Genome wide screen for mutations mediates sensitive to sodium dodecyl sulfate in budding yeast

Chunlei Cao
Jiangnan University

Zhengfeng Cao
Yangzhou University

Peibin Yu
Jiangnan University

Yunying Zhao
Jiangnan University

Research article

Keywords: Budding yeast, SDS, Genetic screening, Genomics, ROS

Abstract

Background: Sodium dodecyl sulfate (SDS) is one of the most widely used anionic alkyl sulfate surfactants. Toxicological information on SDS is accumulating, however, mechanisms of SDS toxicity regulation remain poorly understood. In this study, the relationship between the SDS-sensitive mutants and their intracellular ROS levels has been investigated. Results : Through a genome-scale screen, we have identified 108 yeast single-gene deletion mutants that are sensitive to 0.03% SDS. These genes were predominantly related to the cellular processes of metabolism, cell cycle and DNA processing, cellular transport, transport facilities and transport routes, transcription and the protein with binding function or cofactor requirement (structural or catalytic). We further measured the intracellular ROS (reactive oxygen species) levels of 108 SDS-sensitive mutants treated with 0.015% SDS. The results showed that 85 SDS-sensitive mutants accumulated significantly higher intracellular ROS levels under SDS stress than the wild-type cells. Moreover, SDS could generate oxidative damage and up-regulate several antioxidant defenses genes, and some of the SDS-sensitive genes were involved in this process. Conclusion: This study provides insight on yeast genes involved in SDS tolerance and the elevated intracellular ROS caused by SDS stress. Our findings provide a basis to understand molecular mechanisms underlying the detoxification of SDS by yeast cells.

Background

Surfactants are organic pollutants distributed widely in the current environment, and their toxicity has caused widespread concern. One of the synthetic anionic surfactants, sodium dodecyl sulfate (SDS), or sodium lauryl sulfate (SLS), a product that consists of approximately 70 % sodium dodecyl sulfate and 30 % sodium tetradecyl sulfate, with the formula of $C12H25NaO4S$, has been used in many cleaning and hygiene products such as liquid soaps, shampoos, bubble baths, shower gels, and nearly all toothpastes. SDS is also used in pharmaceutical and food products, as well as in industiral and laboratory applications, i.e, SDS can form complexes with protein through hydrophobic interactions and thus be used in polyacrylamide gel electrophoresis to determine the molecular weight of proteins [1, 2]. The concentration of SDS found in consumer products varies by product and manufacturer but typically ranges from 0.01% to 50% in cosmetic products and 1% to 30% in cleaning products [3]. The lethal dose, 50% (LD50) for SDS is 0.8-1.10 g/kg in rats, SDS concentrations ⩽2% are considered irritating to normal skin in human patch testing, and >5% causes depression, labored breathing, diarrhea, and death (four out of 20 animals) [2].

Safety concerns with SDS application in human include carcinogenicity, skin and eye irritation, and aphthous ulcers. The toxicity of SDS has been demonstrated in bacteria, microalgae, crustaceans, echinoderms, rats, humans and carp. The basis of SDS toxicity seems to be mainly related to the alteration of the cellular ionic balance caused by cellular membrane permeability alterations and to the induction of oxidative stress, that can generate other physiological and biochemical stresses [4]. SDS elicits both physical and biochemical effects on cells, with the membrane the primary target structure, and considered as a a typical cell wall perturbing agent. Effects are concentration dependent and range from loss of barrier function and increased permeability to complete cell lysis. It is suggested that SDS causes elevated the glutathione production, lipid peroxidation as well as changes in carbon metabolism [5], leading to altered cell membrane stability and permeability as well as indirectly to increased accessibility of cell wall [6]. Yeast cell wall serves crucial functions in protecting against osmotic shock stress and mechanical steess, maintaining cell shape, as well as serving a sacffold for cell-surface proteins [7]. SDS interrupts cell membranes and then triggers the Cell Wall Integrity (CWI) signaling pathway, a kinase cascade to maintain cell integrity and can be activated by chemicals that damage the cell wall and membrane in buding yeast [8]. For example, the Slt2/Mpk1, a mitogen-activated protein (MAP) kinase, can be phosphorylated and thus activated by impaired cell integrity [9]. However, deatiled mechanisms of SDS toxicity in microorganisms or higher eukaryotes are poorly understood.

Yeast has been previously used to demonstrate the effect of SDS on biological membranes, showing that micelles of SDS may penetrate the membrane through pores in the yeast cell wall and destroy the membrane [10]. In defense against SDS surplus, yeast cells increase the expression levels of genes involved in oxidative stress which might be caused by its effect on membrane structure, carbon metabolism, or DNA repair [2]. Reactive oxygen spesies (ROS) play an important role in inducing cell death or apotosis in yeast cells by causing damages to proteins, lipids and DNA [11, 12]. In addtion, ROS could induce cell wall damage in yeast cells lacking mitochondrial DNA , making cells to become more sensitive to of SDS stress [13].

As the simplest eukaryotic organism, the budding yeast *Saccharomyces cerevisiae (S. cerevisiae)* has been used to identify the mechanism and regulation of metal ion transport [14]. Here, we used *S. cerevisiae* to explore the SDS effect on eukaryotic cells and compared the oxidative stress (reactive oxygen species, ROS) in cultured cells.To have a global view of SDS stress on eukaryotic cells, we have firstly screened the SDS-sensitive mutants from the yeast nonessential gene deletion library and identified 108 SDS-sensitive mutants. To evaluate whether SDS generates serious oxidative stress to the SDS-sensitive mutant cells, we have then measured the cellular response of cultured yeast cells to SDS in terms of ROS levels. Additionally, we show that SDS can induce oxidative stress and that yeast cells eliminate these oxidative damage by elevating the expression levels of the genes coding for antioxidant defenses.

