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Research Article

Effects of glucose on the cellular respiration in fission yeast expressing Human *GSK3B* gene

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Flycogen synthase kinase 3

Abstract: Glycogen synthase kinase 3β (GSK3B) acts as a signalling and metabolic enzyme and plays a variety of roles in cellular processes such as cell proliferation, DNA repair, cell cycle, signaling, and metabolic processes. GSK3B has been implicated in numerous diseases, including inflammation, neurodegenerative disease, diabetes, and cancer. Yeasts are suitable models for the investigation of various cellular processes because they include homologous genes to human genes. In this study, we transferred the human *GSK3B* gene to *Schizosaccharomyces pombe* (Lindner) cells (*gsk3Δ*) which include a deletion for this gene. Cells with *gsk3* gene deletion and transformant cells with the human *GSK3B* gene that was reversed by genetic complementation were comparatively examined at the level of gene expression for changes in cellular respiration under varying glucose concentration conditions. For this purpose, the expression of *fbp1*, *pka1* and *gsk3* genes were analyzed in cells grown under conditions with different glucose concentrations. We revealed that the *GSK3B* gene was expressed more in glucose starvation conditions than in other conditions. We also observed a decrease in the level of gene expression in mitochondrial respiration when the human *GSK3B* gene was transferred in cells that preferred mitochondrial respiration in the absence of the *gsk3* gene, regardless of ambient conditions.

Özet: Glikojen sentaz kinaz 3β (GSK3B), hücre proliferasyonu, DNA onarımı, hücre döngüsü, sinyalleşme ve metabolik yolaklar gibi çok sayıda hücresel süreçte işlev gören bir serin/treonin kinazdır. GSK3B, iltihaplanma, nörodejeneratif hastalık, diyabet ve kanser dahil olmak üzere farklı hastalıklarda rol oynamaktadır. Mayalar, insan genlerine homolog genler taşıması nedeniyle çeşitli hücresel süreçlerin araştırılmasında kullanılan uygun modellerdir. Bu çalışmada insan *GSK3B* geni, bu gen bakımından delesyonlu *Schizosaccharomyces pombe* (Lindner) (*gsk3Δ*) hücrelerine transforme edildi. *Gsk3* geni bakımından delesyon taşıyan ve insan *GSK3B* geni aktarılarak delesyonu genetik komplementasyon ile geri döndürülen hücreler, değişen glukoz konsantrasyonu koşullarında hücresel solunumda meydana gelen değişiklikler bakımından karşılaştırmalı olarak gen ifadesi düzeyinde incelendi. Bu amaçla farklı glukoz konsantrasyonu içeren koşullarda üretilen hücrelerde *fbp1*, *pka1* ve *gsk3* genlerinin ifadesi analiz edildi. Glukoz açlığı koşullarında *GSK3B* geninin diğer koşullara göre daha fazla ifade edildiği sonucuna vardık. Ayrıca ortam koşullarından bağımsız olarak *gsk3* geni yokluğunda mitokondriyal solunumu tercih eden hücrelerde insan *GSK3B* geni aktarıldığında mitokondriyal solunumda gen ifadesinde azalma gözlemledik.

Introduction

A highly conserved serine/threonine kinase, glycogen synthase kinase 3 (GSK3) is encoded by the genes *GSK3A* and *GSK3B* in mammals. GSK3 is a key enzyme that plays a role in many cellular processes, including glucose metabolism, intracellular signal transduction, DNA repair, and cell division. It has also been shown to play a role in the biological processes of tumor formation, and in various diseases such as inflammation, neurodegenerative diseases, diabetes, and cancer (Lin *et al.* 2020).

GSK3B is a component of multiple cellular signaling pathways, and a number of kinases, phosphatases, and proteases can either directly or indirectly control its activity. GSK3B is an essential player in maintaining cell homeostasis because of the wide range of substrates it can bind to, which includes membrane receptors, cytoskeleton-associated proteins, pro- and anti-apoptotic factors, mitochondrial channels, and glycolytic enzymes. GSK3B activity influences a variety of cellular functions, including amyloid processing, synaptic transmission,

neuroplasticity, energy metabolism, cell survival and proliferation, apoptosis, and membrane polarity (Duda *et al*. 2018). In neurons, GSK3B controls the mitochondrial energy metabolism. GSK3B inhibition increases mitochondrial respiration and membrane potential and changes NAD(P)H metabolism. Thus, its inhibition stimulates mitochondrial energy metabolism (Martin *et al.* 2018).

From simple ascomycetes to humans, many fundamental biological processes, including metabolism, cell division, transcription, translation, protein secretion, and autophagy, are highly similar. Therefore, basic research on yeasts has contributed to numerous fields of medical study (Breitenbach *et al*. 2013). Yeasts, which are single-celled organisms, provide basic information with the expression of recombinant proteins and constitute the primary step to determine the effect of various chemicals (Porzoor & Macreadie 2013). In cases where an ortholog gene is found in studies, that gene is made similarly defective, resulting in a model in which the phenotype of disease caused by a defective gene in humans is experimentally monitored. There are numerous studies in which model organisms have contributed to the development of the treatment of a disease (Wangler *et al.* 2017, Johnston 2020).

