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THE EFFECT OF TOR SIGNALLING PATHWAY ON THE RESERVE CARBOHYDRATE METABOLISM IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Trehalose and glycogen are deposited by *Saccharomyces cerevisiae* yeast cells as storage carbohydrates. Trehalose also serves as a stress protectant and protects yeast cells from physiological stresses. Reserve carbohydrates rapidly degraded to glucose when the yeast cells transferred from nutritionally poor to rich medium. The biosynthesis of trehalose is catalyzed by the TPS complex and the breakdown of trehalose is catalyzed by a neutral trehalase enzyme. The glycogen synthesis requires the activities of glycogenin, glycogen synthase and the branching enzyme. The breakdown of glycogen is catalyzed by glycogen phosphorylase and glucoamylase. TOR (Target of rapamycin) signalling pathway activates many cellular processes and signalling pathways under normal growth conditions in yeast. Besides rapamycin and caffeine treatment, a poor nitrogen source inhibits the TOR pathway. In this research, the effects of the TOR pathway on the reserve carbohydrate metabolism were investigated using *Δtor1* mutant and its isogenic wild-type yeast strain during nitrogen starvation conditions. The trehalose content of wild type and *Δtor1* yeast cells was similar in normal and nitrogen starved growth conditions. The accumulation of glycogen in *Δtor1* mutants was higher than wild type at normal growth conditions. But, the nitrogen starvation triggered the glycogen accumulation 25-fold in wild type and 2-fold in mutant yeast cells. Nutrient replenishment has no considerable effect on the trehalose and glycogen content of mutant and wild type yeast cells. However, the reserve carbohydrate accumulation was continuously increase in non-replenished environment in mutant yeast cells. These results indicate that, TOR signalling pathway is more effective on glycogen metabolism. In addition, deletion of the *TOR1* gene and inactivation of the gene product do not have the same effect on trehalose and glycogen metabolism.

Keywords: Nitrogen starvation, Glycogen, Trehalose, *TOR1*, *Saccharomyces cerevisiae*

1. INTRODUCTION

Trehalose and glycogen are carbohydrates storage in yeast *Saccharomyces cerevisiae*. Trehalose is a nonreducing disaccharide composed of two α-1,1 linked glucose monomers. Glycogen is consisting of α-1,4-linked glucose subunits with α-1,6-linked glucose at the branching points. The trehalose and glycogen contents of yeast cells increase in response to nutrient starvation. Trehalose is stored in response to unfavourable environmental conditions, such as nutrient limitations and stress conditions like oxidative stress and temperature stress (Haunsa et al., 1998; Eleutherio et al., 1993).In yeast, glycogen is formed upon the limitation

of carbon, nitrogen, phosphorous, or sulfur (Lillie and Pringle, 1980). Trehalose biosynthesis is also connected to glucose uptake through trehalose 6-phosphate, which forms during trehalose biosynthesis. It restricts glucose flux by acting on Hexokinase-II (Blazquez et al., 1993). Trehalose synthesis is catalyzed by the trehalose synthase complex encoded by *TPS1*, *TPS2*, *TPS3* and *TSL1* (Bell et al., 1998; De Virgilio et al., 1993). The hydrolysis of trehalose into two glucose molecules is catalyzed mainly by the neutral trehalase Nth1, encoded by NTH1 (Kopp et al., 1993; Nwaka et al., 1995; Van Dijck et al., 1995). The glycogen synthesis is catalyzed by the activities of glycogenin, glycogen synthase and the branching enzyme (Farkas et al., 1991; Cheng et al., 1995; Rowen et al., 1992). Degradation of glycogen is catalyzed by glycogen phosphorylase, which liberates glucose in the form of glucose-1-phosphate from the non-reducing ends of α-1,4 linked chains and glucoamylase, and by a vacuolar glucoamylase, which liberates free glucose (Hwang et al., 1989; Colonna and Magee, 1978; Wilson et al., 2010).

