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MINI-REVIEW

The regulation of *Saccharomyces cerevisiae* Snf1 protein kinase on glucose utilization is in a glucose-dependent manner

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Abstract

Protein phosphorylation catalyzed by protein kinases is the major regulatory mechanism that controls many cellular processes. The regulatory mechanism of one protein kinase in different signals is distinguished, probably inducing multiple phenotypes. The *Saccharomyces cerevisiae* Snf1 protein kinase, a member of the AMP-activated protein kinase family, plays important roles in the response to nutrition and environmental stresses. Glucose is an important nutrient for life activities of cells, but glucose repression and osmotic pressure could be produced at certain concentrations. To deeply understand the role of Snf1 in the regulation of nutrient metabolism and stress response of *S. cerevisiae* cells, the role and the regulatory mechanism of Snf1 in glucose metabolism are discussed in different level of glucose: below 1% (glucose derepression status), in 2% (glucose repression status), and in 30% glucose (1.66 M, an osmotic equivalent to 0.83 M NaCl). In summary, Snf1 regulates glucose metabolism in a glucose-dependent manner, which is associated with the different regulation on activation, localization, and signal pathways of Snf1 by varied glucose. Exploring the regulatory mechanism of Snf1 in glucose metabolism in different concentrations of glucose can provide insights into the study of the global regulatory mechanism of Snf1 in yeast and can help to better understand the complexity of physiological response of cells to stresses.

Keywords Snf1 protein kinase · *Saccharomyces cerevisiae* · Glucose metabolism · Stress response

Introduction

Protein phosphorylation catalyzed by protein kinases is the major regulatory mechanism that controls many cellular processes. The regulatory mechanism of one protein kinase in different signals is changed, which is related to the multiple phenotypes of cells. Snf1 protein kinase is a conserved serine/threonine kinase that exists in *Saccharomyces cerevisiae* (Hedbacker and Carlson 2008). Snf1 has vial important roles in the alleviation of glucose repression and the response of cells to various environmental stresses, ensuring nutrient availability and cell survival (Backhaus et al. 2013; Zhang et al. 2011). Glucose, an important nutrient for life activities of cells, is the preferred raw material component for many industrial productions. Glucose can serve as different signal molecules in varying concentrations. Here the role and the regulatory mechanism of *S. cerevisiae* Snf1 protein kinase in glucose metabolism in different concentrations of glucose are discussed to better understand the role of Snf1 in the regulation of nutrient metabolism and stress response of *S. cerevisiae* cells.

The role of Snf1 in the regulation of glucose metabolism in different concentrations of glucose

Snf1 is best known as the key enzyme in the alleviation of glucose repression, which controls the utilization of alternate carbon sources that are less preferred than glucose, such as sucrose, galactose, maltose, and ethanol (Hong and Carlson 2007). In glucose limitation (at least below 1%), Snf1 is activated and phosphorylates repressor Mig1, thereby abolishing the interaction of Mig1 with the co-repressors Ssn6-Tup1 and promoting the transcription of downstream glucose repressed-genes (Östling and Ronne 1998; Papamichos-Chronakis et al. 2004). Unphosphorylated Mig1 retains in

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the nucleus and interacts with Ssn6-Tup1 when inactive Snf1 exists in the repression of 2% glucose (Östling and Ronne 1998; Papamichos-Chronakis et al. 2004). In this section, the recent studies on the roles of Snf1 in glucose metabolism below 1% (glucose derepression), in 2% (glucose repression), and in 30% glucose (1.66 M, an osmotic equivalent to 0.83 M NaCl) are summarized. As shown in Table 1, the role of Snf1 in regulating glucose metabolism is different in varied glucose. Although Snf1 serves as a positive regulator on the utilization of non-preferred carbon sources, such as maltose, in a derepression state (Zhang et al. 2015), Snf1 has a neutral effect on glucose metabolism. The discrepancy of the role of Snf1 in glucose utilization in the glucose level ranged from 2 to 10% may be due to the differences in the medium and the test methods used.

The mechanism of Snf1 in the regulation of glucose metabolism in different concentrations of glucose