One of the most important features of yeasts used in molecular genetic studies is that they can carry plasmids that contain genetic material that can change the characteristics of a strain. This capability of yeasts facilitates gene cloning (Hoffman *et al.* 2015). *Schizosaccharomyces pombe* (Lindner) has become an increasingly used model for investigating various molecular and cellular processes over the past 50 years. *Schizosaccharomyces pombe* has gained importance as a single-celled "micromammalia" in the study of biological questions. For *in vivo* research of several cellular processes, including cell biology and classical and molecular genetics, this yeast strain offers a potent system (Vyas *et al*. 2021). In *S. pombe*, the *gsk3+/skp1+* gene (SPAC1687.15) and *gsk31+* gene (SPBC8D2.01) are orthologs of the human *GSK3B* gene (Ma *et al*. 2016).

Glucose oxidation is dependent on three main metabolic pathways (the Krebs cycle, the pentose phosphate pathway and glycolysis) in eukaryotic organisms and mammals. The cell dynamically modifies the balance between the three pathways to attain the optimum growth and endurance possible under the circumstances (Nidelet *et al*. 2016, Malecki *et al.* 2020). Even in the presence of oxygen, rapidly reproducing cells use anaerobic metabolism known as aerobic glycolysis, particularly in eukaryotes. However, glucose oxidation only in aerobic respiration maximizes the redox potential stored in glucose and produces the greatest number of ATP molecules. The Warburg effect in cancer cells is a term that is frequently used to describe growth by aerobic glycolysis (Warburg 1956, Lunt & Vander Heiden 2011). Regarding aerobic glycolysis, the Crabtree effect is observed in yeast cells, which actively suppresses carbohydrate oxidation in respiration as long as glucose is present (Turcotte *et al.* 2009). In contrast, the energy metabolism of differentiated human cells or yeast cells, growing in a nutrient-poor environment is changed toward respiration (Lunt & Vander Heiden 2011). According to physiologic and environmental factors, respiration and fermentation levels are thereby rebalanced (Malecki *et al.* 2020).

There are many studies on the relationship of GSK3B with mitochondrial respiration. However, although it is known that GSK3B plays a role in complex processes such as PI3K/AKT/mTOR signaling networks and energy metabolism regulation, there are some points that have not been fully elucidated (Byun *et al*. 2012, Martin *et al*. 2018, Wang *et al*. 2021, Ren *et al*. 2020). These complicated processes can be examined in simpler living organisms and new findings can be obtained. Human proteins can be investigated in yeast by heterologous expression (Winderickx *et al.* 2008, Vanhelmont *et al.* 2010, Coronas-Serna *et al*. 2021, Liu *et al*. 2022). The relationship between GSK3B and cellular respiration will be more easily revealed in yeast cells whose glucose and energy metabolism are relatively better understood. This study is the first to perform heterologous expression of the human *GSK3B* gene in yeast, and also in this study, the effect of different glucose concentrations on cellular respiration in *S. pombe* cells with *gsk3* gene deletion and human *GSK3B* gene transformation was investigated at the level of gene expression.

Materials and Methods

Organisms and experimental conditions

In this study, we used *Schizosaccharomyces pombe* mutant cells with a deletion of the *gsk3* gene (h^+ ade6-M210/ ura4-D18 leu1-32 gsk3), commercially available from BIONEER. *Escherichia coli (E. coli) DH5α* strain containing pREP42 plasmid was obtained from the collection of Department of Molecular Biology and Genetics in Istanbul University. During the study, *gsk3Δ* cells were grown in EMM medium containing adenine, uracil, and leucine (50 µg/ml) (Gutz *et al*. 1974). First, *gsk3Δ* cells were phenotypically controlled by selecting them on selective media containing different combinations of metabolites. In addition, genotype controls of *gsk3Δ* cells were performed by Polymerase Chain Reaction (PCR), using suitable primers for the *gsk3* gene (primer *gsk3*) (Table 1) and "Thermo Scientific Phusion High-Fidelity DNA Polymerase".

In order to determine the middle logarithmic phase of the *gsk3Δ* mutant strain in YNB media, and of transformant cells in Edinburgh Minimal Medium (EMM) containing adenine and leucine, the change in cell growth was measured at 600 nm wavelength optical density in a spectrophotometer device (EON, Biotek Instruments Inc.) for 36 hours at 2-hour intervals. Time-dependent graphs of cell growth were obtained according to the standard graph.

