided into distinct sections in the review. It can be said that the mechanisms of glucose induction and repression are very much integrated [10].

### **Early steps involved in signal transduction**

It is fine that sufficient Gpa2 or Ras activation can produce transcriptome-wide spectrum changes in response to glucose [18]. It is necessary to review the things which are known about these factors and their respective signaling pathways and their immediate activation followed by the glucose appearance [9].

### **Ras/cAMP/PKA pathway**

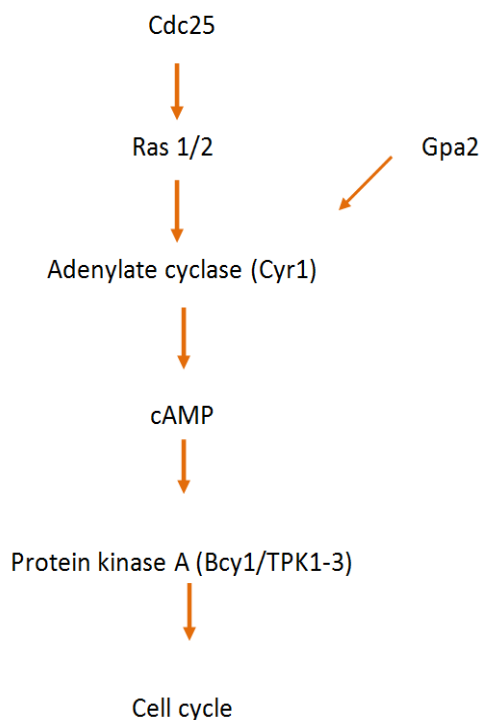
Ras protein, the monomeric GTPase is also called G-proteins which functions as switches; are inactivate when to remain in GDP bound state and becomes active on binding with GTP. The switch from being active to the inactive state involves hydrolysis of bound GTP with the help of intrinsic GTPase and this is stimulated by GAPs (GTPase activating proteins). The reverse switch that is

from inactive to active requires the substitution of bound GDP with GTP which generally gets accomplished with the help of GEFs (guanine nucleotide exchange factors) [19]. The polypeptides which are encoded by two Ras genes in yeast, *S. cerevisiae*, Ras1 and Ras2 are about >70% similar overall and about 90% identical over N-terminal about 180 residues. It contains G1 to G5 boxes that are short stretches of amino acids playing role in recognition of phosphate and guanine nucleotide. Growth on glucose is uninfluenced by the absence of Ras1 or Ras2, although the loss of both Ras1 and Ras2 causes arrest at the G1 phase of the cell cycle [20]. These early findings suggested the feature of nutrient sensing that nutrient-deprived cells arrest in the G1 phase. During the glucose response, some effector molecules modulate Ras activity. Membrane localization and post-translational modification of Ras are important characteristics of these regulatory inputs. For instance, Ras farnesylation contributes to the exchange of nucleotides by GEF Cdc25 [21]. Cdc25 is an important gene and it was originally identified after screening mutations that cause G1 arrest. The stimulation of Ras by RasGEF results in stimulation of the production of cAMP by an essential product of adenylate cyclase gene CYR1 [22]. Ras inactivation results in lower cAMP level and this is facilitated by GAPs Ira1 and 2 [23]. The activity of Adenylate cyclase in yeast depends on Ras proteins. Intracellular acidification and addition of glucose to derepressed cells make an increase in the level of cAMP [24].

### **Downstream effectors of Ras and its signaling pathways in *S. cerevisiae***

The function of both Ras1 and Ras2 is the activation of Adenylate cyclase and it is associated with a protein known as CAP [25]. Adenylate cyclase participates in the synthesis of cAMP from ATP. Then, cAMP binds with Bcy1 protein which functions as a regulatory subunit for protein kinase A (Figure 1). This binding results in activation of PKA. The catalytic subunit of PKA is encoded by 3 genes namely TPK1, TPK2 and TPK3. Substrate phosphorylation leads to regulation of various functions like cell cycle progression. A low-affinity phosphodiesterase which is encoded by gene PDE1  $\alpha$  and also by a high-affinity phosphodiesterase which is encoded by the gene PDE2, hydrolyze the cyclic AMP. Cyclic AMP production is also regulated by Gpa2 (G $\alpha$  protein) which is activated by glucose. cAMP/PKA pathway is known to play various functions of mitochondria [26]. CYR1 gene encodes adenylate cyclase and it encodes a membrane-bound protein which is of 2026 amino acids. 4 domains of this protein have been identified namely C-terminal, catalytic, middle repetitive and N-terminal. The middle-repetitive domains have a repeat of 23 residues, amphipathic, and leucine-rich motif called as LRR domains. The LRR domain is considered as the primary site for interaction with Ras. The N-terminal of LRR domain is known as Ras-associating domain (RAD) [27]. The Ras/cAMP/PKA pathway is involved in the regulation of various processes like cell cycle progression and life span. There are other life processes that are regulated by this pathway [28]. For example, it controls spore morphogenesis and it is shown recently that deregulated Ras

