RNA-seq profiling and construction of transcriptional regulatory network revealed the mechanism of *Sro9* and *Slf1* in oxidation resistance

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Table of Contents

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Abstract

Sro9 and Slf1 are homologous protein and have potential rules in mediating oxidant tolerance at the translational level. Our report focused on the functional complexity of Sro9 and Slf1 and regulatory mechanism in oxidation resistance. RNA-seq and bioinformatics methods were used to screen differential expressed genes by comparing the effect of hydrogen peroxide on wild-type strain, *sro9Δ* and *slf1Δ* mutants *S. cerevisiae* strains. We utilized *Cystoscope 3.8.2* to construct the regulatory network with an emphasis on transcription under oxidative stress based on existing references and combined our RNA-seq data to analyze the effect of *SRO9* and *SLF1* on the expression level. We confirmed that *YAP1* and *MSN4* are the central components governing the oxidative stress resistance process, but they are not the primary pathway in *SRO9*-mediated antioxidative defense. Sro9 directly bind with mRNA of *COX18* and *COX20* under oxidative stress to enhance their mRNA stabilization. Hence, Sro9 participates in aerobic respiration in response to oxidative stress. The results from GO analysis revealed that differential expressed genes in mutants were enriched in RNA transport, mitochondrion function. Multiple pathways, including cell wall integrity pathway, proteasome assembly, cellular response to osmotic stress and thermal stimulus, participated in *SRO9* and *SLF1* mediated oxidative stress response. These observations helps to gain a detailed understanding of the oxidative stress response mechanism in *S. cerevisiae* and has potential value in developing tolerant strains with higher fermentation efficiency in practical application.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright statement

Acknowledgements

First, I am most grateful to my supervisor, Prof Graham Pavitt, for his guidance and selfless assistance. During my essay writing, he consistently encouraged me, pointed out my errors in the report and provided constructive suggestions. I cannot forget zoom meetings we've made every week during the past three months. Though I have asked lots of questions and not familiar with SGD database at the beginning, he was till put up with me and answered patiently.

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Finally, I would like to say thank you to my parents. They provided both emotional support and financial support to me. It's hard to study overseas under this pandemic. Their video calls gave me a lot of motivation.

1. Introduction

Gene expression patterns in eukaryote are tightly controlled in response to diverse environmental stimuli, such as oxidative stress, hyperosmotic response, and heat stresses (Morano et al., 2012). Transcriptional alterations under external stimulus can reflect the mechanism of the cellular stress response. *Saccharomyces cerevisiae* is a preferred eukaryotic model organism and is widely used in ethanol fermentation. During industrial ethanol production, *Saccharomyces cerevisiae* encounters oxidative stress resulted from reactive oxygen species (ROS). Reactive oxygen species represent a type of reactive molecules, such as hydrogen peroxide (H_2O_2), superoxide anion radical and other by-products of aerobic respiration in mitochondria. Environmental stress, ageing and chronic diseases lead to the generation of endogenous ROS. Accumulation of ROS will cause oxidative-damage protein, resulting in dysfunction of the electron transport chain in mitochondria (Costa et al., 2007). In response to oxidative stress, *Saccharomyces cerevisiae* evolved repair systems to defend against ROS, such as various antioxidants, degradation of damaged protein by the proteasome, cell cycle control and catalase(Latella et al., 2001). However, if the dynamic balance between antioxidants and ROS is broken, oxidative stress occurs and eventually induces programmed cell death.

RNA binding proteins (RBPs) mediate gene expression by binding with RNAs to form ribonucleoprotein complexes (RNPs). RBPs are vital determinants in mRNA and non-coding RNAs biosynthesis, selective splicing, modification, transportation(Glisovic et al., 2008; Matera et al., 2007). Consequently, exploration of the mechanism of RBPs in transcription regulation, especially transcription control under external stimulus, has been at the centre of much attention. Many technologies have been applied in this field, including Sequencing of RNA (RNA-seq), RIP（RNA-binding protein immunoprecipitation), CLIP (cross-linking and immunoprecipitation) and derivative technologies such as PAR-CLIP (photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation). RNA-seq can be used for transcriptome analyses. RIP and

CLIP can be used to identify RNA targets of RNA-binding proteins. PAR-CLIP can be used for the identification of RNA-protein binding site(Hrdlickova et al., 2017).

La protein was first discovered as an autoantigen that existed in patients with autoimmune diseases. It now defined as an RNA-binding protein and was ubiquitously detected in all eukaryote cells (Bousquet-Antonelli and Deragon, 2009; Mattioli and Reichlin, 1974; Wolin and Cedervall, 2002). La associates with RNA polymerase III transcripts and binds to the poly(U) tracts of nascent tRNA, rRNA and U6 snRNA to protect nascent transcripts (Bayfield et al., 2021). It has been reported that La protein binds to newly synthesized RNA polymerase II–transcribed small RNAs as well, such as U3 snoRNA (small nucleolar RNAs), to mediate RNA processing (Kufel et al., 2000; Wolin and Cedervall, 2002). In the *S. cerevisiae*, there are only three La proteins, including Lhp1, Slf1 and Sro9 (Bayfield et al., 2021). Lhp1 is a nuclear protein, while Sro9 and Slf1 are mainly cytoplasmic proteins (Sobel and Wolin, 1999). The biological function of La is to stabilize nascent small RNAs from exonuclease digestion and is probably working as a transcription factor. La protein is pivotal in the transcription process in yeast cell (Long et al., 2001). Typical La is modular and contains several domains: the La motif (LAM) at the N-terminal and RNA recognition motifs (RRM) (Wolin and Cedervall, 2002).

The La motif (LAM) is the most conserved domain that existed in the La protein. Slf1 and its paralog Sro9 have atypical-La motif (LAM) containing RNA binding proteins, but do not have RRM. By conducting BLASTP (https://blast.ncbi.nlm.nih.gov/Blast.cgi) similarity search, Sro9 and Slf1 are about 35% homologous in amino acid level. Both belong to *LHP1* family protein and contains the only functional domain, LHP1. *SRO9* or *SLF1* deletion strains do not perform the phenomenon of synthetic lethality, but a deletion in *SRO9* manifest a slow-growth phenotype (Kagami et al., 1997; Sobel and Wolin, 1999). In previous studies (Kershaw et al., 2015), Sro9 and Slf1 were proposed to be potential trans-acting factors mediating mRNA stabilization. Recently, Sro9 and Slf1 had reported engaging in transcriptional control of *S. cerevisiae* in response to

oxidizer stimulation. Transcript altered more in *SRO9* deletion strain than *slf11Δ* strain, but the fold change of *SLF1* deletion strain under oxidative stress showed a greater change. Researchers concluded that Slf1 played a more significant role in maintaining the mRNA steady-state than Sro9. The life span of *sro9Δ* strain under oxidative stress is shorter than the wild-type strain, but longer than *slf1Δ* strain (Kershaw et al., 2015). Overproduction of Slf1 was described to suppress the *CUP14* mutation involved in copper homeostasis by stabilizing its mRNAs. Deletion of *SLF1* cause the absence of copper detoxification ability in $CuSO_4$-containing medium. Accumulation of cupric ion leads to overproduction of ROS and eventually result in cooper-induced cell apoptosis (Yu et al., 1996).

Until now, studies of the function of Sro9 and Slf1 at the transcriptional level under oxidative stress are limited. Although researchers confirmed the necessity of Sro9 and Slf1 in response to oxidative stress, the mechanism of Sro9 and Slf1 in regulating transcription of oxidation tolerance is not yet fully understood. Recently, RNA-seq has become an alternative method to RNA-microarray in transcriptomic studies. Therefore, we performed RNA-seq analysis and in combination with RNA-protein binding data to identify transcriptional changes of sro9Δ and slf1Δ mutant strains compared to wild-type strain under hydrogen peroxide treatment.