The activation of Snf1

Snf1 is phosphorylated and activated by increased cellular AMP: ATP ratios and three upstream protein kinases Sak1, Tos3, and, Elm1 in glucose derepression (Hong et al. 2003; Wilson et al. 1996). Although Sak1 appears to be the major one, any of the three kinases is sufficient to activate Snf1 (Liu et al. 2011). The cyclic AMP (cAMP)-dependent protein kinase A (PKA) pathway negatively regulates the activation of Snf1 via phosphorylation of Sak1 in glucose limitation; however, Sak1 is not the only target of cAMP-PKA because Tos3 and Elm1 also have the PKA recognition domain (Barrett et al. 2012). Another way of the cAMP-PKA pathway affecting Snf1 is to regulate the localization of Sip1 β subunit of the Snf1 complex (Shashkova et al. 2015). Snf1 is also phosphorylated and activated in many environmental stresses, such as alkaline pH, sodium ion, and oxidative stresses, but not in sorbitol and heat shock stresses (Hong and Carlson 2007). The *SNF1* mutant of *S. cerevisiae* laboratory strain could resist sorbitol stress, but the homolog mutation of filamentous fungus *Pestalotiopsis microspore* exhibited hypersensitivity (Wang et al. 2018), suggesting that the role of Snf1 is different in various microorganisms and environments. Sorbitol did not active Snf1 of *S. cerevisiae* (Hong and Carlson 2007), whereas high osmolarity due to glucose activated Snf1 (Meng et al. 2020). This may be attributed to the discrepancy of strength of osmotic pressure and genetic background of yeast strains and the native attribute of Snf1 in different signal molecules. The Elm1 protein kinase is likely to be the primary one regarding activating Snf1 in alkaline pH and multidrug stresses (Casamayor et al. 2012; Souid et al. 2006). Accordingly, it could be speculated that the mode of activation of Snf1 in 30% glucose is different from that in the glucose derepression condition. The specific activation pathways of Snf1 in 30% glucose need to be studied. Snf1 is unphosphorylated and inactivated in glucose repression, in which Snf1 is targeted to the protein phosphatase Glc7 by the regulatory subunits Reg1/Reg2 (Ludin et al. 1998; Rubenstein et al. 2008). This process is regulated by glucose via the changed level of ATP, ADP, and AMP (Gowans and Hardie 2014; Gowans et al. 2013). In addition, Glc7-Reg1 is one of the targets of the cAMP-PKA pathway in the control of Snf1 activity (Shashkova et al. 2015). SUMOylation is another way that can inhibit the activity and function of Snf1. SUMOylated Snf1 losts functionality via two ways: by interacting the SUMO anchored at lys549 with the SUMO-interacting domain near the active site of Snf1; by using the directed SUMO ubiquitin ligase to target Snf1 for inhibition. Snf1 is SUMOylated via Mms21, a small ubiquitin-like modifier protein SUMO (E3) ligase, in 2% glucose (Simpson-Lavy and Johnston 2013).

Table 1 The role of Snf1 in the regulation of glucose metabolism in different concentrations of glucose

The concentration of glucose	The regulation on glucose metabolism[a]
Below 1%	Not obvious (Zhang et al. 2015)
1%	Positive (Martinez-Ortiz et al. 2019)
2%	Not obvious (Meng et al. 2020; Nicastro et al. 2015)
5%	Negative (Nicastro et al. 2015)
7%	Not obvious (Meng et al. 2020)
10%	Not obvious (Martinez-Ortiz et al. 2019)
30%	Positive (Meng et al. 2020)

[a]Snf1 protein kinase is a complex that contains an α catalytic subunit Snf1, a γ regulatory subunit Snf4, and one of the three alternative β regulatory subunits Sip1, Sip2, or Gal83 (Daniel and Carling 2002). In this section, the recent studies on the role of the catalytic subunit Snf1 in the regulation of glucose metabolism in different concentrations of glucose is summarized

The valid Snf1 form in the light of β subunits

The β subunits, including Sip1, Sip2, and Gal83, are responsible for the linkage and intracellular localization of the Snf1 protein kinase (Vincent et al. 2001). Gal83 is the major β subunit of the Snf1 complex of yeast in glucose and makes the greatest contribution to the activity of Snf1 in glucose limitation (Hedbacker et al. 2004). However, the glycogen binding domain (GBD) of Gal83 interacts with the γ regulatory subunit Snf4 and consequently strengthens the glucose inhibition of Snf1 activity in glucose limitation (Momcilovic et al. 2008). GBD interacts with the Glc7-Reg1 phosphatase complex, leading to the Snf1 inactivation in high glucose condition (Momcilovic et al. 2008). Therefore, Gal83 plays