TOR complex regulates the transcription of many genes involved in growth and metabolism in response to nitrogen and carbon source availability (Beck and Hall, 1999). TOR pathway (Target of rapamycin) is inhibited by rapamycin, caffeine and poor nitrogen source, proline (Wullschleger et al., 2006; Reinke et al., 2006; Stracka et al., 2014). Either low glucose or low nitrogen concentration activates the TOR pathway (Rohde et al., 2008). Although proline is both low nitrogen and low carbon source, it is used only as a nitrogen source by *S. cerevisiae*. In good nitrogen source (such as ammonium sulfate and glutamate), Tor1 protein is activated and triggers the activation of the Tap42 protein. In a poor nitrogen sources (such as Proline) or the presence of rapamycin, Tor1 protein becomes inactive and terminate Tap42 activation. It has been observed that yeast cells incubated with rapamycin cause important physiological changes such as the arrest of G1 phase, inhibition of protein synthesis, increase in autophagy and glycogen accumulation (Rohde et al., 2008). Decreased TOR activity triggered the accumulation of storage carbohydrates and enhanced stress resistance (Barbet et al. 1996; Powers et al. 2006). Therefore, the aim of this study is to analyze the role of TOR signalling pathways on the reserve carbohydrate metabolism during nutrient limitation in *S. cerevisiae*.

2. MATERIALS AND METHODS

The *TOR1* deletion strain Y06864 (MATa; *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*; *YJR066w::kanMX4*) and its isogenic wild type strain BY4741 (MATa, *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*) were used in this study. *S. cerevisiae* BY4741 strain has no known mutations relevant to trehalose metabolism. All yeast strains were purchased from EUROSCARF (European *Saccharomyces cerevisiae* Archive for Functional Analysis).

S. cerevisiae strains were grown in YNBD-HLMU culture (Yeast Nitrogen Base without amino acid and ammonium sulfate; 0.5% Ammonium sulfate; 2% Dextrose; 0.2% Histidine; 0.3% Leucine; 0.2% Methionine; 1% Uracile), till logarithmic stage (OD600: 0.5-0.7) with constant shaking and temperature (30°C). Once the yeast cultures reach to log stage, the yeast cultures were divided into four parts and one part was harvested and used for enzyme assays. The second and third cultures were washed and transferred to YND-HLMU and YND-HLMU supplemented with 0.5% ammonium sulfate and 0.1% proline, respectively. The last part of the culture was allowed to grow without any treatment. At the end of 4 hours of incubation, yeast cultures were harvested and used for determining trehalose and glycogen accumulations. Yeast cultures were grown in triplicate in all experiments. Enzyme assays were done in triplicates and all experiments were repeated at least twice under the same experimental conditions.

Trehalose and glycogen accumulation of yeast cells was determined as described previously (Parrou and François, 1997). Yeast cells (50-60 mg cell wet mass) were removed and washed with ice-cold water and then resuspended in 250 µl of 0.25 M Na2CO3 and boiled for 2 h.

Then 150 µl of 1M acetic acid and 600 µl of 0.2 M sodium acetate pH 5.2 were added and divided into two parts. One half of the cell mixture was incubated at 37 °C for 18 h in the presence of 3mU trehalase enzyme (Sigma, T8778, 0.25U ml-1) for trehalose assay. The second half of the suspension was incubated at 57 °C for 18 h with continuous shaking in the presence of 1 U/ml amyloglucosidase enzyme from Aspergillus niger (Sigma, 10115) for the glycogen assay. The amount of the liberated glucose was determined enzymatically via the glucose oxidase-peroxidase system (GOD-POD assay) using a commercial kit (Fluitest®-GLU, Biocon, Germany) (Goldstein and Lampen, 1975). The determined trehalose and glycogen contents of yeast cells were given as a microgram of glucose equivalent per milligram of wet mass (µg/mg) of the yeast cells. The standard error values are less than 10%, so the error bars were not displayed in the graphs.