2. Materials and methods

2.1 Strains, yeast culture

The haploid S. cerevisiae strain BY4741, derived from the S288C strain, was used for the generation of *Sro9Δ* and *Slf1Δ* mutants and was used as wild-type control. Hydrogen peroxide with 0.4 mM in concentration was used to stimulate *S. cerevisiae* cells generating oxidative stress response. Wet lab work, including mutagenesis, cell culture and library construction, was processed by Dr. Martin Jennings (Martin.Jennings-3@manchester.ac.uk). We set three comparison groups: (1) The difference of transcriptome performance between wild-type *saccharomyces cerevisiae*

strains after hydrogen peroxide treatment and untreated wild-type strain; (2) The *sro9Δ* strain after hydrogen peroxide treatment and isogenic strain without H_2O_2 treatment; (3) The *slf1Δ* mutant after hydrogen peroxide treatment and isogenic strain without H_2O_2 treatment. The RNA-seq experiment followed the Illumina short-read RNA-seq workflow. All yeast cells were cultured under a tightly controlled environment to eliminate variability between batches. Using a standard workflow, each experiment was performed twice to produce two independent biological replicates in each group.

2.2 Gene expression analysis

Fastq format data processing and mapping were processed by Dr. Martin Jennings. The method *DEGseq* in the R package *DESeq2* version 1.44.0 was used to identify differential expression genes (DEGs) between each group(Wang et al., 2009). It took the raw count table as input and generated a normalized count matrix. Benjamini-Hochberg procedure, which controls the false discovery rate (FDR), was adopted for multiple testing of p-value correction(Benjamini and Hochberg, 1995). FDR of 0.05 and Log_2 (fold change) of 0.7 was set as the threshold for DEGs. Principal component analysis (PCA) plot was made by *plotPCA* in *DESeq2* R package to evaluate the differential expression between biological duplicates in RNA-seq data. Boxplot was generated in GraphPad Prism version 9.0.0 for windows (www.graphpad.com).

2.3 GO and KEGG enrichment analysis

Gene ontology enrichment analysis (GO analysis) was implemented in the online tool *PANTHER* version 14 (http://www.geneontology.org/) (Ashburner et al., 2000; Mi et al., 2019). Up-regulated and down-regulated differential expressed genes were analyzed separately. We set *S. cerevisiae* as the species parameter. The annotation data set was set to *PANTHER GO-slim*. Test type was set to Fisher's Exact test and used FDR calculation for correction. GO terms with Adjusted p-value (FDR) < 0.05 were regarded as significant enrichment. R version 4.0.4 was used for the generation of heatmap by the package of *pheatmap* version: 1.0.12 *(https://github.com/raivokolde/pheatmap)*.

Bioconductor annotation packages org.Sc.sgd.db (Carlson, 2019) was downloaded within R for transformation from systematic name to Entrez ID (accessed March, 2021). *KOBAS 3.0* (accessed March, 2021) *(http://kobas.cbi.pku.edu.cn/)* was implemented for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (Kanehisa et al., 2009). Benjamini–Hochberg procedure was used for multiple correlations to control false positive result. KEGG pathways with adjusted p-value (FDR) <0.05 were set as the threshold for significantly enriched pathways.

2.4 Transcription network construction

SGD, YEASTRACT and STRING database was used. The "rank by TF" tool from Yeast Search for Transcriptional Regulators And Consensus Tracking database (YEASTRACT; http://www.yeastract.com/) (accessed March, 2021) was applied to search for transcription factors regulating DEGs. We uploaded up-regulated DEGs and down-regulated DEGs to the website separately. The "Check for all TFs" option was selected, and others parameters were set to the default value. Transcription factors regulating more than 30% of up-regulated or down-regulated DEGs were chosen as nodes in the transcription regulatory network.

The "Search for Associations" tool (Monteiro et al., 2019) from YEASTRACT was applied to identified regulatory associations, including direct association (DNA-protein interaction demonstrated by Chip-sequencing) and indirect association, showed by RNA-seq or microarray, between transcription factors and corresponding genes. The oxidative stress subgroup was set as the environmental condition.

The "Multiple Proteins by Names / Identifiers" tool from STRING 11.0 (https://string-db.org/) was used for protein-protein interaction data collection. The organism parameter was set as *saccharomyces cerevisiae*. We excluded the text-mining option and only chose protein-protein interaction demonstrated by experiment result as interaction sources. Combined the above data and mRNA-protein interaction data gain from RNA-Seq and PAR-CLIP, the regulatory network was generated by Cytoscape version 3.8.2 (Shannon et al., 2003).

3. Results

3.1 Overview of the transcriptome data

From RNA-Seq data, we compared transcriptomic profiles in the wild-type strain and two mutants under the effect of hydrogen peroxide to transcriptomic profiles in corresponding isogenic strains without H_2O_2 treatment. As shown in Fig.1A, PCA plot visually displayed the differences between two different biological replicates. It was performed with a count table as the input. The figure showed that samples clustered together in specific manner, with only minor variations between each independent biological replicates. PC1 (76% variance) separated stressed cells from non-stressed cells and confirmed that the majority of responses were intact in the mutants.

More than 1000 genes were differentially expressed in response to H_2O_2 in all strains, but the number of significantly changed genes was various in different experimental groups. In wild-type strains, there were about the same number of differentially expressed genes up-regulated (1088 genes) and down-regulated (1039 genes) after H_2O_2 treatment. The number of differentially expressed genes under H_2O_2 treatment in mutants were less than the wild-type strain. After H_2O_2 treatment, only 547 and 358 genes were significantly up-regulated, and 348 and 132 genes were down-regulated in *sro9Δ* and *slf1Δ* mutants, respectively. In Fig.1B, only DEGs with FDR less than 0.05 were taken into account. The log2FC showed a similar distribution in wild-type strain and

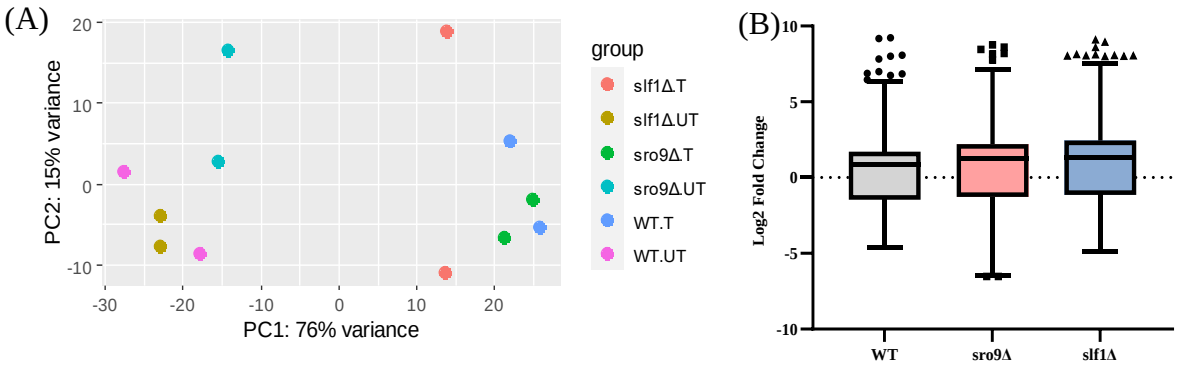

Fig. 1. Response of DEGs in each comparison samples to oxidative stress.
(A) The PCA result.
(B) Box plot of RNA-Seq result
WT represented comparison between the expression level of DEGs in wild-type under oxidative stress and the control strain without H_2O_2 treatment. *SRO9Δ* stood for *SRO9* deletion mutant treated with H_2O_2 (compared with SRO9Δ mutant untreated). *SLF1Δ* represented *SLF1* deletion mutant treated with H_2O_2 (compared with untreated SRO9Δ mutant). Expression level was shown as Log2 fold enrichment. DEGs were filtered (FDR<0.05). Tukey's multiple comparison test was adopted to plot whiskers and outliers.

mutants. The *slf1Δ* mutant had the largest median of fold enrichment value in all three strains, but no median in all sample was large than 1.5. This indicated that, overall, more DEGs in *slf1Δ* mutant changed its expression level than sro9Δ, but less than wild-type strain.

In RIP-Seq and PAR-CLIP experiments, researchers identified 1671 mRNA targets of Sro9 before H_2O_2 treatment and 1905 mRNA targets of Sro9 with H_2O_2 treatment (corrected FDR < 0.05). Slf1 had 488 mRNA targets before H_2O_2 treatment and 1052 mRNA targets with H_2O_2 treatment. There were some overlaps in Sro9 and Slf1 targets. With hydrogen peroxide treatment, 589 RNA were targets of both Sro9 and Slf1, 1316 RNA were Sro9 targets only, and 463 were Slf1 targets only. The number of Slf1 targets increased under oxidative stress and varied more obviously than that of Sro9 targets. This may produce evidence supporting the deduction that Slf1 contributes more to the stabilization of mRNA than Sro9 in response to oxidative stress.