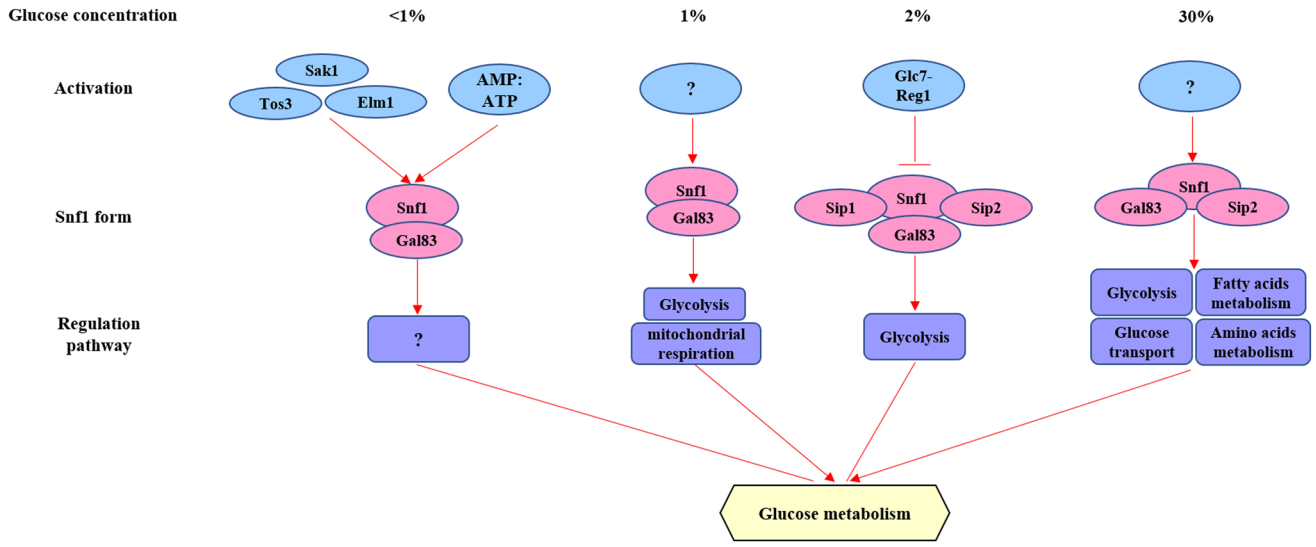

Fig. 1 Mechanism of Snf1 regulation on glucose utilization in varied glucose. Red arrow line: positive regulation. Red straight line: no obvious effect. Red flat end line: negative regulation

multiple roles in regulating Snf1 (Coccetti et al. 2018). In 2% glucose, the Snf1 complex dominated by any of the three β subunits alone served as a negative contributor to glucose utilization. This could be attributed by the disturbance on yeast growth (Meng et al. 2020). Overexpression of *SIP2* or *GAL83* could enhance the utilization of glucose in 30% glucose, suggesting that nonunique Sip1 isoform of Snf1 participated in the regulation of glucose metabolism in high glucose stress (Meng et al. 2020).

The regulatory pathway of Snf1

Snf1 positively regulates the transcription of glucose-repressed genes via controlling the phosphorylation status of the repressor Mig1 in glucose limitation (García-Salcedo et al. 2014). The *SNF1* gene is necessary to maintain the glycolytic flux in 1% glucose, which could be related to the variation of NAD(P)H, *HXK2* (encoding for hexokinase 1) expression level, and mitochondrial respiration (Martinez-Ortiz et al. 2019). Overexpression of *SNF1* up-regulates the expression of genes involved in glycolysis without affecting the glucose transport and decomposition in 2% glucose (Meng et al. 2020). In other words, Snf1 mediates the transcriptional adaptation of yeast cells to the metabolic re-arrangement with changed expression of glycolytic genes in an inhibited status (Nicastro et al. 2015). Snf1 commonly regulates the transcription of hexose transporters via controlling the nuclear localization and phosphorylation of the key components of Rgt2/Snf3 signaling pathway in high glucose (Pasula et al. 2007). This is not incompatible with the results of Meng et al. (2020), which could not exclude the possibility of changed expression of other hexose transporter genes except *HXT1*. Snf1 regulates the composition/proportion of fatty acids and the accumulation of amino acids, conferring tolerance of yeast cells to 30% glucose stress (Meng et al. 2020). Simultaneously, glucose transport and glycolysis improved in *SNF1* overexpression through up-regulating the mRNA level of genes involved in these two processes when coping with 30% glucose (Meng et al. 2020).

In summary, Snf1 regulates glucose metabolism in a glucose-dependent manner, which is associated with the different regulation on activation, localization, and signal pathways of Snf1 by varied glucose (Fig. 1). With regard to the three aspects of regulation on Snf1 mentioned above, at least the following topics need to be studied: (1) the activator of Snf1 in high glucose (2) the intracellular localization, abundance, and signaling specificity of the β subunits in response to high glucose (3) the mechanism of Snf1 regulation on the metabolism of downstream cell protectants in high glucose. Exploring the regulatory mechanism of Snf1 in glucose metabolism in the varied level of glucose can help to deeply understand the role of Snf1 protein kinase in the regulation of nutrient metabolism and stress response of yeast, which provides insights into the study of the global regulatory mechanism of Snf1 protein kinase in yeast.

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