3. RESULTS AND DISCUSSION

The effect of Tor1 on storage carbohydrates, the trehalose and glycogen content of yeast cells was enzymatically hydrolysed into glucose and released glucose was measured. The amount of trehalose and glycogen were given as microgram glucose measured in mg wet mass (µg glucose/mg wet cell mass) of yeast cells. To analyze the effects of the TOR pathway on the reserve carbohydrate accumulations, logarithmically grown yeast cells were transferred to a nutrient-poor environment, which includes proline instead of ammonium sulfate.

The amount of trehalose measured in wild type yeast cells (428.4±51.2 µg glucose/mg cell wet weight) was 3-fold higher than starved yeast cells (1239.1±162.3 µg glucose/mg cell wet mass) (Figure 1). However, the glycogen content of wild type yeast cells (183.6±34.2 µg glucose/mg cell wet weight) was 25-fold lower than starved yeast cells (4717.5±196.2 µg glucose/mg cell wet mass). The trehalose level of mutant yeast cells (451.3±54.2 µg glucose/mg cell wet mass) was 2-fold lower than the starved mutant yeast cells (1051.1±102.3 µg glucose/mg cell wet mass) (Figure 2). Similarly, the glycogen content of mutant yeast cells (1066.4±98.6 µg glucose/mg cell wet weight) was 2-fold lower than starved yeast cells (2460.3±186.3 µg glucose/mg cell wet mass).

The content of trehalose was nearly equal in wild type and mutant yeast cells growing in both strong (ammonium sulfate) and poor (proline) nitrogen sources. At normal growth conditions, the accumulation of glycogen in *Δtor1* mutant yeast cells was greater (6-fold) than wild type yeast cells. But, the glycogen accumulation decreased nearly two-fold in *Δtor1* yeast cells under nitrogen starvation with respect to wild type yeast cells. As shown that the deletion of the *TOR1* gene affects

mainly glycogen metabolism. So, these results indicate that the Tor1 protein is essential for glycogen metabolism both in normal and nutrient li

miting growth conditions.