3.2 GO enrichment analysis

Many genes related to oxidative tolerance were altered at the transcriptional level under H_2O_2 exposure in all of the tested strains. Differentially regulated genes (DEGs) included *SRX1, HSP30, OYE3* significantly up-regulated in all three strains and were reported to facilitate oxidative stress resistance. *SRX1* is encoding for sulfiredoxin. It forms a complex with thioredoxin peroxidase, Tsa1, and reduces cysteine-sulphenic acid form of Tsa1 generated under oxidative stress with the presence of ATP to Cys–SH form. This process is vital in peroxidase function (Biteau et al., 2003). From the result of PAR-CLIP and RIP-Seq experiment, it has been attested that *SRX1* is the target of Sro9 and Slf1. Although under oxidative stress, the fold change of its expression level was up-regulated in both wild-type and mutants, wild-type strain changed slightly greater than mutants. This suggested that Sro9 and Slf1 may increase the abundance of Srx1 in response to oxidative stress. Therefore, we speculated that Sro9 and Slf1 directly mediating copper metabolism. *HSP30* is a heat shock protein. It involves in multiple stress response processes, including ethanol treatment, thermal stimulus, organic acid treatments, and response to osmotic stress. *HSP30* located

in *Saccharomyces cerevisiae* plasma membrane and negatively regulates ATPase activity(Piper et al., 1997). Oye3 is NADPH dehydrogenase involves in antioxidative defense and arrests hydrogen peroxide-induced programmed cell death. The null mutant of Oye3 resulted in decreased resistance to chemicals; however, under hydrogen peroxide treatment, overproduction of Oye3 lead to cell apoptosis (Odat et al., 2007). Oye3 was the mRNA target of Sro9, but its expression level in the wild-type strain was not distinct from two mutant strains. Accordingly, we could speculate that Sro9 inhibit the expression of Oye3 under oxidative stress and prevent Oye3-induced cell apoptosis.

DEGs were categorized into two groups by their expression level, including up-regulated DEGs(log2 fold changes>0) and down-regulated DEGs(log2 fold changes<0). Gene ontology enrichment analysis (GO analysis) was conducted, and DEGs were independently assigned to

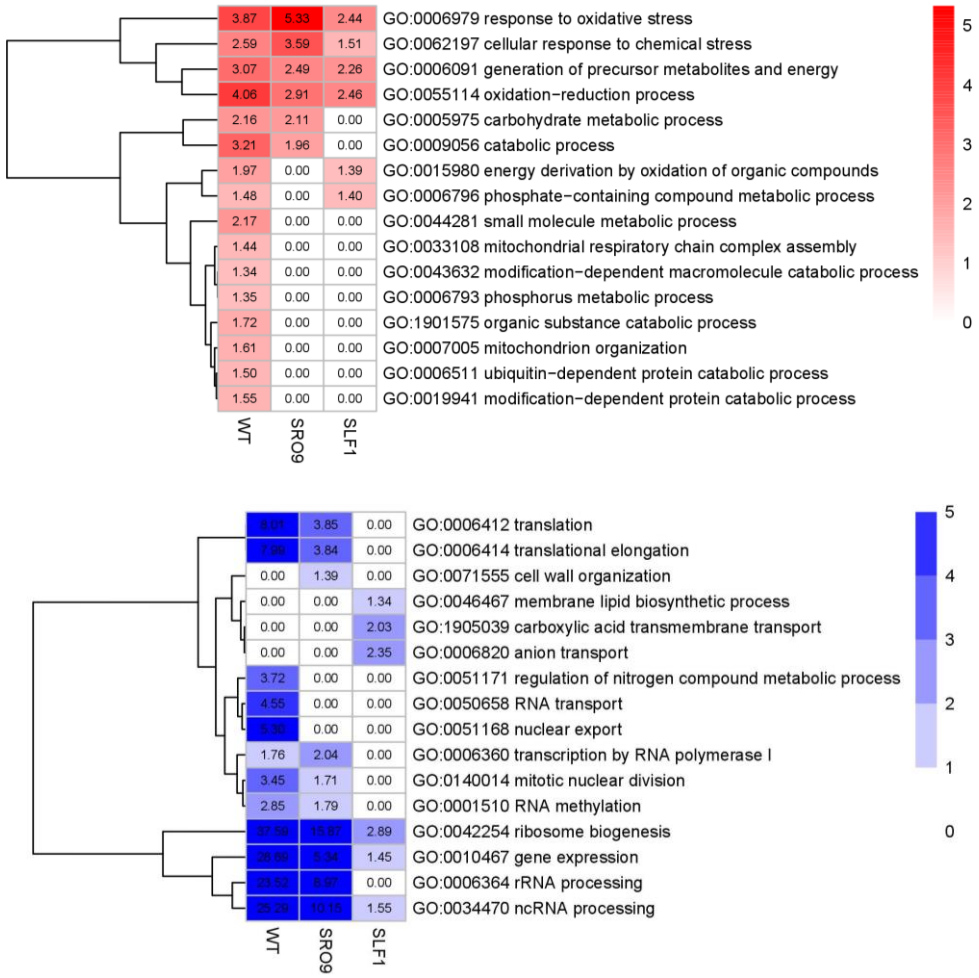

Fig. 2. Enriched GO terms in biological processes (BP) ontology aspect
Confidence of each GO term was shown as the $\log_{10}$ (Bonferroni adjusted p value). Sample groups were indicated in the bottom. GO term enrichment was allocated with different colour. Red indicates GO terms enriched in up-regulated DEGs, while blue indicates GO terms enriched in down-regulated DEGs.

different GO terms associated with biological process (BP), molecular function (MF) by their biological properties. K-means method was used for clustering the transcriptional level of DEGs under different experimental conditions. Clustering was applied to detect the potential relationship between each GO terms based on the log10(FDR). *Sro9Δ* and *slf1Δ* mutants showed a similar expression pattern, but they were distinct from the wild-type strain.

In the biological process aspect, GO terms enriched in up-regulated DEGs was shown in Fig.2. Up-regulated DEGs were significantly enriched in all three strains in two subcategories, including response to oxidative stress (GO:0005979) and metabolic process. GO terms including mitochondrial respiratory chain complex assembly (GO:0033108), small molecule metabolic process (GO: 0044281) and ubiquitin-dependent protein catabolic process (GO:0006511) were significantly up-regulated in wild-type. However, those GO terms were not enriched in mutants. Functional analysis in a previous study revealed that mRNA targets of Slf1 and Sro9 were significantly enriched in mitochondrial functions(Kershaw et al., 2015). Therefore, it is not surprising that mitochondrion organization (GO: 0007005) were not enriched in *sro9Δ* and *slf1Δ* strains. Genes involved in mitochondrion organization, including *PET100, ATG7, FIS1, COX18, ATG9, CBP6, FUN14, QCR2, COR1, MIX17, COA1, ATG33, COX20, OM14* and *MIC26,* exclusively up-regulated in wild-type strain, but not in mutants. From PAR-CLIP and Rip-Seq data, we confirmed that *PET100* and *COR1* are Sro9 target. From *Saccharomyces* Genome Database (SGD; https://www.yeastgenome.org/), we can find that *PET100* and *COR1* deletion phenotype showed alleviated resistance to oxidative stress. *COR1* is responsible for coding a 44-kDa core protein of reductase in cytochrome C maturation. The mitochondrial cytochrome c pathway response to both external and mitochondrial-induced oxidative stress. The pathway is highly related to programmed yeast cell apoptosis as well (Tzagoloff et al., 1986). *FIS1, COX18, COA1* and *COX20* are mRNA target of both Sro9 and Slf1, and their null mutants also decrease resistance to oxidant. As Cytochrome c oxidase, Cox18 is essential in mitochondrial biogenesis and construction of mitochondrial reparatory. Cox18Δ mutant strain reduced the steady-state level of Cox2, which

is one of the largest subunits in the catalytic core of cytochrome oxidase(Fiumera et al., 2009). Cox20 is a novel chaperone involves in the assembly and maturation of cytochrome c oxidase(Hell et al., 2000). The function of Cox20 needs further exploration. We can conclude that Sro9 and Slf1 mediate oxidation resistance through regulating mitochondrial pathways.