signaling settles DNA damage checkpoint recovery [21]. It is also discovered that various pathway by which PKA gets activated also result in the distinct phenotypes [29]. For instance, deletion of the inhibitor of Ras/PKA that is Ira2 results in xylose growth and its metabolism [30]. Ras signaling pathway in yeast *S. cerevisiae* is shown in figure 1 which, as explained above.



**Figure 1: Ras signaling pathway in yeast *S. cerevisiae***

### The Gpa2/Gpr1 pathway

Heterotrimeric G proteins function as signaling molecules consists of  $\alpha$ ,  $\beta$  &  $\gamma$  subunits representing a group of factors which can bind to guanine nucleotides. The  $G\alpha$  protein Gpa2 and its negative regulators (GAP) Rgs2 to take part in glucose signaling in yeast *S. cerevisiae* [9] [31]. The identification of gene Gpr1 was done by the interaction screen of a hybrid protein with Gpa2. Gpr1 gene encodes a member of seven transmembrane G protein-coupled receptor superfamily. This Gpr1 is situated on the cell surface of yeast. It has been suggested that Gpa2 acts in the downstream direction from Gpr1 in signaling pathway: In *S. cerevisiae*, cAMP accumulation is controlled by the pathway. Intracellular acidification results in Ras-mediated cAMP increase. Glucose additions to cells which are grown on non-fermentable carbon sources activate a transitory burst in the level of intracellular cAMP. This glucose instigated cAMP signal depends on the  $G\alpha$  protein Gpa2 [10]. Studies had shown that Gpr1 interacts with Gpa2 and is needed for stimulating cAMP synthesis by glucose. It is also found that Gpr1/Gpa2 also play a role in sensing of glucose and signal transduction during the process of pexophagy in the yeast. It is the process of selective degradation peroxisomes by autophagy [32].

**Ras and Gpa2 activation by glucose**

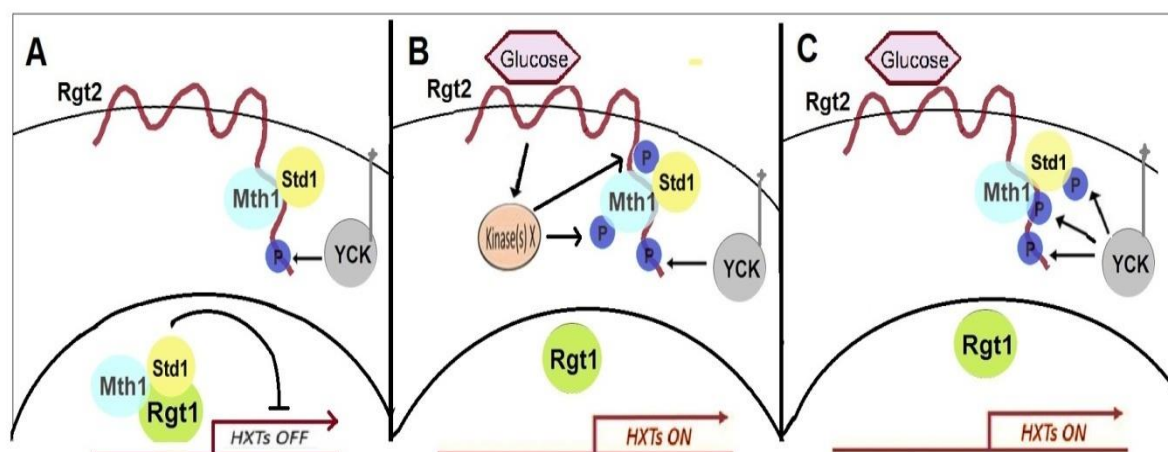
The question arises here that how do Gpa2 and Ras activate in wild yeast cells in the presence of sugar. So, it is differentiated in 2 categories:

**Extracellular and intracellular sensing**

Out of 18 hexose transporter (HXT1 to 17 & GAL2) genes in yeast *S. cerevisiae* if 7 genes from them get deleted results in transport and growth hamper. So, in the absence of these genes, constitutive expression of gene GAL2 which encodes galactose permease reinstate glucose-induced synthesis of cAMP. From this, it becomes clear that hexose transporters only take part in transport and not required for glucose signaling. Gpr1, the transmembrane protein may sense extracellular glucose and hence activate Gpa2 but not needed to signal Ras. The deletion of Gpr1/Gpa2 does not stop the glucose-induced increase in the level of Ras2-GTP. In fact, the increase in Ras2-GTP is absent in the strains which are glucose phosphorylation-deficient. Thus, it can be said that Ras gets activated by intracellular phosphorylated-glucose and not by extracellular glucose and Gpr1 gets activated on extracellular glucose.

**Extracellular sensing by Rgt2/Snf3**

The glucose sensors Rgt2 and Snf3 are somehow identical (~60%) to each other and also hexose transporters that are found in various organisms. It consists of 12 (predicted) transmembrane spanning domains. Rgt2 and Snf3 do not show their involvement in glucose signaling but rather sense and signal its presence [33]. The transcriptional response of these Rgt2/Snf3 target genes generates in the absence of glucose by expressing hyperactive Gpa2 or Ras. These two sensors seem to either play a redundant role in initial glucose detection or act upstream of the Ras/cAMP/PKA pathway. Rgt2 and Snf3 both have different affinity for glucose. Snf3 has a high and Rgt2 has a low affinity for glucose [34]. The reason for different affinities is the different arrangement of amino acid residues of sensors which form the glucose binding sites. So, it has been confined that Rgt2 is a low-affinity glucose receptor so senses a high concentration of glucose and Snf3 is a high-affinity glucose receptor that is why it senses a low concentration of glucose [35]. This pathway is also known as sensor/receptor-repressor pathway (SRR) pathway and involves the presence of glucose sensors that are Rgt2 and Snf3, repressors such as Mth1, Std1 and Rgt1 and Yck1 and 2 protein kinases [36]. Rgt1 repressor, in the absence of glucose, binds to HXT genes promoters and recruits the corepressors Mth1 and Std1 to repress the expression of HXT genes (Figure 2A) [37]. Glucose binds to the plasma membrane-bound sensors Snf3 (high affinity) and Rgt2 (low affinity) and these sensors undergo phosphorylation in Yck dependent manner at C-terminal tail (Figure 2B) [38]. SCF<sup>GRR1</sup> ubiquitin ligase recognizes the phosphorylated corepressors and subsequently ubiquitinated and then targeted for proteasomal degradation. So, in the absence of corepressor, Rgt1 is not able to bind the promoters of HXT genes which lead to derepression of expression of HXT genes (Figure 2C) [39].



**Figure 2: Schematic of the sensor/receptor-repressor pathway signaling. (A).** In the absence of glucose, the corepressors bind to the promoters of HXT genes and block its expression. Rgt2 (sensor present at plasma membrane) undergoes phosphorylation in Yck dependent manner. This leads to the generation of interaction sites for corepressors at plasma membrane results in bringing all the signaling components together and the system is ready for glucose response. **(B).** Rgt2 binds with glucose and results in activation of the downstream signaling cascade. Phosphorylation occurs by some kinases (B) or by Ycks (C) then ubiquitinated and targeted for degradation by the proteasome. The cellular pool of corepressors gets depleted and Rgt1 cannot bind to HXT gene promoter thereby relieving repression.