Table 1. Primer sequences used in the study

	Primer sequences	Tm (°C)	Product size
gsk3	F: 5' TCCGGATCCACTCCACGTTCATCAC 3' R: 5' CCCGGGTCGACTAATGACTCTACCAAA 3'	60°C	~ 2355 bp
GSK3B	F: 5' TTTCATATGATGTCAGGGCGGCC 3' R: 5' GCGTCGACTCAGGTGAAGTTGGAA 3'	55°C	~ 1256 bp
qGSK3B	F: 5' TCCACAGATCAAGGCACATC 3' R: 5' GGCGTGTACTCCAACAATCT 3'	55°C	~120 bp
act1	F: 5' TGCTCAATCTTCCTCCCTTG 3' R: 5' CAAAGCTGAGGGTTGGAAAA 3'	55°C	~120 bp
pka1	F: 5' TGGGGTACTTTCCAAGATGC 3' R: 5' CTTTTTCGGGAAATCGATGA 3'	55°C	~120 bp
fbp1	F: 5' GTATGGTGCTTCGGCTCATT 3' R: 5' TTCATGTTTCGATGGGTCAA 3'	55°C	~120 bp

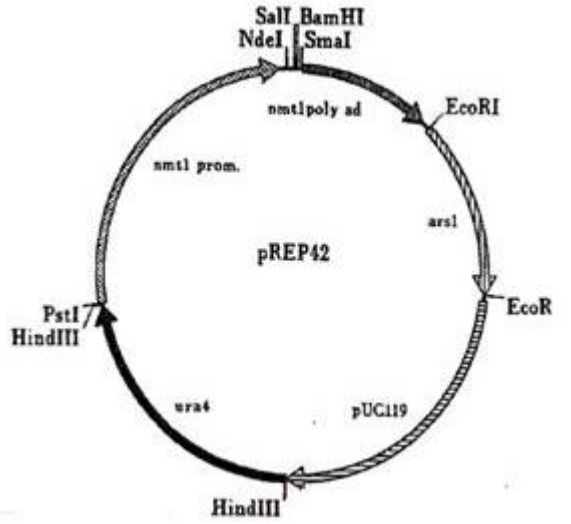

Fig. 1. pREP42 restriction map (Karaer 2001).

EMM medium containing adenine and leucine was used for the production of transformant cells. The *nmt1* (no massage in thiamine) promoter can be repressed by thiamine and is most commonly used to manipulate the expression of genes in fission yeast. Since the *GSK3B* gene, which was transferred to the pREP42 plasmid (Fig. 1) was under the control of the *nmt1* promoter, it was grown in the EMM medium containing thiamine (15 µM), adenine and leucine for 24 hours and then in thiamine-free EMM medium containing adenine and leucine for 20 hours. Then, transformant cells washed with Phosphate-Buffered Saline (PBS) were harvested by growing for 4 hours in the EMM medium containing 0.5%, 2%, and 5% glucose and proper metabolites (Brandis *et al.* 2006).

Cloning of the human GSK3B gene into pREP42 plasmid

The cDNA sequences of the *GSK3B* gene were determined in accordance with the data obtained from the NCBI database, and then codon optimization was performed for *S. pombe* using IDT (Integrated DNA Technologies) Codon Optimization Tool (https://www.idtdna.com/CodonOpt). The *GSK3B* gene was obtained synthetically and purchased commercially (GenScript, USA). Amplification of the *GSK3B* gene was performed using these synthetic sequences. Reverse and forward primers suitable for the *GSK3B* gene (primer *GSK3β*) were designed to amplify the entire gene sequences. In addition, the recognition sequences of the *Nde*I and *Sal*I restriction endonucleases were added into these primers to form the appropriate ends required for the ligation of the gene into the plasmid (Table 1).

The *GSK3B* gene PCR product and pREP42 plasmid were cut with *Sal*I and *Nde*I restriction endonucleases (Thermo Scientific) to form suitable ends, and the cut products were extracted from the gel with the "Thermo Scientific GeneJET Gel Extraction Kit" according to the manufacturer's instructions. The *GSK3B* gene and the pREP42 plasmid were ligated by Invitrogen™ T4 DNA Ligase (1 U/µL) at the ratio of 3:1 (insert/vector). The ligation product was transferred into *E. coli DH5α* cells by heat-shock method (Inoue *et al.*, 1990). Colony PCR was performed with *GSK3B* primers by using transformant *E. coli DH5α* cells grown in the selective medium containing ampicillin antibiotic. Then, the obtained recombinant plasmid was amplified and isolated from *E. coli*. In addition, the isolated recombinant plasmid was confirmed by cutting with *Hind*III restriction endonuclease.

Transformation of the pREP42 containing the GSK3B gene into S. pombe

pREP42 (~3.5 µg) containing the *GSK3B* gene was transferred to the *S. pombe gsk3Δ* mutant strain used as the host strain by using the transformation method suggested by Kanter-Smoler and colleagues (Kanter-

Smoler *et al.* 1994). Transformation of empty pREP42 plasmid was also performed to obtain a control group. Transformant cells (pREP42+*gsk3Δ* "control group" and *GSK3B*.pREP42+ *gsk3Δ* "experimental group") grown on selective medium (EMM+AL) were confirmed by colony PCR using *GSK3β* primers (Table 1).