Compared to 37 genes enriched control strain, fewer up-regulated DEGs were enriched in the oxidation-reduction process in mutant strains (24 DEGs in *Sro9Δ* and 23 DEGs in *Slf1Δ*). The majority of DEGs enriched in the oxidation-reduction process was the same in three strains. *QCR7* encodes for the subunit of cytochrome-c reductase (Complex III) in the mitochondrial respiratory chain(De Haan et al., 1984). Evidence showed that the absence of *QCR7* increases resistance to apoptosis induced by cupric ion. The mRNA of *QCR7* was the target of both Sro9 and Slf1, but was uniquely up-regulated in slf1Δ mutant. The PAR-CLIP experiment had identified the binding site of Slf1 in QCR7 mRNA. It indicated that Slf1 mediate electron transfer in the mitochondrial electron transport chain by binding to *QCR7*.

Down-regulated DEGs were significantly enriched in ncRNA processing (GO: 0034470), gene expression (GO:0010467), ribosome biogenesis (GO: 0042254). Compared with the wild-type strain, RNA transport (GO:0050658) and regulation of nitrogen compound metabolic process(GO: 00051171) were not enriched in mutants. There were 35 down-regulated DEGs in wild-type strain enriched in the mitotic nuclear division pathway, while 14 of those DEGs also occurred in down-regulated DEGs dataset in *sro9Δ* mutants. Two genes, *MCM2* and *BUB1*, were down-regulated in *sro9Δ* strain, but not in *slf1Δ* or wild-type strain. Interestingly, *BUB1* was the mRNA target of Sro9, while *MCM2* was not. Depressed transcriptional level of *BUB1* is likely to decrease the proliferative activity of yeast under oxidative stress by spindle checkpoint control. This indicated that *BUB1* may directly be regulated by Sro9. Although *BUB1* is a non-essential gene, deletion of *BUB1* strain was confirmed to arrested cell cycle progression (Goto et al., 2011). Regarding *MCM2*, it is an essential gene encoding for Mcm2 protein which is the main component in the helicase complex

and contributes to DNA replication (Giaever et al., 2002). The expression level of *MCM2* is likely to be indirectly down-regulated by Sro9.

GO terms related to mitochondrial function (Fig.2), including ribosome biogenesis (GO: 0006364) and rRNA processing (GO: 0006364), were more significantly enriched in down-regulated DEGs in wild-type and *sro9Δ* strain, but was less significantly enriched in *slf1Δ* mutants. There were 127, 51 and 23 down-regulated DEGs enriched in ribosome biogenesis in wild-type strain, *sro9Δ* strain and *slf1Δ* strain, respectively. In previous studies, without hydrogen peroxide treatment, 99 genes

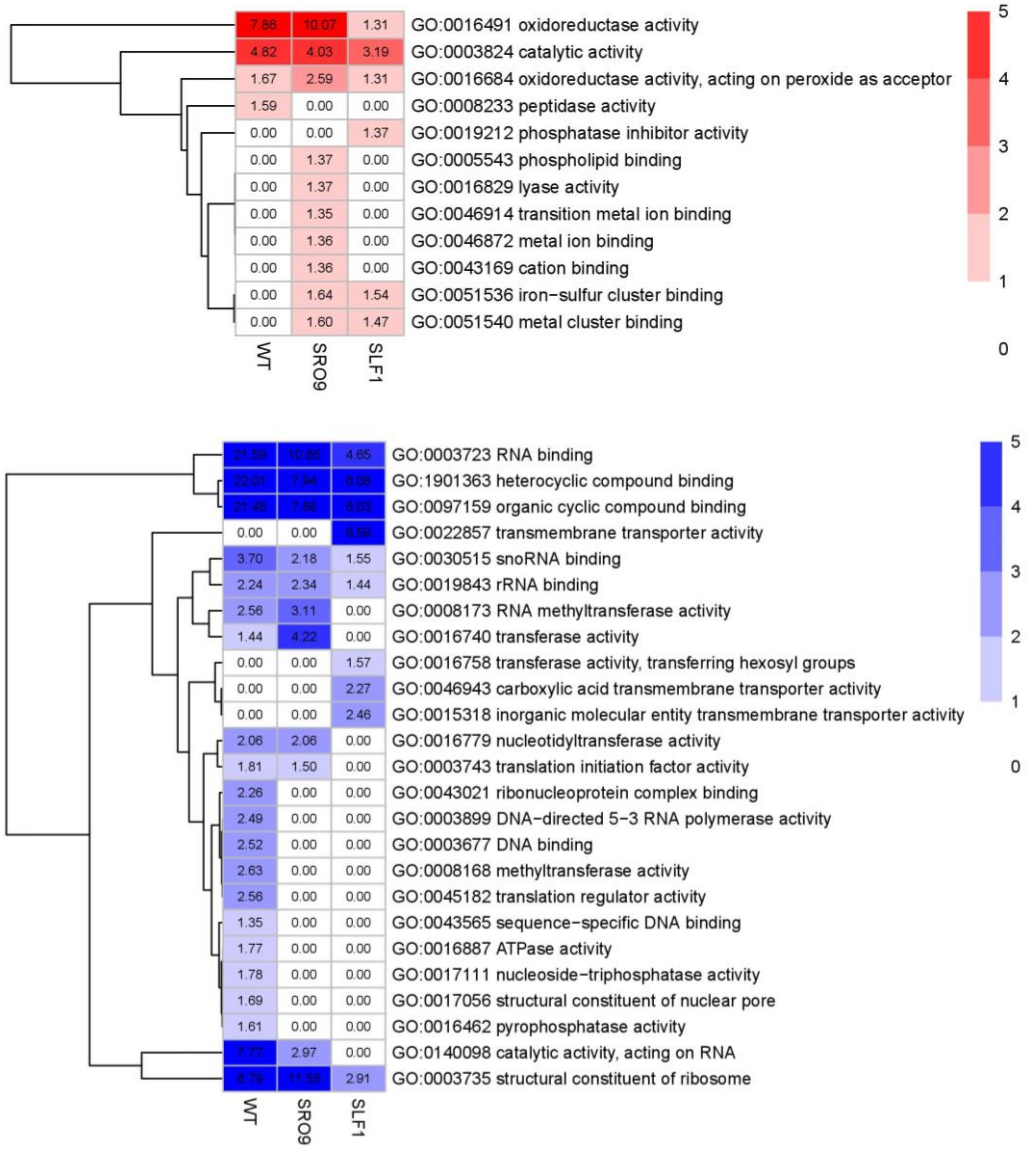

Fig. 3. Enriched GO terms in Molecular Function (MF) ontology aspect
DEGs were clustered based on the $\log_{10}$ (adjusted p value). Sample groups were indicated in the bottom. GO terms was allocated with different colour. Enrichment GO terms identified in up-regulated DEGs were showed in red, while down-regulated DEGs were shown in blue. (corrected P -value <0.05)

with a down-regulated relative transcript abundance in *SLF1* deletion strain than wild-type parental strain (BY4741) were also enriched in ribosome biogenesis (Kershaw et al., 2015). This indicates that the function of Slf1 is related to ribosome assembly. Unexpectedly, the transcription level of *RPS16A*, *RPS21B* and *RPS14B*, which encode for protein component in ribosomal subunit, was only down-regulated in *sro9Δ* strain. However, they were mRNA target of both Sro9 and Slf1. *RPS14A*, the paralog of *RPS14B*, was only down-regulated in *slf1Δ* strain, but is the target of both Sro9 and Slf1.

Within the category of molecular function (Fig.3.), up-regulated DEGs in two mutants significantly enriched in iron-sulfur cluster binding (GO:0051536) and metal cluster binding (GO: 0051540), while was not in the wild-type strain. This finding can be verified by previous research, which demonstrated that oxidative stress and the copper and iron homeostasis shared a portion of regulating genes. Copper is defined as a redox active metal and is able to induced toxic effect by oxidative damage (Schenk et al., 2012). *Slf1Δ* strain also characterized by increased sensitivity to cadmium stimuli(Kershaw et al., 2015). As expected, our dataset identified oxidoreductase activity as one of the top enriched categories affected by H_2O_2 treatment. Genes related to this category were highly expressed at the transcriptional level after H_2O_2 treatment. By analyzing down-regulated DEGs, ribonucleoprotein complex binding (GO:0043021) and translation regulator activity (GO: 0045182) were significantly enriched in wild-type but not in mutants. This verified from another side that SRO9 and SLF1 mediate various pathways in transcription level.