### Signal reception – glucose repression and induction

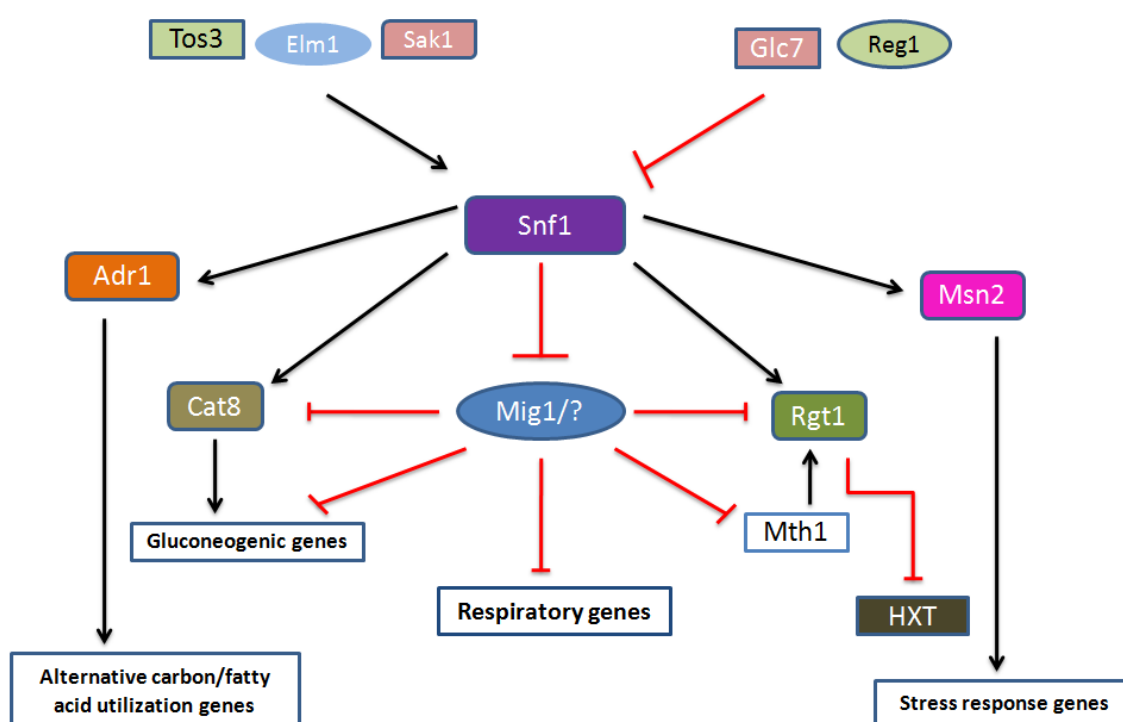
Glucose repression is the phenomenon in which the cells which grow on glucose represses the expression of other genes which are responsible or needed for the metabolism of other carbon sources such as sucrose, galactose and other non-fermentable carbon sources like glycerol and ethanol. This repressive outcome of glucose gets conveyed to cellular machinery by the process of interlinked signaling pathways and regulatory interactions. Results of these molecular activities not only show their effects at the transcriptional level but at the post-transcriptional and post-translational level also [40]. In *S. cerevisiae*, the preferred carbon source is glucose and its extracellular sensing and level of its metabolized forms are foremost for coordination of carbon metabolism in yeast. Yeast cells possess diverse mechanisms according to the level of glucose present extracellularly and intracellularly. Snf3/Rgt2 signaling pathway, a sensory cascade works in the direction of detecting extracellular glucose levels [41]. The Snf1 protein kinase signaling is the major one in the functionality of repression of glucose Snf1 kinase also plays a dual role, both activator and repressor [42]. However, it is important for maintaining energy homeostasis; snf1 also regulates certain metabolic enzymes responsible for lipid metabolism, carbohydrate storage, GCN4 translation and amino acid biosynthesis [43]. Glucose repression is of high relevance in yeast and



here are the key pathways involved in the process.