Figure 1. Trehalose and glycogen accumulation of wild type yeast cells

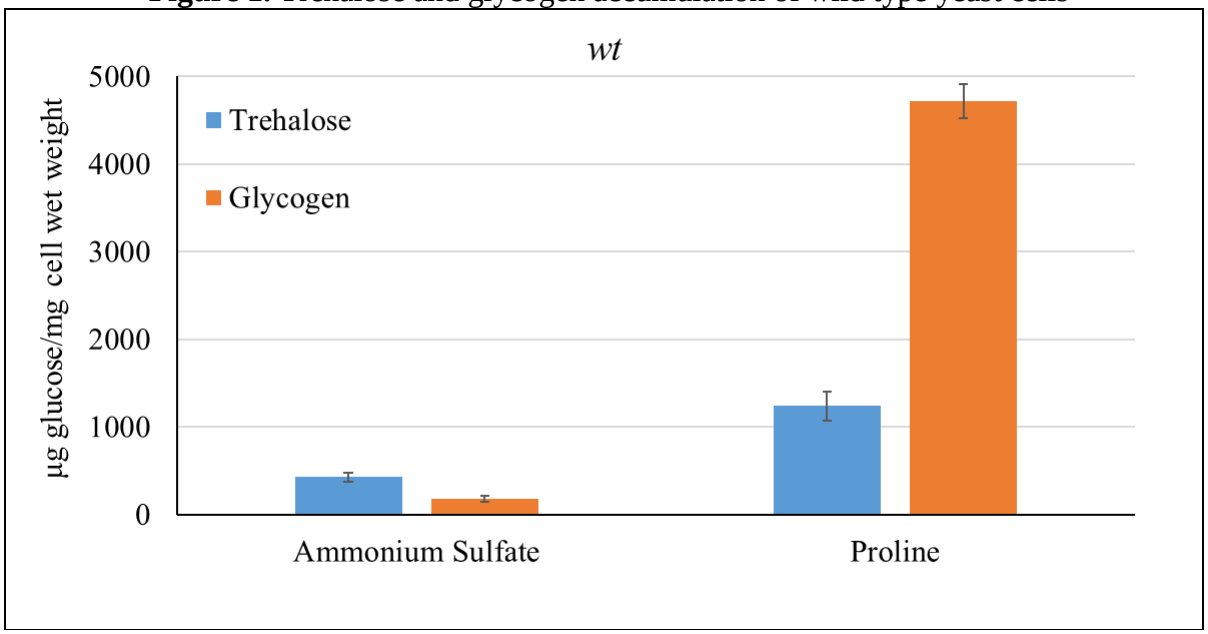
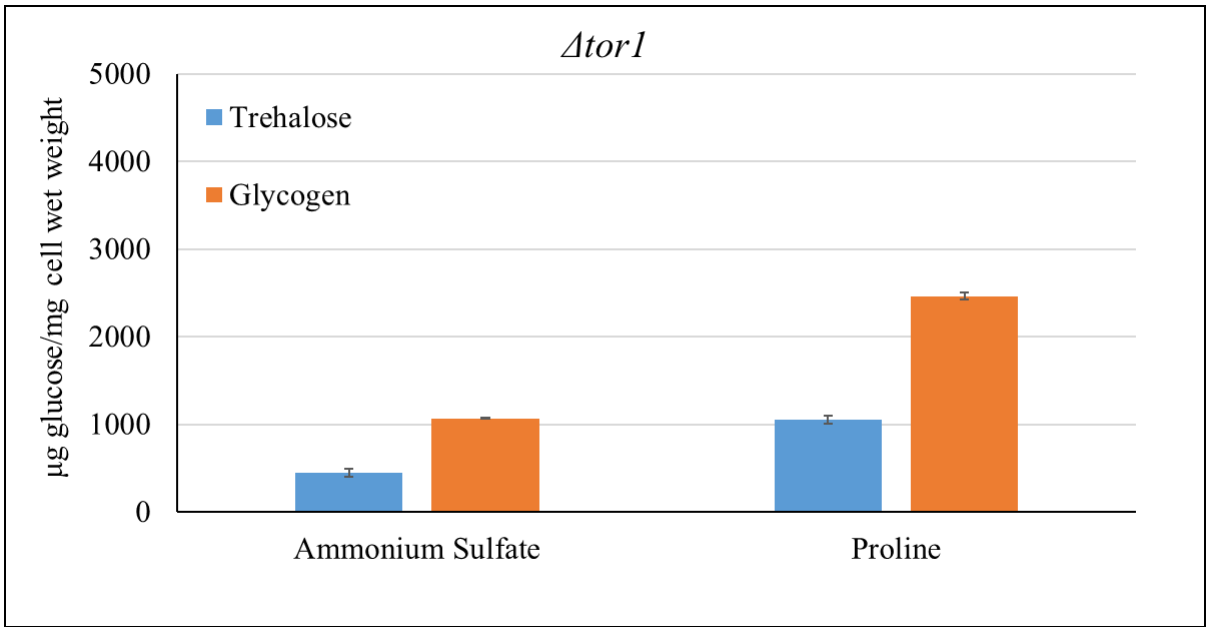