3.3 KEGG analysis

In the wild-type strain, up-regulated DEGs under oxidative stress involved in 16 significantly enriched KEGG pathways (corrected P -value <0.05), including biosynthesis of secondary metabolites (sce01110), proteasome(sce03050), carbon metabolism(sce01200), starch and sucrose metabolism(sce00500) (Fig.4). 15 and 7 of these pathways were significantly enriched in *sro9Δ* mutants and *slf1Δ* mutant, respectively. DEGs enriched in glyoxylate and dicarboxylate metabolism

(sce00630), tyrosine metabolism (sce00350), butanoate metabolism (sce00650) and alanine, aspartate and glutamate metabolism(sce00250) indicated the variation of gene expression patterns in mutant strains under oxidative stress, because these pathways were only enriched in *sro9Δ* and *slf1Δ* mutants. This indicated that deletion of *SRO9* or *SLF1* increased the sensitivity of *S. cerevisiae* response to oxidative stress via various pathways, especially metabolic pathways. In proteasome pathway (sce03050), 16 up-regulated DEGs in the wild-type strain and 8 up-regulated

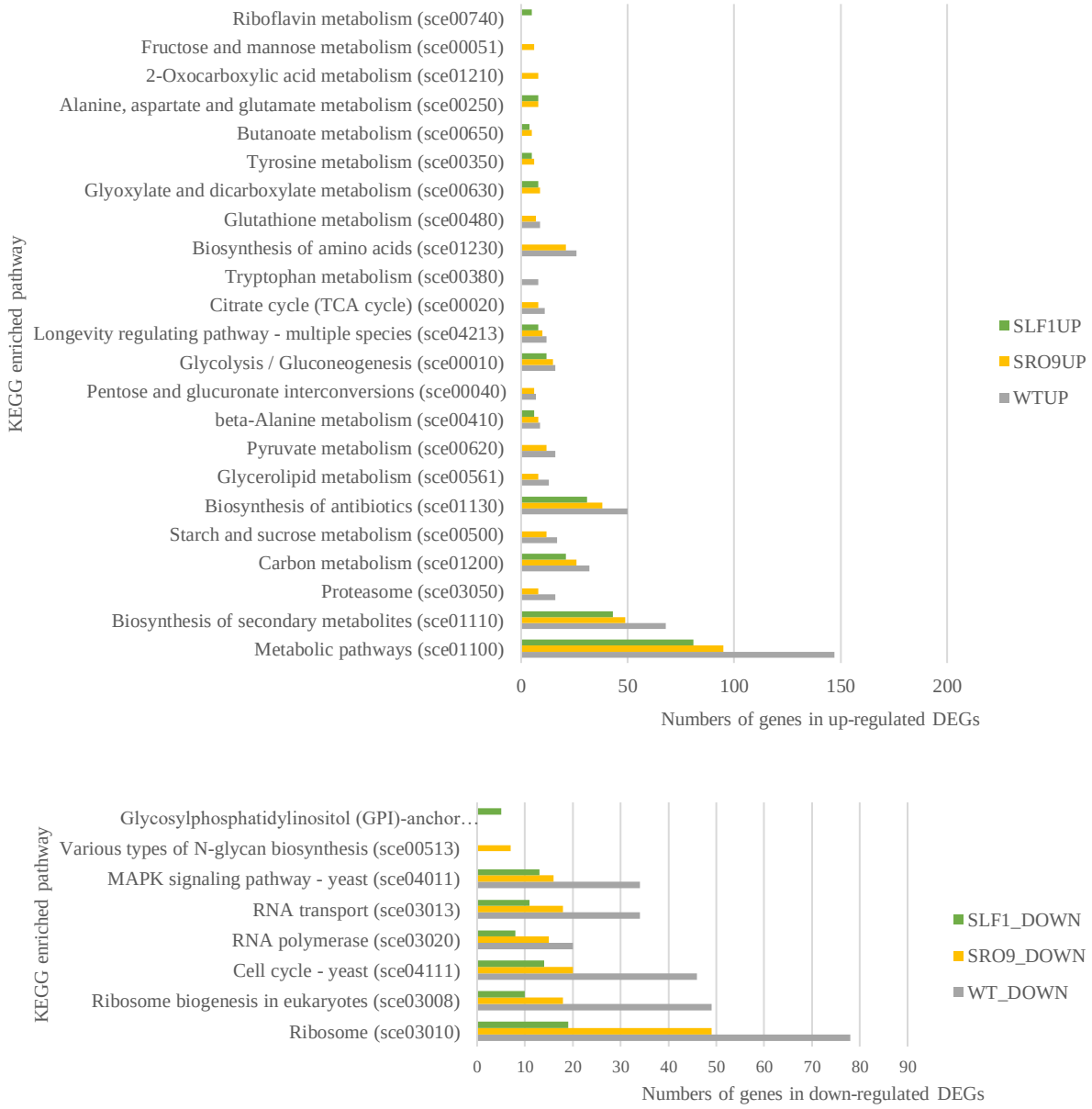

Fig. 4. Enriched KEGG pathway in up-regulated DEGs and down-regulated DEGs
Numbers of DEGs enriched in specific KEGG pathways were shown in the figure. Hypergeometric test was used as statistical test method (corrected P -value <0.05). FDR correction method used Benjamini and Hochberg procedure for multiple testing.

DEGs in *sro9Δ* mutant were enriched, while *slf1Δ* was not enriched in the pathway. The main property of proteasomes is degradation of misfolded protein by the ubiquitin-proteasome system (Hiller et al., 1996). Therefore, the lack of Slf1 may repress the degradation of incorrectly folded proteins under oxidative stress and reduce the tolerance of yeast cells to external stimuli. In *sro9Δ* strain, three up-regulated DEGs, including *RPN3*, *RPN8* and *SCL1*, were essential genes involved in ubiquitin-dependent protein metabolism, but were not up-regulated in other cell types. PAR-CLIP experiment demonstrated that they were mRNA target of Sro9.

In down-regulated DEGs, many pathways, including ribosome, ribosome biogenesis in eukaryotes, cell cycle, RNA polymerase, RNA transport and MAPK signaling pathway (mitogen-activated protein kinases) were enriched. However, genes involved in the MAPK pathway were slightly different: *HKR1* only existed in *sro9Δ* mutant, while *MSG5* specifically existed in *slf1Δ* mutant. Interestingly, both *HKR1* and *MSG5* are mRNA target of Sro9, but none of them was the mRNA target of Slf1.

3.4 Regulatory network analysis at the transcriptional level

Gene expression of yeast under oxidative stress relied on the concerted interplay between transcription factors (TFs). We first mined existing data using the YEASTACT database to identify transcription factors regulating more than 30% of our DEGs in our dataset (Table 1 and Table 2). We identified 37 and 28 TFs that are essential in regulating up-regulated DEGs and down-regulated DEGs. Then, we investigated regulatory relationships showed by experiments between each transcription factors at the transcriptional level. We made an overall regulatory network in *S cerevisiae* under oxidative stress in combination with protein-protein interaction data gain from the STRING database. The network contained 36 nodes (transcription factors) with 2.875 as the average number of neighbours and 71 edges (Fig.5). Transcription factors, which did not interact with others, were removed from the network. The transcription factor Yap1 unveiled to be the node with the highest betweenness centrality in our network and Msn2 is the second one. Yap1 is the

major transcription factor activated in response to oxidative stress. Heat shock transcription factor Hsf1 and stress-responsive transcription activator Msn2/4 are mRNA target of Sro9. The expression level of *HSF1*, as well as *MSN2* and its homolog *MSN4*, altered under oxidative stress and sublethal temperatures (Morano et al., 2012). This network also involved *RPN4*, which is a transcription factor involved in the ubiquitin-proteasome system (Karpov et al., 2008), and general stress-response transcription factor Msn2 (Vlahakis et al., 2017). We confirmed that the transcription factors including Yap1, Msn4, Msn2, Gln3 found to be essential for H_2O_2-induced stress response in yeasts, but transcription factors such as Pdr1, Pdr3 has fewer interactions with other nodes.