Real-time PCR

Total RNA isolation was performed from transformant cells grown under different glucose conditions by using PureLink® RNA Mini Kit (Ambion®by Life Technologies) according to the manufacturer's instructions. Before using the kit, the cells were mechanically homogenized with glass beads and PBS. Subsequently, isolated RNAs were converted to cDNA by Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit, according to the manufacturer's instructions. Expressions of *pka1* (protein kinase A subunit), *fbp1* (fructose-1,6-biphosphatase) and *GSK3B* (glycogen synthase kinase-3) genes were analyzed by using the Thermo Scientific Applied Biosystems PowerUp SYBR™ Green PCR Master Mix. *Act1* gene was used as housekeeping gene. We designed the primer of the *GSK3B* gene (primer *qGSK3β*) in the IDT Primer Quest Tool (https://eu.idtdna.com/Primerquest/Home/Index) program (Table 1). "Bio-Rad CFX96" was used for real-time PCR. Analyzes were performed with the "Bio-Rad CFX Manager Software".

Statistical Analysis

The results of the qPCR analysis were evaluated with the "Pfaffl" method (Pfaffl 2001). Statistical analyzes were performed with Two-way ANOVA in GraphPad Prism 7 Software.

Results

Control and Reproduction of the Cells

Phenotypes of *S. pombe gsk3Δ* mutant strain cells were controlled on selective media (Fig. 2a). Only one of the colonies grown on an EMM medium containing adenine, leucine, and uracil was selected. Genotypes of *gsk3Δ* cells were checked by performing PCR with primers suitable for *S. pombe gsk3* gene (primer *gsk3*) (Fig. 2b).

Optical densities (OD) of the *gsk3Δ* mutant strain and transformant strains were measured in YNB medium and in EMM medium containing adenine and leucine, respectively, at 600 nm wavelength for 36 hours at 2-hour intervals. Time-dependent graphs of cell growth in cultures were obtained by using the standard graph created according to the OD values of various dilutions with known cell numbers per milliliter (Fig. 2c). The generation time of the *gsk3Δ* mutant cells was found to be approximately 4 hours in YNB medium containing adenine, uracil and leucine, and the generation time of the transformant cells (pREP42+*gsk3Δ* and *GSK3B*.pREP42+ *gsk3Δ*) was approximately 5 hours in EMM medium containing adenine and leucine.