### **Snf1 signaling**

Snf1 plays a significant role in the control of metabolic changes by regulating various activators and repressors and glucose availability regulates the functioning and activity of Snf1 in glucose repression [44]. When the level of glucose is optimal, Snf1 becomes inactive and get excluded from the nucleus. This results in the non-phosphorylation of the transcription repressor Mig1 which is a major downstream target of Snf1 [13]. And if there is any drop in the glucose concentration, it activates Snf1 which results in phosphorylation and deactivation of the transcription repressor Mig1 which alleviates repression of glucose. The Snf1 of yeast is a conserved serine/threonine kinase found in eukaryotes which are needed for cellular energy homeostasis. Snf1 kinase has a Heterotrimeric structure having an  $\alpha$  catalytic subunit (Snf1), a  $\gamma$  regulatory subunit (Snf4) and 3  $\beta$  subunits (Gal83, Sip1 and Sip2). In the presence of high glucose concentration, Snf1 kinase complex become inactive because of autoinhibition happening due to the interaction between C terminal regulatory domain and N terminal catalytic domain of Snf1. But in the presence of low glucose concentration, this autoinhibition gets removed and interactions between Snf1 catalytic subunits and Snf4 regulatory subunits are promoted. And activation of Snf1 requires phosphorylation by the protein kinases namely Sak1, Elm1 and Tos3 of the conserved residue at Thr<sup>210</sup> in the activation loop of Snf1 [45]. Snf1 is regulated by ADP which makes sure a direct link between this key regulator and energy metabolism [46]. These protein kinases play important functions as their deletion or absence abolishes the activation of Snf1. It is still unknown about the mechanism of activation of kinases of snf1 by glucose signals but each and every activating kinases functions differently in different situation of availability of carbon source (Figure 3). Sak1, the most important activating kinase of Snf1 as its interaction is the most stable one and performs its function at growth condition on alternative carbon sources. The  $\beta$  subunits present in the structure of Snf1 also give specificity for particular upstream activating kinase under different conditions. Snf4 plays an important role in the regulation of Snf1 [47]. Dephosphorylation of Snf1 is considered as another level of control carried by Glc7/Reg1 phosphatases and has been hypothesized as the main regulator of the activity of Snf1 since it does not affect the phosphorylation of Snf1 by upstream kinases by variations in glucose concentrations. And it is also shown that dephosphorylation of the kinase is correlated with the availability of glucose in surrounding or environment. Although glucose fluctuations in the environment do not affect the activity of Reg1/Glc7 phosphatase [48], there has been a hypothesis that attainability of Snf1 for dephosphorylation by the enzyme phosphatases varies. Further research revealed about the glucose addition leads to a rapid increase in activity of Reg1 which causes inactivation of Snf1.

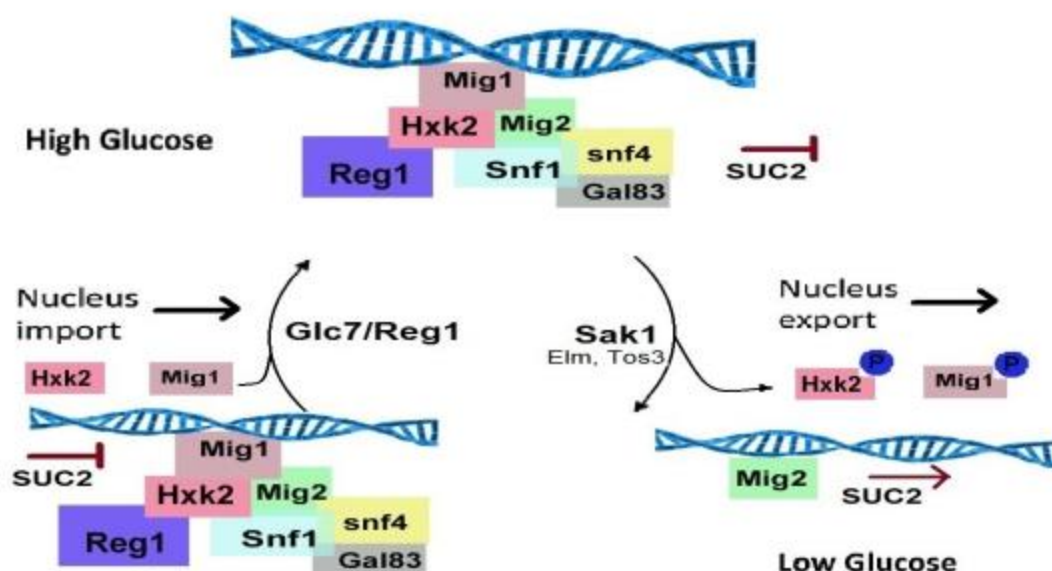


**Figure 3: Showing that Snf1 protein kinase is mainly involved in glucose repression pathway. It regulates glucose repression by inactivating or activating expression of genes of gluconeogenesis and respiration [49].**