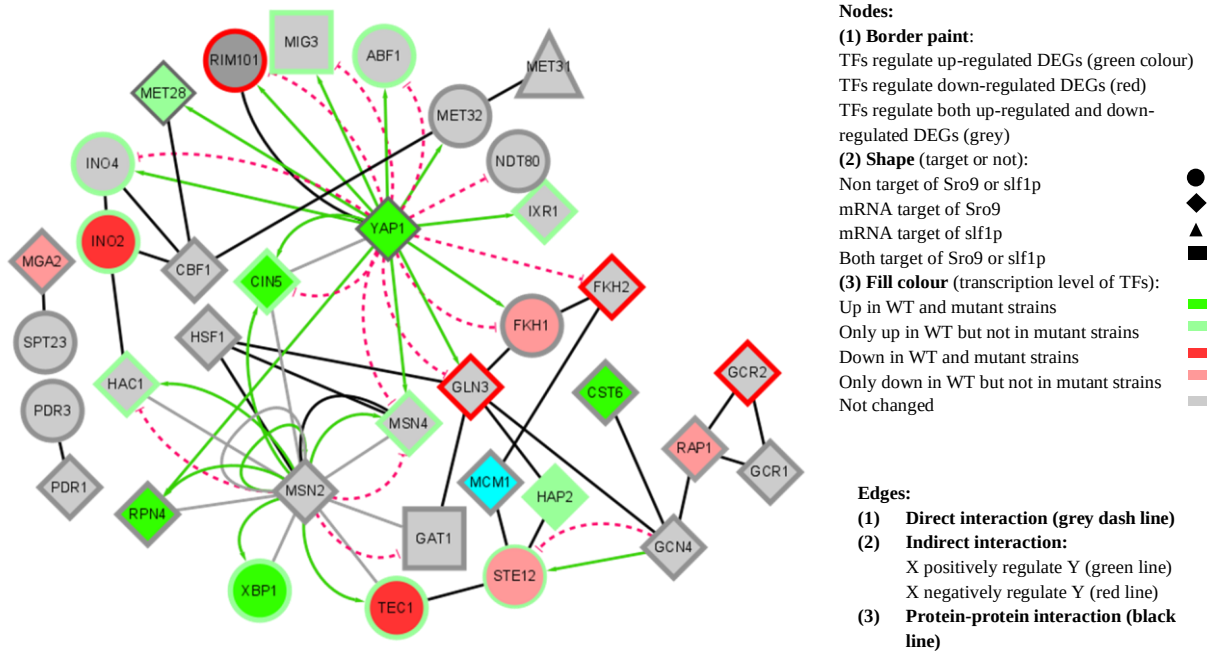

Fig.5. Regulatory network under oxidative stress

Edges (node X → node Y) shown in lines indicates that TFs(X) regulates the gene encoding another TFs(Y). Directed interaction (DNA-protein binding experiment evidence) was showed by Chip-seq or Chip-on-chip method. Indirect interactions between each TFs was showed by RNA-Seq or microarray experiments. Solid black lines illustrated protein-protein interactions from STRING database. Some transcription factors, including Sok2p, Bas1p, Xbp1p,Tup1p and Hap2p are not showed in the figure, because they do not have experimentally proved connection with other transcription factors.

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Systematic name	Transcription factors			FC			MJ Par-CLIP				CK RIP		
	WT	SRO9:	SLF1	WT	Sro9	Slf1	Sro9UT	Sro9T	Slf1ut	Slf1t	RIP SRO9	RIP SLF1	RIP SLF1 T
YIR017C	Met28p	Met28p	Met28p	**2.6207**	1.6226	1.3899	YES	NO	NO	NO	YES	NO	NO
YJR060W	Cbf1p	Cbf1p	Cbf1p	0.3317	0.2931	0.2101	YES	YES	NO	NO	NO	NO	NO
YPL038W	Met31p	Met31p	Met31p	-0.0001	0.3929	0.0198	NO	NO	NO	NO	NO	YES	YES
YGL013C	Pdr1p	Pdr1p	Pdr1p	-0.0339	0.0048	-0.0015	NO	YES	NO	NO	NO	NO	NO
YDL020C	Rpn4p	Rpn4p	Rpn4p	**1.7913**	**1.7443**	**1.7607**	NO	YES	NO	NO	NO	NO	NO
YEL009C	Gcn4p	Gcn4p	Gcn4p	-0.2363	0.8728	0.9922	YES	YES	NO	NO	NO	NO	NO
YMR016C	Sok2p	Sok2p	Sok2p	-0.2272	0.6703	0.8048	NO	YES	NO	NO	YES	NO	NO
YML007W	Yap1p	Yap1p	Yap1p	**1.4099**	**1.6499**	**1.4983**	YES	YES	NO	NO	YES	NO	NO
YIR033W	Mga2p	Mga2p	Mga2p	**-1.0908**	-0.5595	-0.4009	NO	YES	NO	NO	YES	NO	NO
YBL005W	Pdr3p	Pdr3p	Pdr3p	-0.5338	0.0573	-0.2814	NO	NO	NO	NO	NO	NO	NO
YKL062W	Msn4p	Msn4p	Msn4p	0.0144	0.2794	0.0918	YES	YES	NO	NO	NO	NO	NO
YIL036W	Cst6p	Cst6p	Cst6p	**0.8375**	**1.2981**	**1.2181**	YES	YES	NO	NO	NO	NO	NO
YKR099W	Bas1p	Bas1p	Bas1p	**-2.8751**	-1.2757	-0.8561	NO	NO	NO	NO	YES	NO	NO
YFL021W	Gat1p	Gat1p	Gat1p	0.3459	-0.4628	-0.4970	NO	NO	NO	NO	YES	NO	YES
YPR104C	Fhl1p	Fhl1p	Fhl1p	**-2.0073**	-1.2689	-1.1450	NO	NO	NO	NO	NO	NO	NO
YFL031W	Hac1p	Hac1p	Hac1p	0.6416	0.5024	0.5960	YES	YES	NO	NO	NO	NO	NO
YGL073W	Hsf1p	Hsf1p	Hsf1p	0.2201	0.0213	0.1597	NO	YES	NO	NO	YES	NO	NO
YIL101C	Xbp1p	Xbp1p	Xbp1p	**3.8293**	**2.2869**	**3.6065**	NO	NO	NO	NO	YES	NO	NO
YMR043W	Mcm1p	Mcm1p	Mcm1p	0.1521	**1.4068**	**1.2033**	YES	YES	NO	NO	NO	NO	NO
YER028C	Mig3p	Mig3p	Mig3p	-0.3366	0.3417	-0.6501	NO	NO	NO	NO	YES	NO	YES
YLR403W	Sfp1p	Sfp1p	Sfp1p	-0.5692	-0.3450	0.1440	YES	NO	NO	NO	YES	NO	NO
YOR162C	Yrr1p	Yrr1p	Yrr1p	**1.8322**	**1.9776**	**1.4514**	NO	NO	NO	NO	NO	NO	NO
YMR037C	Msn2p	Msn2p	Msn2p	0.3397	0.9422	0.9554	NO	YES	NO	NO	NO	NO	NO
YKL032C	Ixr1p	Ixr1p		0.2166	0.3001	0.0422	NO	YES	NO	NO	NO	NO	NO
YDR253C	Met32p	Met32p		-0.2021	1.4513	-2.0135	NO	NO	NO	NO	NO	NO	NO
YNL199C		Gcr2p		-0.6222	0.1785	0.6463	NO	NO	NO	NO	YES	NO	NO
YPL075W		Gcr1p		-0.2701	0.6238	0.4587	NO	YES	NO	NO	YES	NO	NO
YBR083W	Tec1p			**-2.1004**	**-1.8507**	**-2.1572**	NO	NO	NO	NO	NO	NO	NO
YNL216W	Rap1p			**-0.8283**	-0.4632	-0.2799	YES	YES	NO	NO	NO	NO	NO
YOR028C	Cin5p			**4.5538**	**5.0500**	**5.4934**	NO	YES	NO	NO	NO	NO	NO
YHR084W	Ste12p			**-2.3521**	-1.0245	-1.0971	NO	NO	NO	NO	NO	NO	NO
YIL131C	Fkh1p			**-1.5177**	**-0.9866**	-0.1532	NO	NO	NO	NO	NO	NO	NO
YKL020C	Spt23p			0.5931	0.7034	0.3588	YES	NO	NO	NO	NO	NO	NO
YHR124W	Ndt80p			0.1602	-0.0055	0.5830	NO	NO	NO	NO	NO	NO	NO
YOL108C	Ino4p			0.4558	0.4795	0.2676	NO	NO	NO	NO	NO	NO	NO
YDR123C	Ino2p			**-2.8168**	**-2.4308**	**-2.1036**	NO	NO	NO	NO	NO	NO	NO
YKL112W	Abf1p			-0.5681	-0.4003	0.1679	NO	NO	NO	NO	NO	NO	NO