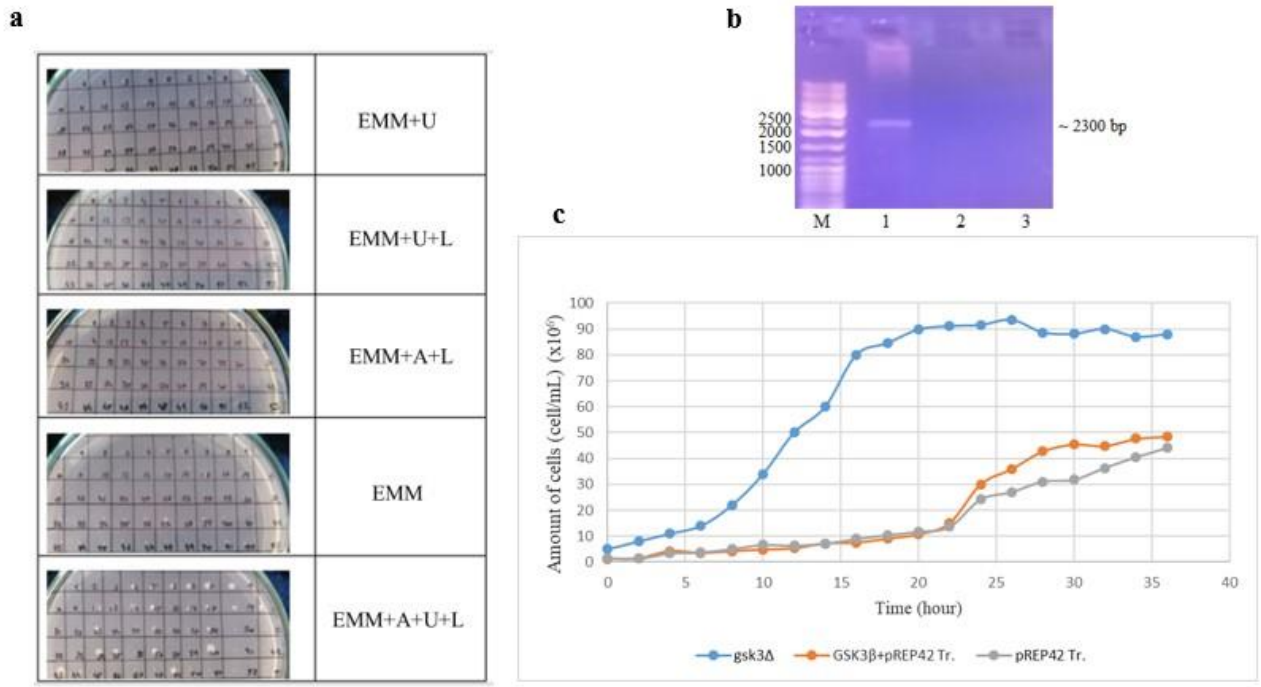

Fig. 2. Reproduction and phenotypic and genotypic control of cells, a. growth and phenotypic control of *gsk3Δ* mutant cells on selective EMM medium, b. agarose gel image of genotype control of *gsk3Δ* mutant cells. M: GeneRuler 1 kb DNA Ladder; 1: *972h*$^{-}$ wild type *S. pombe* genome; 2: *gsk3Δ* mutant cell genome; 3: non-template control, c. time-dependent growth curve of *gsk3Δ* cells in YNB medium containing adenine, leucine, and uracil, and transformant cells in EMM medium containing adenine and leucine.

pREP42 Plasmid Containing the GSK3B Gene

First, the pREP42 plasmid was confirmed by digesting with *Eco*RI restriction endonucleases (Fig. 3a). The *GSK3B* gene was amplified by PCR with gene-specific primers using commercially pEX-A258-GSK3B plasmid DNA containing the *GSK3B* gene (Fig. 3b). The PCR product was observed to be ~1256 bp as expected.

Before ligation, the pREP42 plasmid and the *GSK3B* gene PCR product were cut with *Sal*I and *Nde*I restriction endonucleases to form the appropriate end, and then they were extracted from agarose gel for purification (Fig. 3c). After agarose gel extraction, pREP42 plasmid and *GSK3B* gene were ligated and ligation product was transferred into *E. coli DH5α* cells. Colony PCR was performed using *E. coli DH5α* cells grown in the selective medium containing ampicillin antibiotic and transformant cells that received the *GSK3B* gene were determined (Fig. 3d).

The accuracy of the pREP42 plasmid containing the *GSK3B* gene, controlled by PCR, was confirmed by cutting with *Hind*III restriction endonuclease since there is also a recognition point inside the *GSK3B* gene (Figs 3e, f).

Schizosaccharomyces pombe Cells Containing the GSK3B Gene

pREP42 containing the *GSK3B* gene, and pREP42 were transferred into *S. pombe gsk3Δ* mutant cells. After transformation, twelve positive transformant colonies (two of them *GSK3B*.pREP42 and ten of them pREP42 transformant) were selected in EMM medium containing adenine and leucine. Positive transformant *gsk3Δ* colonies were controlled by colony PCR using primers specific to the *GSK3B* gene (Fig. 4).

Schizosaccharomyces pombe Cells Containing the GSK3B Gene

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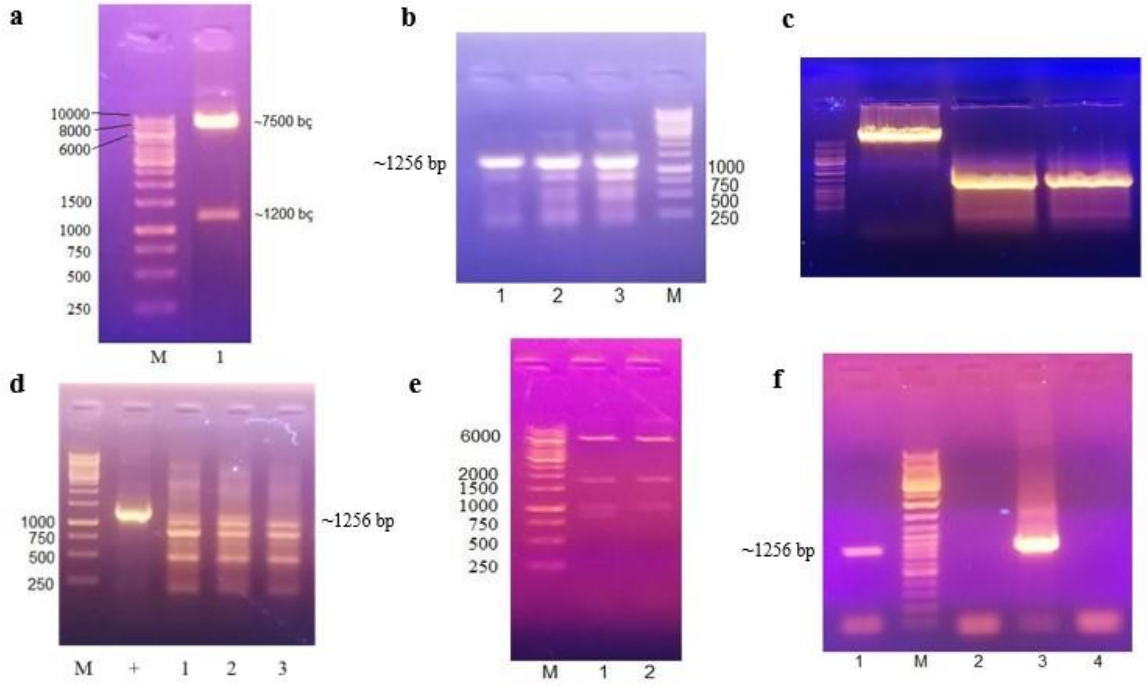

