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RNA-Seq analysis of global transcriptomic changes suggests a roles for the MAPK pathway and carbon metabolism in cell wall maintenance in a *Saccharomyces cerevisiae FKS1* mutant

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ABSTRACT

FKS1 encodes a β-1,3-glucan synthase, which is a key player in cell wall assembly in *Saccharomyces cerevisiae*. Here we analyzed the global transcriptomic changes in the *FKS1* mutant to establish a correlation between the changes in the cell wall of the *FKS1* mutant and the molecular mechanism of cell wall maintenance. These transcriptomic profiles showed that there are 1151 differentially expressed genes (DEGs) in the *FKS1* mutant. Through KEGG pathway analysis of the DEGs, the MAPK pathway and seven pathways involved in carbon metabolism were significantly enriched. We found that the MAPK pathway is activated for *FKS1* mutant survival and the synthesis of cell wall components are reinforced in the *FKS1* mutant. Our results confirm that the FKS1 mutant has a β-1,3-glucan defect that affects the cell wall and partly elucidate the molecular mechanism responsible for cell wall synthesis. Our greater understanding of these mechanisms helps to explain how the *FKS1* mutant survives, has useful implications for the study of similar pathways in other fungi, and increases the theoretical foundation for the regulation of the cell wall in *S. cerevisiae*.

1. Introduction

The cell wall of fungi is essential for its osmotic integrity; defines the cell shape during growth, mating, sporulation, and pseudohyphae formation; and protects the cell from various environmental stresses such as oxidative stress [1]. Currently, the study of fungal cell wall synthesis has been applied to develop antifungal agents and to increase the resistance of strains under conditions of stress [2–4]. *Saccharomyces cerevisiae* is a preferred model organism for the study of fungal cell walls because of its well-understood genetic background and complete genome annotation [5]. The cell wall of *S. cerevisiae* consists of β-1,3- and β-1,6-glucans (30–60%), a small amount of chitin (1–2%), and many different mannoproteins (35–40%) [6]. These components become cross-linked in various ways to form higher-order complexes.

As the major component, β-1,3-glucan is present as a reticulate structure that provides mechanical strength for the organism and attachment sites for mannoproteins and chitin in *S. cerevisiae* [6]. β-1,3-glucan is normally synthesized on the surface of the cytomembrane in which GTPase and β-1,3-glucan synthase (GS) work together [7]. The GS is usually encoded by *FKS1* and consists of a 215-kDa polypeptide predicted to be an integral membrane protein with 16 transmembrane helices. The disruption of *FKS1* results in a significant reduction in GS activity as well as the inhibiting of the synthesis of β-1,3-glucan to some extent [8,9]. Many scientists have carried out the independent researches related to *FKS1* mutation to gain insights into how the mechanism of the cell wall synthesis and maintenance work in *S. cerevisiae*. Based on microarray analyses, the expression of five genes encoding glycosylphosphatidylinositol (GPI)-attached proteins up-regulated in response to cell wall damage caused by disruption of *FKS1* [10]. Analyses of the genome-wide responses to five deletion strains related to cell wall defects, including the *FKS1* mutant, revealed upregulation of a “cell wall compensatory cluster” of 79 co-regulated genes whose products include a range of proteins involved in wall synthesis and remodeling [11]. Through phenotype and protein expression analyses, the chitin biosynthesis process has been characterized as a compensatory mechanism in which chitin synthase III (CSIII) activity

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showed a significant increase and high rates of chitin synthesis in the *FKS1* mutant [12]. In addition, many changes in the cell wall of the *FKS1* mutant are proved to be associated with the cell wall integrity (CWI) pathway, which is reported that would not be triggered unless obstruction of the synthesis of the cell wall happened, including cell wall perturbation resulting from drug exposure, cell wall-weakening mutations, osmotic shock, and temperature changes [10–12,13]. It is, however, difficult to connect the these identified or changed genes or proteins to one another and to analyze the relationships among them as well as how the CWI signaling pathway activated from these previous research, because of lack of adequate genes with up/down-regulated expression or with detectable changes in expression by microarray analyses.

RNA-Seq has become a powerful tool in transcriptomics for its advantages over microarray analyses, including the independence of the reference genome, reduced background noise, a broader detection range, and greater reproducibility [14]. This approach allows global changes in intracellular gene expression to be identified, enabling data-driven identification of the pathways affected by the single gene mutation [15,16]. In this study, we first used RNA-Seq technology to analyze the transcriptomic profile response in the *FKS1* mutant. Genes differentially expression in the *FKS1* mutant, along with KEGG pathway enrichment analysis were analyzed. The global transcriptomic profiling in the *FKS1* mutant and the molecular mechanisms of cell wall maintenance were further investigated.

2. Materials and methods

Microbial strains and culture conditions. Saccharomyces cerevisiae strain BY4743 (MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0) was used to generate the *FKS1* mutant. *S. cerevisiae* BY4743 genomic DNA was used to amplify the upstream and downstream sequences of the mutation region of *FKS1*. The plasmid pUG6 was used to amplify the KanMX resistance fragment. BY4743 cultures were grown in YPD medium (1% yeast extract, 2% peptone, and 2% glucose) containing 100 μg/ml of the antibiotic G418 as needed.