Table1. Transcription factors regulating up-regulated DEGs

The table summarized transcription factors regulating up-regulated DEGs in each experimental group. “FC” represents the logarithm (to basis 2) of the fold change gained from DESeq2 result. Red indicates that the expression level of transcription factors was down-regulated, while green indicates up-regulated expression. Two experimental methods were used to identify Sro9 and Slf1p RNA-targets in S. cerevisiae “MJ Par-CLIP” column shows PAR-CLIP data (unpublished results) collected by Dr. Martin Jennings. “CK RIP” shows RNA immunoprecipitation data collected by Christopher J. Kershaw(Kershaw et al., 2015). In RIP-Seq experiment, TAP-tagged Slf1p and tagged Sro9 were used to bind with their target mRNAs. “Sro9UT” indicates the experimental group was not treated with hydrogen peroxide, while the sample from “Sro9T” was the yeast cells treated with hydrogen peroxide.

Systematic name	Transcription factors			FC			MJ Par-CLIP				CK RIP		
	WT: TFs	SRO9: TFs	SLF1: TFs	WT	Sro9 Δ	Slf1Δ	Sro9UT	Sro9T	Slf1ut	Slf1t	RIP SRO9	RIP SLF1	RIP SLF1 T
YML007W	Yap1p	Yap1p	Yap1p	**1.4099**	**1.6499**	**1.4983**	YES	YES	NO	NO	YES	NO	NO
YEL009C	Gcn4p	Gcn4p	Gcn4p	-0.0734	-0.9837	0.0000	YES	YES	NO	NO	NO	NO	NO
YBL005W	Pdr3p	Pdr3p	Pdr3p	-0.5338	0.0573	-0.2814	NO	NO	NO	NO	NO	NO	NO
YDL020C	Rpn4p	Rpn4p	Rpn4p	**1.7913**	**1.7443**	**1.7607**	NO	YES	NO	NO	NO	NO	NO
YJR060W	Cbf1p	Cbf1p	Cbf1p	0.3317	0.2931	0.2101	YES	YES	NO	NO	NO	NO	NO
YIR017C	Met28p	Met28p	Met28p	**2.6207**	1.6226	1.3899	YES	NO	NO	NO	YES	NO	NO
YLR403W	Sfp1p	Sfp1p	Sfp1p	-0.5692	-0.3450	0.1440	YES	NO	NO	NO	YES	NO	NO
YFL021W	Gat1p	Gat1p	Gat1p	0.3459	-0.4628	-0.4970	NO	NO	NO	NO	YES	NO	YES
YIL036W	Cst6p	Cst6p	Cst6p	**0.8375**	**1.2981**	**1.2181**	YES	YES	NO	NO	NO	NO	NO
YPR104C	Fhl1p	Fhl1p	Fhl1p	**-2.0073**	-1.2689	-1.1450	NO	NO	NO	NO	NO	NO	NO
YGL073W	Hsf1p	Hsf1p	Hsf1p	0.2201	0.0213	0.1597	NO	YES	NO	NO	YES	NO	NO
YCR084C	Tup1p	Tup1p	Tup1p	-0.3738	-0.5391	-0.1859	YES	YES	NO	NO	YES	NO	NO
YGL237C	Hap2p	Hap2p	Hap2p	**1.2273**	1.0564	1.1461	NO	YES	NO	NO	NO	NO	NO
YGL013C	Pdr1p	Pdr1p		-0.0339	0.0048	-0.0015	NO	YES	NO	NO	NO	NO	NO
YIR033W		Mga2p	Mga2p	**-1.0908**	-0.5595	-0.4009	NO	YES	NO	NO	YES	NO	NO
YPL075W		Gcr1p	Gcr1p	-0.2701	0.6238	0.4587	NO	YES	NO	NO	YES	NO	NO
YMR043W	Mcm1p		Mcm1p	0.1521	**1.4068**	**1.2033**	YES	YES	NO	NO	NO	NO	NO
YER040W		Gln3p		0.0598	0.1593	0.2569	YES	YES	NO	NO	NO	NO	NO
YPL038W	Met31p			-0.0001	0.3929	0.0198	NO	NO	NO	NO	NO	YES	YES
YDR253C	Met32p			-0.2021	1.4513	-2.0135	NO	NO	NO	NO	NO	NO	NO
YOR162C	Yrr1p			**1.8322**	**1.9776**	**1.4514**	NO	NO	NO	NO	NO	NO	NO
YHL027W	Rim101p			0.4050	0.1214	0.6926	NO	NO	NO	NO	NO	NO	NO
YKL020C	Spt23p			0.5931	0.7034	0.3588	YES	NO	NO	NO	NO	NO	NO
YMR037C	Msn2p			0.3397	0.9422	0.9554	NO	YES	NO	NO	NO	NO	NO
YNL216W	Rap1p			**-0.8283**	-0.4632	-0.2799	YES	YES	NO	NO	NO	NO	NO
YIL131C	Fkh1p			**-0.5177**	**-0.9866**	-0.153	NO	NO	NO	NO	NO	NO	NO
YNL068C	Fkh2p			-0.7978	-0.1869	-0.1800	YES	YES	NO	NO	YES	NO	NO
YHR124W	Ndt80p			0.1602	-0.0055	0.5830	NO	NO	NO	NO	NO	NO	NO

Table2. Transcription factors regulating down-regulated DEGs

The table summarized transcription factors regulating down-regulated DEGs in each experimental group. "FC" represents the log2 ratios of mean normalised counts gained from DESeq2 result. Two experimental methods were used to identify mRNA targets of Sro9 and Slf1p in S. cerevisiae. "MJ Par-CLIP" column shows PAR-CLIP data (unpublished results) collected by Dr. Martin Jennings. "CK RIP" shows RNA immunoprecipitation data collected by Christopher J. Kershaw (Kershaw et al., 2015). "Sro9UT" indicates the experimental group was not treated with hydrogen peroxide, while the sample from "Sro9T" was the yeast cells treated with hydrogen peroxide.

4. Discussion and conclusion

Transcriptional regulation of oxidation resistance mediated by SRO9 and SLF1 integrated multiple pathways. In this experiment, we systematically analysed transcriptome data collected by Illumina RNA-seq. Transcriptional profiling revealed that the total number of differential expression genes in wild-type strain after H_2O_2 treatment was higher than *sro9Δ* and *slf1Δ* mutants. Many DEGs, such as *YAP1*, *YRR1* and *SKN7*, were responsible for encoding for transcription factors. Table 1 and Table 2 summarized transcription factors regulating more than 30% of total DEGs in wild-type and mutant strains. Transcription factors analysis revealed that most transcription factors regulated both up-regulated DEGs and down-regulated DEGs in response to oxidative stress. Transcription factors that expression level altered only in wild-type strain were more likely to involve reprogramming of transcriptional regulation in Sro9 or Slf1 mediated oxidation tolerance. We combined RNA-seq data with previous RNA-protein interaction data (RIP-Seq and PAR-Clip) to quantify and dissect the function of Sro9 and Slf1 in response stress. We could speculate that a potential repair system existed in Sro9-mediated response to oxidative stress, because some GO terms, such as oxidation-reduction process and ribosome biosynthesis, enriched in both wild-type and *sro9Δ* strain. However, cellular response to chemical stress, carbohydrate metabolic process, mitochondrial function related GO terms were not or less enriched in *slf1Δ* strain. In combination with the fact that there were mRNA targets of Slf1 than Sro9 targets, we could hypothesise that the deletion of *SLF1* led to more severe stress response to hydrogen peroxide stimuli at the transcriptional level.