Fig. 3. Agarose gel electrophoresis images. **a.** Cleavage of the pREP42 plasmid with *Eco*RI restriction endonuclease. M: GeneRuler 1kb DNA Ladder; 1: cut product of the pREP42 plasmid, **b.** agarose gel image of the *GSK3B* gene amplified with different primer annealing temperatures. 1: 60°C; 2: 58°C; 3: 57°C; M: GeneRuler 1 kb DNA Ladder. **c.** Agarose gel image of pREP42 plasmid and *GSK3B* gene for gel extraction after digestion with restriction endonucleases, **d.** agarose gel image of colony PCR product performed with transformant *E. coli* cells and primers of *GSK3B* gene, **e.** agarose gel image of pREP42 plasmid, and pREP42 plasmid containing the *GSK3B* gene after cutting with *Hind*III restriction endonuclease. M: GeneRuler 1 kb DNA Ladder; 1: pREP42 plasmid containing the *GSK3B* gene (recombinant pREP42); 2: pREP42 plasmid, **f.** agarose gel image of the PCR product performed with the recombinant pREP42 plasmid and *GSK3β* primers. M: GeneRuler 1 kb DNA Ladder; 1: pREP42 plasmid containing the *GSK3B* gene; 2: pREP42 plasmid; 3: commercial vector containing the *GSK3B* gene, positive control; 4: non-template control.

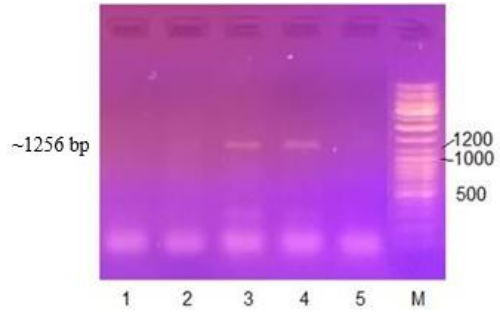

Fig. 4. Agarose gel image of colony PCR product performed with transformant *S. pombe* cells and primers of *GSK3B* gene. 1: *S. pombe gsk3Δ* cell; 2: *S. pombe gsk3Δ* cell with pREP42; 3,4: *S. pombe gsk3Δ* cell with pREP42 containing the *GSK3B* gene; 5: Non-template, negative control; M: GeneRuler 1 kb DNA Ladder.

Gene Expression Level Analyses

Total RNA was isolated from transformant cells grown in the EMM medium containing different glucose concentrations (0.5%, 2%, 5%) and appropriate metabolites (adenine, leucine) and then qPCR was performed from the synthesized cDNAs.

When transformant cells grown under optimum glucose concentration (2%) conditions were compared at the level of gene expression in terms of *pka1*, *fbp1*, and *GSK3B* genes, a 1.6-fold increase was observed in the *fbp1* gene involved in gluconeogenesis, in the transformant containing the *GSK3B* gene, while a 12.8-fold increase was observed in the *pka1* gene (Fig. 5a). When cells grown in media containing glucose at different concentrations (0.5% and 5%) were compared in terms of the *pka1* and *fbp1* gene expressions, a 7.1-fold increase in expression of the *pka1* gene was observed in experimental group cells grown in media containing 0.5% concentration of glucose, while a 20.1-fold increase was observed in the *fbp1* gene expression (Fig. 5b). When compared with the control group cells, a 6-fold increase in expression of the *pka1* gene was observed in experimental group cells grown in a medium containing 5% glucose, and a 17.1-fold decrease in *fbp1* gene expression was observed (Fig. 5c).

Considering the expression of the *pka1* and *fbp1* genes at the level of gene expression in control group cells, the *pka1* gene expression decreased by 3.2 times in cells grown in medium containing 0.5% glucose, compared to cells grown in medium containing 2% glucose, while a decrease in the *fbp1* gene expression was observed by 2.2 times. In control group cells grown in a medium containing 5% glucose, a 24.8-fold decrease was observed in *pka1* gene expression, while a 3.1-fold increase was observed in the *fbp1* gene expression, compared to cells grown in medium containing 2% glucose (Fig. 6a).

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When expression of the *pka1*, *fbp1*, and *GSK3B* genes in experimental group cells was compared with cells grown in the medium containing 2% glucose, the *pka1* gene expression did not change in cells grown in the medium containing 0.5% glucose, while the *fbp1* gene expression was decreased by 3 times and expression of the *GSK3B* gene increased by 52 times. In cells grown in a medium containing 5% glucose, a 6.6-fold increase in the *pka1* gene expression and a 9.2-fold decrease in the *fbp1* gene expression were observed, while no change was observed in the expression of the *GSK3B* gene (Fig. 6b).