#### **How hexokinase Hxk2 is involved in glucose response**

Glucose serves as the signaling molecule for the regulation of central pathways. The very first fate of intracellular metabolism of glucose is its phosphorylation. There is involvement of three enzymes encoded by Hxk1, Hxk2 and GLK1 in catalyzing this reaction and only Hxk2 gets highly expressed in the presence of glucose [50]. Earlier it was shown that Hxk2 lesions such as null and point mutations can cause failures in the repression of glucose. But after various controversies, it is concluded that the catalytic activity of Hxk2 is very much involved in intracellular glucose signaling pathway [51]. It is postulated that after the beginning of the phosphoryl transfer reaction, the Hxk2 conformation is altered by a steady transition intermediate and it also mediates regulatory functions in changing the expression of the target gene. However, this model failed to explain certain facts such as glucose metabolized in the Hxk2 independent manner can induce transcriptional repression in comparison to those which are observed after glucose addition to wild type cells or on Ras or Gpa2 activation. It is important that evidence of the regulatory role of nuclear Hxk2 needs an unambiguous demonstration about its role in glucose response depends upon the relatively small amount of protein which is present in the nucleus. When the level of glucose is high, the repressor protein Mig1 becomes major transcription factor which is responsible for the repression of genes required for use of alternate fermentable carbon sources. The repressor protein Mig1 binds to the

DNA and stops the transcription of SUC2 and about 350 other genes (Figure 4). Scientists have also demonstrated that in various cases, this process of inhibition of transcription also needs the Hxk2 protein [52]. The level of glucose availability controls the expression of Hxk2 gene and their expression is mediated by the transcription factors Rgt1 and Med8 which is responsible for the repression of Hxk2 expression in the presence of low glucose [53]. Hxk2 also plays its role in glucose-induced repression of gene Hxk1 while Hxk1 protein is involved as a negative factor in the expression of Hxk2 gene. Transcriptional analysis of a mutant strain showed the scientists a notable upregulation of genes having binding sites for Mig1. Additionally, Hxk2 is also involved in controlling sugar transporters genes and many others. Thus, the Hxk2 protein is regarded as a regulator of carbohydrate metabolism which is necessary for mediating glucose repression signals. Since Hxk2, glucose kinase is important for regulation of gene transcription in the nucleus, it is obvious that to carry out the functions Hxk2 needs to go in and out of the nucleus but the protein is large in size and so it needs a carrier protein for its transport across the nuclear envelope. The mechanism which is involved in the entry and exit of Hxk2 around the nucleus is mediated by  $\alpha/\beta$ -importin pathway and also Xpo (crm1) carrier protein [54]. The direction of transport is regulated by Gsp1 and Snf1 kinase Glc7 phosphatase. Both the proteins work together to regulate the phosphorylation of serine 14 of Hxk2 and its transport. Snf1 kinase also plays an important role in the regulation of glucose repression signaling pathway as mentioned above. It gets activated in low glucose condition and modifies the state of phosphorylation of Hxk2 and at least 4 serine residues of Mig1 [55]. The phosphorylation of serine 311 in Mig1 is shown to be sufficient for the inhibition of repressor activity of Mig1 protein and induction of translocation of protein from the nucleus to the cytoplasm [56]. In yeast, Reg1 targets the Glc7 protein phosphatase to Hxk2 and Mig1 and in high glucose condition, Hxk2 and Mig1 are dephosphorylated by the action of Glc7-Reg1 protein phosphatase complex and thus, repress transcription [57]. It is established that low-level expression of SUC2 is mainly due to the repression by Mth1-Rgt1 complex in the absence of glucose [58].