Construction of the mutant strain of FKS1. All primers used for strain construction were designed according to the sequence published in the NCBI genome database and are listed in Table S1 (see supporting information). The mutation region of *FKS1* (gene ID: 851055) corresponded to that which encodes the amino acids responsible for enzymatic activity (~2160bp), as described [17]. The upstream and downstream arm of the mutation area (1062 bp and 1014 bp, respectively) and the KanMX resistance fragment were amplified from BY4743 genomic DNA and pUG6 using the primers *FKS1* P1/*FKS1* P2, *FKS1* P5/*FKS1* P6, and *FKS1* P3/*FKS1* P4. These three fragments were designed to have a 34-bp overlap within their primer sequences and were integrated to become an Upstream arm–KanMX–Downstream arm cassette using *FKS1* P7/*FKS1* P8 through the fusion PCR. The cassette was transformed into BY4743 to mutate the *FKS1* activity area by the LiAc/SS carrier DNA/PEG method [18]. PCR with two pairs of primers, one of which consisted of *FKS1* P9, located upstream of the deleted fragment, and Pf, located within the deleted fragment, and the other of which consisted of *FKS1* P9 and Pk, located within the KanMX fragment, were used to verify the *FKS1* mutant.

Total RNA extraction, library preparation, and transcriptome sequencing. Total RNA was extracted from the mutant and control *S. cerevisiae* strains by the acid/phenol method [19]. RNA degradation and contamination were checked on 1% agarose gels. In addition, the $OD_{260/230}$ ratio and the RNA integrity number were used for assessing RNA quality and purity using a NanoDrop (Thermo Scientific, USA) and an Agilent 2100 Bioanalyzer system (Agilent Technologies, USA). Library preparation and transcriptome sequencing were performed as described [20]. RNA-Seq was performed by the Beijing Genomics Institute (BGI) using the BGISEQ-500RS to generate 100-bp paired-end reads.

RNA-Seq analysis. The raw data were cleaned by removing reads containing adapter sequences, reads containing poly-N sequences, and reads of low quality using SOAPnuke (version 1.5.2, parameters: -l 15 -q 0.2 -n 0.05). Available reads were aligned to the *S. cerevisiae* S288C reference genome (version R63-1-1) using Bowtie2 (version 2.2.5, parameters: -q –phred64 –sensitive –dpad 0 –gbar 99999999 –mp 1,1 –np 1 –score-min L,0,-0.1 -I 1 -X 1000 –no-mixed –no-discordant -p 1 -k 200), and the expression levels of the resulting genes and transcripts were determined using RSEM (version 2.2.5, default parameters). DEGs (differentially expressed genes) detection were performed with DEseq2 (Parameters: Fold Change ≥ 2.00 and Adjusted Pvalue ≤ 0.05) as described [21]. KOBAS software was used for the statistical enrichment of DEGs among KEGG pathways [22].

Ion exchange chromatography determination of cell wall polysaccharides. Cells were pre-incubated in 5 ml of YPD liquid medium for 24 h, and then the cells were collected and equal numbers were transferred into 50 ml of fresh YPD medium for 14 h (logarithmic phase) at 30 °C, 250 rpm. We collected ~150 OD_{600} cells to extract cell wall polysaccharides and quantified it using ion exchange chromatography as described previously with some modifications [23].

Validation of RNA-Seq data by quantitative PCR (qPCR). The RNA extracted for library preparation and transcriptome sequencing was separated and preserved independently at −20 °C and was used as a template for validation of RNA-Seq data according the previous method [3]. The primer sequences listed in Table S2 (see supporting information).

3. Results

As described in material and methods, the 'Upstream arm–KanMX–Downstream' arm cassette was transformed into *S. cerevisiae* strain BY4743 to construct *FKS1* gene mutant strain. To characterize the *FKS1* mutant at the transcriptome level, we used high-throughout sequencing of isolated RNA to generate 6.9 Gb raw reads from each of the samples, including two independent samples for the *FKS*1 mutant and control strain. The gene expression levels were normalized as FPKM (Fragments Per Kilobase Million) mapped reads to compare gene expression between different samples. According to the gene expression level of each sample, we detected the DEGs using the DEseq2 method to verify the significance of gene expression differences. Based on these criteria, 1151 genes were differentially expressed in the *FKS1* mutant strain as compared with the control. Among the DEGs, 662 genes were up-regulated and 489 genes were down-regulated. Full details are given in File S1 (see supporting information). The RNA-Seq data was verify to be reliable by quantitative PCR and the results showd in Table S3 (see supporting information).

To further analyze changes in the specific pathways in the *FKS1* mutant cells, we mapped DEGs to reference canonical pathways in the KEGG (Kyotoencyclopedia of genes and genomes) database. Full details are given in File S2 (see supporting information). The DEGs were significantly enriched among mainly 20 pathways, in which the MAPK pathway (ko04011) and seven pathways involved in carbon metabolism including starch and sucrose metabolism (ko00500), fructose and mannose metabolism (ko00051), glycolysis/gluconeogenesis (ko00010), galactose metabolism (ko00052), glyoxylate and dicarboxylate metabolism (ko00630), amino sugar and nucleotide sugar metabolism (ko00520), and pentose