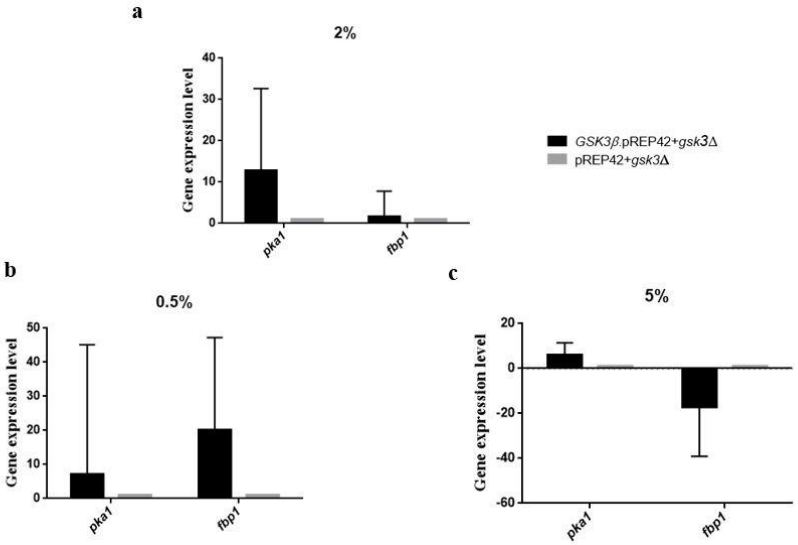

Fig. 5. Gene expression analysis of transformant strains under different glucose concentration conditions. Comparative analysis of expression of the *pka1* and *fbp1* genes. **a.** In experimental strain versus control strain, **b.** in cells grown in medium containing 0.5% glucose and **c.** in cells grown in medium containing 5% glucose.

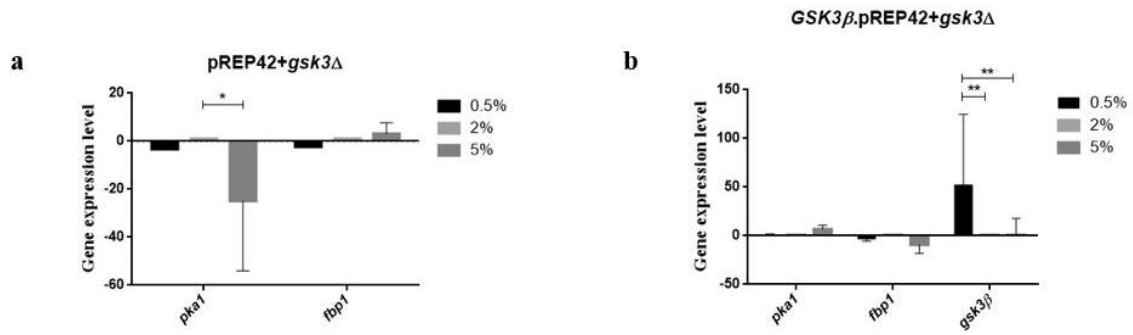

Fig. 6. Gene expression analysis of transformant strains. **a.** Comparative expression analyses of *pka1* and *fbp1* genes in control group cells grown at different glucose concentrations (*P* value <0.05*), **b.** comparative expression analyses of *pka1*, *fbp1* and *gsk3* genes in experimental group cells grown at different glucose concentrations (*P* value <0.01**).

Discussion

The role of GSK3 activity in insulin resistance, obesity, neurodegenerative disorders, stress response, inflammation, and energy metabolism has recently been re-evaluated (Wang *et al.* 2021). Given that GSK3B is involved in the biological processes that lead to carcinogenesis and that GSK3B inhibitors have been shown to have beneficial effects when combined with chemotherapy and radiation in the treatment of several cancer types, it is likely that GSK3B will be significant in the development of cancer therapies (Lin *et al.* 2020). According to Avila (2008), GSK3 is a crucial molecule in the progression of Alzheimer's disease and is involved in the synthesis of paired helical filament (PHF)-Tau. In the brains of people with Alzheimer's disease, GSK3B induces neurofibrillary tangle formation, tau hyperphosphorylation, synaptic loss, neuronal death, and memory deficiency (Kimura *et al.* 2008, Kamat *et al.* 2014).

In the presence of glucose, many organisms repress mitochondrial respiration, preferring aerobic glycolysis or fermentation, which is sufficient for ATP production. In glucose repression, the use of alternative carbon sources, aerobic respiration, stress response pathways and sporulation are repressed, while glycolysis and fermentation are activated (Johnston, 1999). However, fission yeast cells perform partial respiration for rapid proliferation under fermentation conditions. Differentiated mammalian cells or yeast cells grown in a nutrient-poor environment carry out their energy metabolism by mitochondrial respiration. Thus, the balance between respiration and fermentation is maintained (Malecki *et al.* 2020).