Figure 2. Trehalose and glycogen accumulation of mutant yeast cells

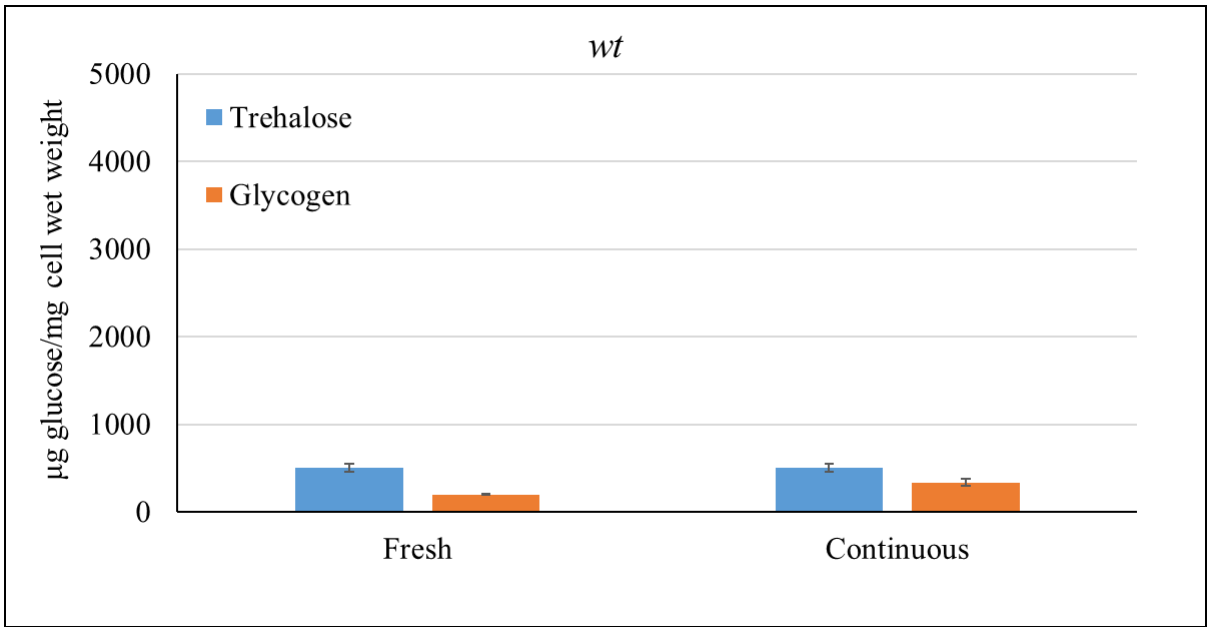


Glutamine, glutamate, asparagine and ammonium are preferred nitrogen sources for *S. cerevisiae* yeast cells, while proline and urea are weak and poor nitrogen sources. Yeast cells growing in poor nitrogen sources activate the transcription of a group of genes. Tor1 protein is inactive in poor nitrogen source and Msn2/Msn4 transcription factors, repressed by Tor1p, activate

the transcription of STRE-dependent genes (Schreve et al., 1998; Zähringer et al., 2000; De Wever et al., 2005; Conrad et al., 2017). The promoter of genes involved in trehalose and glycogen metabolism include STRE sequences and regulated by Msn2/Msn4 transcription factors. In our research Tor1 kinase was inactivated with proline in wild type yeast cells and also we used mutant yeast cells including *TOR1* gene deletion. In *TOR1* deleted yeast cells trehalose and glycogen levels in normal growth conditions were nearly four times less than the wild type yeast cells growing in a nitrogen starved environment. This result indicates that Tor1 kinase is more essential during stress conditions. Also, the deletion of the *TOR1* gene is more effective on glycogen metabolism than the inactivation of Tor1 kinase with a poor nitrogen source.

To determine the effect of replenishment on reserve carbohydrate accumulation, the exponentially growing yeast cells were shifted to a fresh medium. Shifting of wild type yeast cells to fresh culture caused no change in the content of both trehalose and glycogen (Figure 3). The glycogen content of yeast cells in continuous culture (no replenishment applied) increased slightly (from 198.1±9.1 to 366.9±41.8 µg glucose/mg cell wet weight). However, the accumulation of trehalose and glycogen in continuous culture increased 4-fold and 3-fold, respectively. Trehalose content of mutant yeast cells increased from 396.3±36.1 to 1632.7±152.9 µg glucose/mg cell wet weight, and the glycogen level increased from 745.3±58.6 to 2257.6±168.9 µg glucose/mg cell wet weight (Figure 4). In contrast to wild-type yeasts, the amount of glycogen in mutant yeasts is higher than that of trehalose. In the absence of Tor1 protein, the accumulation of glycogen was increased.