As the most important transcription factors involved in oxidative stress tolerance, Yap1 regulated more than 90% of both up-regulated DEGs and down-regulated DEGs in

wild-type and two mutant strains. Transcriptomic profiles showed that Yap1 is a transcriptional regulatory protein and is implicated in response to multiple cellular stresses. The transcriptional expression level of *YAP1* showed a substantial increase with H_2O_2 treatment. A large-scale survey showed that the null mutant of YAP1 showed limited oxidative stress resistance(Stephen et al., 1995). Yap1 is able to shuttle between the nucleus and the cytoplasm. After oxidant treatment, Yap1 is activated, forms disulfide bonds with oxidant and accumulates in the nucleus, therefore promotes gene transcription in response to oxidative stress (Gulshan et al., 2005). Several genes encoding for nuclear pore proteins (Wente et al., 1992), including *NUP133, NUP100, NUP145, NUP2, NUP170, NUP1, NUP157, NUP82* and *NUP188*, were only up-regulated in the wild-type strain. These genes were enriched in RNA transport and nuclear transport, and all of them were documented regulated by Yap1. Null mutants of those genes showed a phenotype with enhanced oxidative stress resistance(Brown et al., 2006). Although some of them were mRNA target of Sro9, such as *NUP100* and *NUP145*, none of them were identified as the targets of Slf1. This indicated that Sro9 and Slf1, especially Slf1, were principally regulated those nuclear export genes indirectly.

MET28 is the target of Sro9 and its transcriptional level was only significantly increased in the wild-type strain. The transcription of *MET28* was shown to be positively regulated by Yap1 by microarray analysis under sodium selenite-induced and acrolein-induced oxidative stress (Ouyang et al., 2011; Salin et al., 2008). Met28 works as an accessory factor that forms the Cbf1-Met4-Met28 complex, promote Cbf1 binding to its target and involves in sulfur metabolism (Kuras et al., 1997). TFs including Met31, Msn4, Bas1, Hac1, Xbp1, Yrr1, Met32, Gcr1, Fkh1, Hap2 and Fkh2 potentially regulate

MET28, because they contain the binding site of the promoter sequence of *MET28*. The implied regulators in regulating *MET28* under oxidative stress needs further studies.

Msn2 and Msn4 are transcriptional factors that activate and induce gene expression in response to multiple stress conditions(Boy-Marcotte et al., 2006). In our dataset, Msn2 and Msn4 regulated more up-regulated DEGs than down-regulated DEGs. *MSN2* and *MSN4* were mRNA targets of Sro9. However, although Msn2 and Msn4 regulated more than 30% of DEGs, the expression level of *MSN2* and *MSN4* did not significantly alter in our transcriptomic analysis in the wild-type and mutant strains after H_2O_2 treatment. This indicated that Msn2 and Msn4 might be not essential in regulating H_2O_2-induced oxidative stress.

Rpn4 is a crucial transcription factor because the transcription of *RPN4* was significantly up-regulated in all three strains. Rpn4 regulated both up-regulated DEGs and down-regulated genes. It acts as a transcriptional activator of genes encoding proteasomal subunits, and it is the targeted gene of both Yap1 and Msn2/4 (Dohmen et al., 2007). It has been revealed that, upon oxidative stress, Yap1 bonds to the promoter of *RPN4* and mediates mRNA up-regulation of *RPN4* (Harbison et al., 2004; Zhang et al., 2016). Because the transcriptional level of RPN4 was not significantly altered in mutants, suggesting that Sro9 and Slf1 may not play a decisive effect in mediating its transcriptional output as well.

The ubiquitin-proteasome system is the main pathway of removing misfolded proteins by protein degradation(Hiller et al., 1996). Evidence showed that, in the wild-type strain, the ubiquitination of ribosome protein occurs as an immediate response to oxidative stress (Shcherbik and Pestov, 2019). In our GO functional analysis, ubiquitin-dependent protein catabolic process (GO:0006511) was only enriched in up-regulated

DEGs in the wild-type strain. The expression level of *PRE10*, which codes for the mRNA binding subunit of proteasome, was only up-regulated in the wild-type strain. *PRE10* was the mRNA target of both Sro9 and Slf1. Although the effect of H_2O_2 on *pre10Δ* strain has not yet been verified by experiment, decreased resistance to chemicals(bortezomib) in *pre10Δ* strain had been confirmed(Hoepfner et al., 2014). Therefore, we can speculate that Sro9 and Slf1 accelerate the degradation of misfolded protein stimulated by hydrogen peroxide and prevent cell apoptosis in this way.

Another GO term, cell wall organization (GO:0071555), was only enriched in down-regulated DEGs in sro9Δ strain. Cell wall is the primary barrier to extreme conditions. Dysfunction in the cell wall integrity pathway (CWI) will result in decreased resistance to environmental distractions. In *Saccharomyces cerevisiae*, the formation and regulation of cell wall are stringently controlled by over 1200 genes(Klis et al., 2002). In our RNA-seq result, the expression level of two DEGs, *PMT1* and *ECM7*, encoding for O-Mannosyl transferase and integral membrane protein, respectively, were only down-regulated in *sro9Δ* strain under oxidative stress (Lussier et al., 1997; Strahl-Bolsinger et al., 1993). Both of them are mRNA targets of Sro9. It has been reported that the MAPK signaling pathway plays an essential role in regulating transcriptional responses to cell wall defects in CWI (García et al., 2019). We found that, in all identified genes enriched in MAPK signal transduction pathway, *HKR1* only existed in *sro9Δ* mutant, while *MSG5* was the target of both Slf1 and Sro9 and specifically existed in *slf1Δ* mutant. Hkr1 is transmembrane mucin involved in the HOG (high-osmolarity glycerol) pathway in yeast and works as an osmosensor to transfer signals of extracellular osmolarity variations. Hkr1 also involves in cell wall organization (Tatebayashi et al., 2007). More surprisingly, *HKR1* was detected to be the mRNA target of Sro9 by RIP-Seq and was significantly down-regulated in *sro9Δ* mutant but

not in others, suggesting Sro9 binding to *HKR1* and stabilizing its RNA directly. This revealed *HKR1* mediated by Sro9 has a hypothetical role in regulating oxidative stress tolerance. Therefore, the involvement of HKR1 in various pathways shows some overlaps of transcription factors in both oxidative stress and osmosensor signaling pathway.

In summary, our result verified that Sro9 and Slf1 participate in response to oxidative stress, especially at the transcriptional level. Paradoxically, another conclusion of the present work was that there is no significant difference in the expression level of essential TFs regulating *S. cerevisiae* response to oxidative stress, such as *YAP1*, *MSN2/4*, *SKN7*, between the wild-type and mutant strains. Therefore, traditional transcription factors involved in regulating oxidation response may not play a dominant role in Slf1 and Sro9-mediated translational response to oxidative stress. The enrichment analysis results suggested that Sro9 and Slf1 were well characterized for regulating RNA transport, nuclear transport, and mitochondrion function. Surprisingly, in our BP ontology analysis, the GO term, nuclear export, only occurred in the wild-type strain. Therefore, Sro9 and Slf1 may have the ability to shuttles between the nucleus and the cytoplasm or have potential rules in regulating genes involves in nuclear transport. We speculated that the utility of Sro9 and Slf1 depends on their subcellular localization. Sro9 and Slf1, in response to oxidative stress, were highly related to RNA transport from the nucleus to the cytoplasm. Sro9 and Slf1 also have the capacity in regulating mitochondrial function in response to oxidative stress.

One limitation of this experiment is that the data reliability has not been fully validated. Real-time quantitative RT-PCR (qRT-PCR) or RNA microarray to verify DEGs identified from RNA-seq data. Another drawback is that the gene ontology terms

chosen in the report are subjective. Gene ontology is a loosely hierarchical structure, and one term is likely to be allocated to several parent terms. Therefore, it is hard to find overlap terms between wild-type and mutant strains accurately. The screening process of attributes may introduce subjectivity into the experiment, and we may underestimate the importance of some pathways. In further studies, we can integrate data from proteomics and dig into the mechanism of Sro9 and Slf1 in specific pathways. In addition, additional investigation of lower-ranked GO terms could yield new insights into the function of Sro9 and Slf1. We can also further explore the potential application of overexpression of Sro9 and Slf1 in oxidation resistance in the alcoholic fermentation process.

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