In *S. pombe*, glucose is first detected via the G protein-coupled receptor (GPCR), and then signal transduction occurs via cAMP-dependent protein kinase A (PKA) (Hoffman & Winston 1991, Byrne & Hoffman, 1993, Hoffman 2005). Glucose repression signal suppresses expression of *fbp1* gene by activation of cAMP-dependent PKA pathway (Hoffman & Winston 1990), while glucose starvation signal increases expression of *fbp1* gene via activation of MAPK (Walker 1998). The *fbp1* gene encodes the fructose-1,6-bisphosphatase enzyme, which is an important enzyme in gluconeogenesis (Hoffman & Winston 1991).

Under glucose starvation conditions, cells tend to mitochondrial respiration and the gluconeogenetic process occurs metabolically. Accordingly, expression of the *fbp1* gene increases. Protein kinase A (PKA) encoded by the *pka1* gene is a gene that works against gluconeogenesis, and in this case, the expression of the *pka1* gene is downregulated. Since *pka1* and *gsk3* are in a positive genetic interaction, a decrease in the expression of the *gsk3* gene is expected (Rallis *et al.* 2017). Under glucose repression conditions, the cells tend to fermentation, and glycolytic flow is accelerated. In this case, a decrease in *fbp1* gene expression is expected. There is an increase in the *pka1* gene, which has inverse interaction with gluconeogenesis (Hoffman & Winston 1991, Byrne & Hoffman 1993, Hoffman 2005).

When heterologous expression of the *GSK3B* gene was performed in mutant cells with a deletion of the *gsk3* gene under optimum conditions, we observed an increase in the expression of the *pka1* gene, while the alteration in the expression of the *fbp1* gene was very low. In glucose starvation conditions, the expression of the *pka1* gene increased in the *GSK3B* gene-transferred cells compared to the control group. However, expression of the *pka1* gene was at lower levels in the experimental group compared to the optimum conditions, and expression of the *fbp1* gene was increased. In glucose starvation conditions, an increase in the *pka1* gene is expected, as stated by Walker (Walker 1998). An increase in expression of the *fbp1* gene, which is involved in gluconeogenesis, was observed in the *GSK3B* gene transferred cells. In the glucose repression conditions, we observed that expression of the *pka1* gene increased while expression of the *fbp1* gene decreased. In our study, in cells expressing the *GSK3B* gene, we found that a decrease in mitochondrial respiration occurred in the level of gene expression in accordance with the findings of Hoffman and Winston under glucose repression conditions (Hoffman & Winston 1991).

When the control group is examined, the host strain has a deletion in terms of the *gsk3* gene and mitochondrial respiration is triggered in cells with a deletion of the *gsk3*

gene (Martin *et al.* 2018). When the expression graph of the *pka1* and *fbp1* genes in the host strain was examined, the expression of the *pka1* gene was characterized by a decrease. This indicates that the activity of the PKA pathway in the cell is reduced. However, the situation here is due to the absence of the *gsk3* gene product rather than the changing environmental conditions. Owing to the absence of the gsk3, we observed that even under glucose repression conditions, the cell prefers mitochondrial respiration.

In the experimental group cells expressing the *GSK3B* gene, expression of the *pka1* gene did not change under glucose starvation conditions, while expression of the *fbp1* gene was increased. In the glucose repression conditions, the increase in the expression of the *pka1* gene and the associated decrease in the expression of the *fbp1* gene indicate that the cell begins to regulate respiration in accordance with the environmental conditions after the transfer of the *GSK3B* gene to the host cells containing the *gsk3* gene deletion. Expression of the *GSK3B* gene was increased most under glucose starvation conditions. Overexpression of the *GSK3B* gene under glucose starvation conditions may be associated with increased expression of the *pka1* gene, with which it has positive genetic interaction, and thus seems to cause decreased the *fbp1* gene expression. However, contrary to our findings, Martin *et al.* reported low levels of the *GSK3B* gene expression in the brain of monkeys when calorie restriction was present (Martin *et al.* 2018).

In this study, we revealed that the *GSK3B* gene was expressed more in glucose starvation conditions than in other conditions. In addition, we observed a decrease in the level of gene expression in mitochondrial respiration when the *GSK3B* gene was transferred in cells that preferred mitochondrial respiration in the absence of the *gsk3* gene, regardless of ambient conditions. In the absence of the *gsk3* gene, changing glucose concentration conditions had no effect on cells, with cells preferring mitochondrial respiration even under glucose repression conditions. When the *GSK3B* gene-containing transformant cells we obtained were compared with the cells with the deletion, cellular respiration metabolism reversed to the expected state after the *GSK3B* gene transfer. We have performed the cloning of the *GSK3B* gene, which is associated with various diseases (Parkinson's, Alzheimer's, Diabetes, *etc.*) and plays a key role in many cellular pathways, into *Schizosaccharomyces pombe* for the first time. These transformant cells we obtained can be used as models to better understand the role of the GSK3B in molecular processes and the changes in the expression of this gene depending on different environmental conditions. Further studies with the GSK3B will also contribute to the elucidation of the molecular mechanisms in which kinases, which have important roles in intracellular signalling, play a role.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: Data available on reasonable request.

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