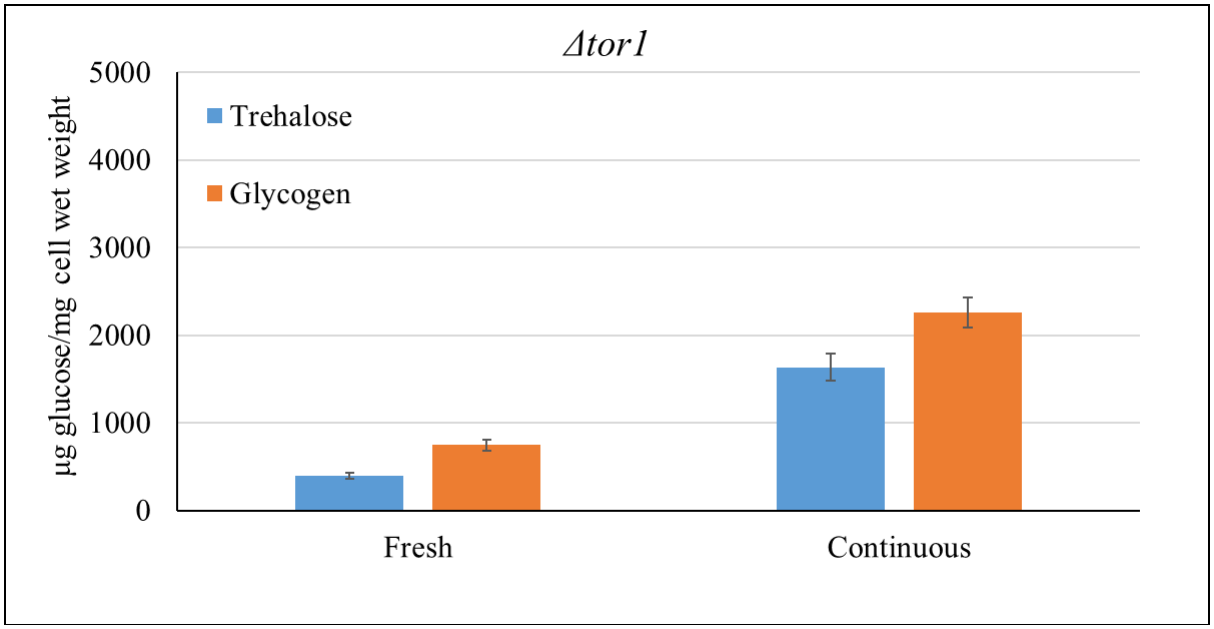
Figure 3. The effect of nutrient replenishment on trehalose and glycogen accumulation of wild type yeast cells



TOR signalling pathway activates many cellular processes and signalling pathways under normal growth conditions in yeast. It is known that trehalose and glycogen were rapidly degraded to glucose when the yeast cells transferred from poor to nutritionally rich medium (Attfield, 1987). TOR complex in *S. cerevisiae* involves the activation of several pathways when the yeast cells are grown in a nutritionally replenished medium (Loewith and Hall, 2011). Trehalose and glycogen were rapidly recycled to glucose when stress conditions

terminated (Nwaka et al., 1995). In our research, in the absence of Tor1 protein, the trehalose and glycogen level was 3-fold and 7-fold higher than wild type yeast cells in continuous culture, respectively. Also, the glycogen level in mutant yeast cells was four times higher than wild type yeast cells after the replenishment period. These results indicate that complete deletion of gene creates a more different effects than the inactivation of related gene products.

Figure 4. The effect of nutrient replenishment on trehalose and glycogen accumulation of mutant yeast cells



In conclusion, *TOR1* gene deletion and Tor1 kinase inactivation do not have similar effects on trehalose and glycogen metabolism. In addition, the TOR signalling pathway is more effective in glycogen metabolism than trehalose metabolism.

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