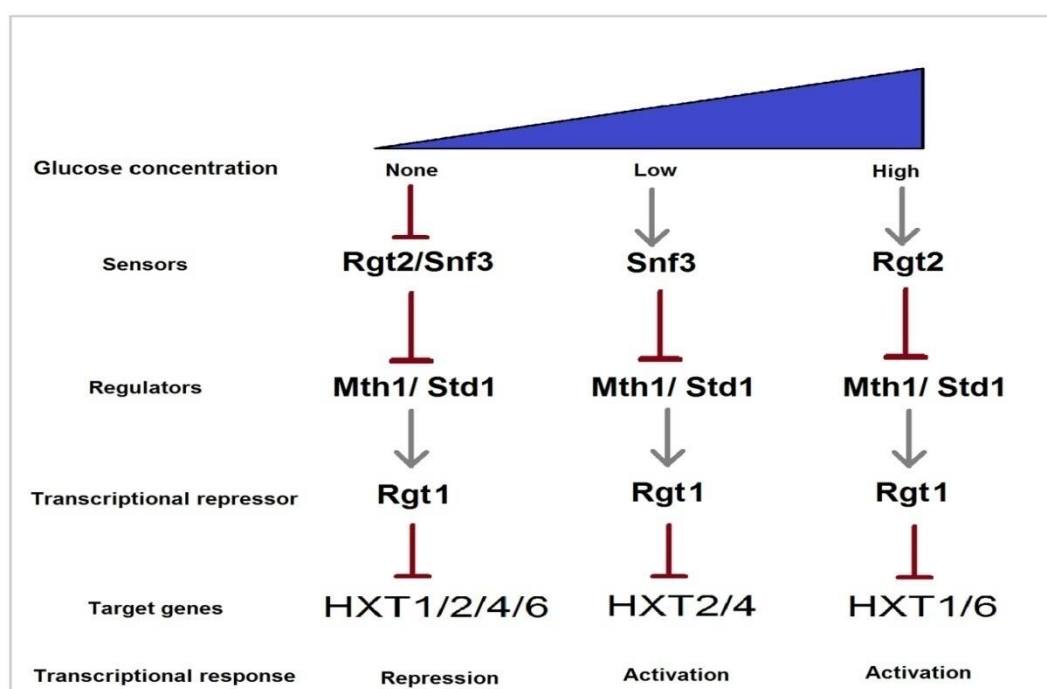


**Figure 4:** This figure demonstrates that in high glucose condition Hxk2 is needed for stabilizing SUC2 repressor complex and for the inhibition of Mig1 phosphorylation by Snf1 kinase. In low glucose condition, the interaction of Hxk2 with Mig1 is terminated by Snf1 dependent Hxk2 phosphorylation and increased interaction between Snf1 and Mig1 is seen which stimulated the Hxk2 and Mig1 phosphorylation by Snf1 kinase at serine 14 and 311 respectively. This resulted in the export of protein from the nucleus to cytoplasm and repressor complex disassembled at SUC2 promoter. Here, regulatory proteins such as Snf1, Snf4, Gal83, Mig2 and Reg1 are part of repressor complex at SUC2 promoter of the gene.

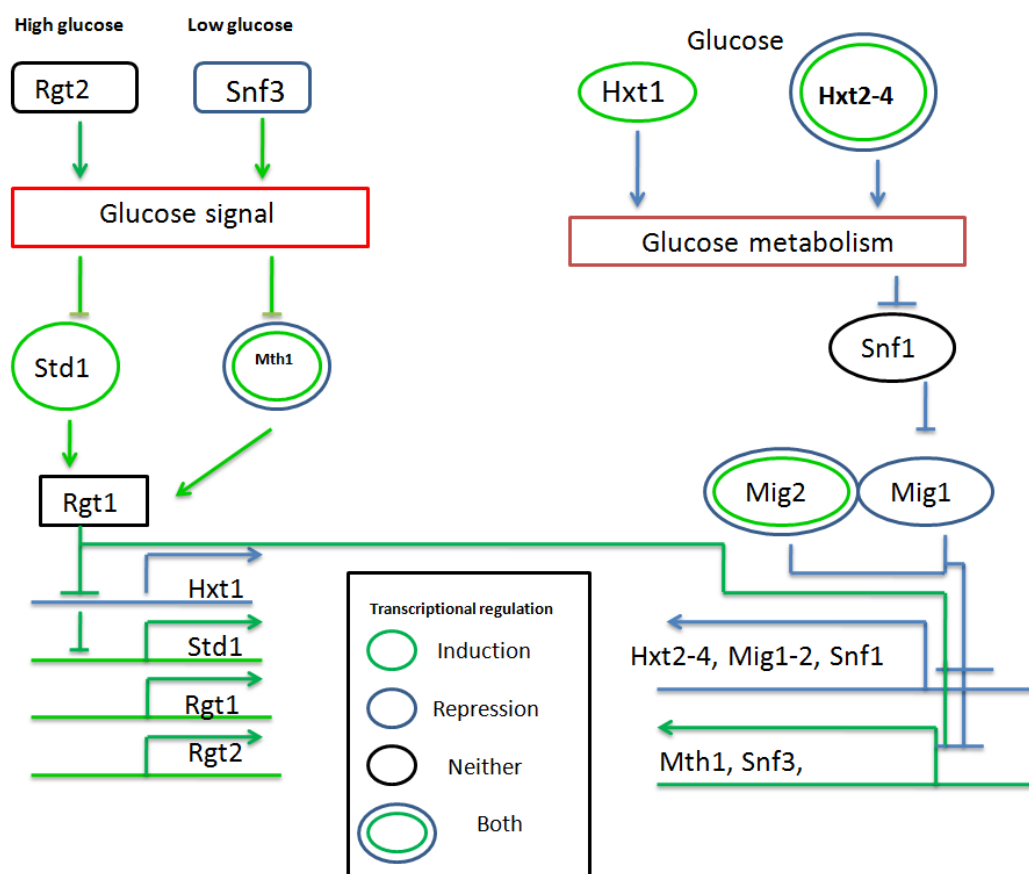
### Snf3/Rgt2 signaling

This is the signaling pathway which mainly senses the changing available glucose concentration in the environment. The Rgt2 and Snf3 sensors basically belong to the HXT gene family along with HXT1-17 and Gal2 protein. Since Snf3 and Rgt2 are structurally similar to HXTs but they cannot transport glucose. Snf3 functions as a sensor for the low level of extracellular glucose and Rgt2 senses a high level of glucose. The transcriptional regulation and HXT proteins expression are coordinated by the intracellular signals which are generated by the detection of the attainable amount of glucose. If the glucose is present in abundance, low-affinity hexose transporters like HXT1 are activated and expressed where, in such conditions, expressions of high-affinity glucose transporters are repressed. Rgt2 and Snf3 are also able to sense the internal to the external ratio of the concentration of glucose which helps in adjusting the uptake of glucose and maintaining the intracellular environment. Snf3/Rgt2 pathway regulates the transcriptional activity and it allows the yeast *S. cerevisiae* to nicely coordinate the uptake of glucose according to the availability of hexose sugar (Figure 5). The membrane attached kinases Yck1 and Yck2 are activated when Snf3 and Rgt2 transmembrane proteins sense the extracellular glucose. These active kinases are needed for

degrading the Mth1 and Std1. Earlier it was thought that Mth1 and Std1 were phosphorylated directly by Yck1 and 2 for degradation but recent data states that degradation of the Mth1 occurs in the nucleus independent of localization of Yck1 and 2. So, this finding suggests that there is a protein to which glucose sensors transmit a signal which promotes the phosphorylation and degradation of Mth1. After phosphorylation, Std1 and Mth1 are targeted for other modification like ubiquitination and proteosome degradation [59]. Moreover, when glucose is present in abundance, the expression of Mth1 is repressed by Mig1 for maintaining glucose repression of HXT genes [39]. On the contrary, when the Std1 is degraded, it increased its expression ensuring the orderly expression of HXT genes when the glucose is depleted. After Std1 and Mth1 degradation, protein kinase A removes Rgt1 (transcriptional repressor) by hyperphosphorylation which is allowed by the translocation of PKA which is induced by glucose [60]. This results in HXT gene expression and optimal consumption of glucose. Exhaustion of glucose provide Std1 and Mth1 present for Rgt1 interaction which results in concealing PKA phosphorylation sites present on Rgt1 and due to this repressors do their work by binding to promoters, repressing HXT genes expression when glucose is absent. Snf1 phosphorylation of transcriptional repressor Rgt1 induces its repressor activity and its tendency of binding DNA. The interaction between Rgt1 and Snf1 kinase is important for HXT expression and overall glucose repression.



**Figure 5: Schematic representation of Snf3/Rgt2 glucose sensing pathway showing expression level of HXT genes at various glucose concentration**



**Figure 6: This is the qualitative representation of Snf/Rgt/Mig network, showing the glucose repression pathway in blue lines and glucose induction pathway in green lines. Coloured spheres are indicating that which pathway is regulating the transcription of mRNA of each & every protein (blue-repression, green-induction, black-neither) [61].**

## 2. CONCLUSION

Few aspects of glucose signaling in yeast *Saccharomyces cerevisiae* is well established in this review. The reaction cascade induced by the binding of glucose to its various sensors like Rgt2/Snf3 under different glucose concentration has been focussed here showing the activity of several proteins or corepressors such as Yck1/2, Std1/Mth1 against inhibition of Rgt1 of transcription of several genes. And also the role of Hxk2 is dealt that it is essential for glucose to affect transcription of few genes and it is not so essential for other genes. Increase in PKA activity mediated by Gpr1 and signaling pathway is shown to be important or enough to amend the motif of gene transcription resulting in activation of some genes and repression of other genes. It is also clearly evident that no single system works on either induction or repression of transcription of genes by glucose rather it depends upon the type and combination of signals. Although there is similarity in glucose signaling pathways and their regulatory elements such as Hxk2, Snf1 etc. in yeast cells and eukaryotic/mammalian cells but they act and function differently in different systems.

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**CONFLICT OF INTEREST**

Authors declare no conflicting interests.

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