Results

An overview of genes involved in the SDS sensitivity of yeast cells

To obtain a global view of genes involved in the sensitivity of yeast cells to 0.03% SDS, we screened the yeast diploid nonessential gene deletion library. The results show that 108 gene deletion mutants (2.3% of the screened 4757 gene deletion mutants) were identified as sensitive to 0.03% SDS (Fig. 1 and Table 1), indicating that they possess a weakened cell membrane or cell wall. The genotypes of these 108 mutants were confirmed by PCR with the forward primer derived from the promoter region of each correspondent gene and a reverse primer KanMX4-R (Additional file 1: Table S1 and Additional file 2: Figure S1) derived from the ORF region of KanMX4. The functional categories of these 108 genes are involved in metabolism (17), cell cycle and DNA processing (15), transcription (14), cellular transport, transport facilities and transport routes (28), biogenesis of cellular components (6), cellular communication / signal transduction mechanism (2), protein with binding function or cofactor requirement (structural or catalytic) (10), as well as unclassified proteins (16) (Table 1). We showed that mutants for genes related to the functions of metabolism and ellular transport, transport facilities and transport routes were most sensitive to SDS stress (Table 1). We listed some genes as the representative genes of their categories as below.

Genes involved in cellular transport and transport routes are associated with SDS tolerance

The largest functional category of these 108 identified SDS-sensitive genes is the cellular transport, transport facilities and transport routes (Table 1), including 28 genes identified. Notably, 11 out of 63 nonessential vacuolar protein sorting (VPS) genes in the genome of *S. cerevisiae* being identified [15]. Namely, mutants for *VPS1, VPS16, VPS20, VPS24, VPS25, VPS33, VPS36, VPS38, VPS51, VPS63*, and *VPS64* were identified being sensitive to 0.03% SDS (Table 1; Fig. 1). The results suggest that the VPS pathway involved in protein trafficking and membrane fusion plays an important role in the response of yeast cells to SDS stress.

The H$^+$-ATPase localized in the membrane of vacuole (V-ATPase) is composed of the catalytic V1 subcomplex and the proton-translocating membrane V0 subcomplex, playing crucial roles in the organelles acidification and other intracellular activities [16, 17]. The V1 complex is composed of at least eight subunits (A-H) encoded by eight *VMA* (Vacuolar Membrane ATPase) genes: *VMA1, VMA2, VMA4, VMA5, VMA7, VMA8, VMA10* and *VMA13*, respectively. The V0 complex is composed of at least five subunits (a, c, d, c' and c") encoded by six *VMA* genes: *VPH1, STV1, VMA3, VMA6, VMA11*, and *VMA16*, respectively. Three genes *VMA12, VMA21* and *VMA22* encode proteins that are required for the biogenesis of a functional V-ATPase [18]. V1 subcomplex and V0 subcomplex are responsible for ATP hydrolysis and proton translocation, respectively. Mutants for the VMA genes showed growth defects in response to oxidative stress, such as H$_2$O$_2$ [19]. In this study, four mutants for *VMA3, VMA5, VMA13*, and *VMA21* were sensitive to 0.03% SDS (Table 1; Fig. 1). *VMA5* and *VMA13* encodes the V1 complex subunit C and H [20, 21], respectively. *VMA3* encodes the subunit c of the V0 complex [22]. *VMA21* is not an actual component of the V-ATPase complex, but encodes proteins functioned in the assembly of the V-ATPase [23]. These results indicate that the V-ATPase is critical for *S. cerevisiae* cells in responding to SDS in the environment.

Mutants for genes involved in cell cycle and DNA processing render yeast cells sensitive to SDS stress

There are 15 genes identified in our study that are involved in cell cycle and DNA processing. *SLX5* and *SLX8* encode the subunit of Slx5-Slx8 ubiquitin-like modifier (SUMO)-targeted ubiquitin ligase (STUbL) complex [24-26]. Mutants for *SLX5* or *SLX8* were sensitive to 0.03% SDS (Table 1 and Fig. 1), suggesting that STUbL complex is involved in SDS tolerance of yeast cells. The small SUMO-targeted ubiquitin ligase complex is a nuclear ubiquitin ligase complex that specifically targets sumoylated proteins. It is formed of homodimers or heterodimers of RING finger protein 4 family ubiquitin ligases and is conserved in eukaryotes [25]. Three genes, *MSH1*, *FYV6* and *XRS2*, encode three proteins required for the DNA repair process [27-29], has been identified in this study. The other six genes, *EAF1*, *ARP5*, *RSC1*, *RSC2*, *DCC1* and *CTF4* associated with chromatin modification, remodeling and cohesion [30-34], are all required for SDS tolerance. Interestingly, *SIT4* and *PHO85* coding for a serine/threonine-protein phosphatase and a cyclin-dependent kinase, respectively, were both screened in our study. The serine/threonine-protein phosphatase Sit4 controls the processes of mitochondrial function, lifespan, cellular traffic, cell growth and cell cycle progression by regulating the phosphorylation levels of many proteins [35, 36], while the kinase Pho85 is involved in regulating the cellular responses of cell cycle progression, autophagy, response to DNA damage, phosphate and glycogen metabolism, establishment of cell polarity, as well calcium-mediated signaling. Therefore, deletion of the *SIT4* or *PHO85* cause a decreased resistance to oxidative stress, chemicals, toxin, utilization of carbon and nitrogen [37-41]. In addition, we have identified two genes, *NEM1* and *CDC50*, which are required for normal nuclear envelope morphology and sporulation, or cell division, respectively [42, 43]. Taken together, these results suggests that SDS can affect the cell cycle and DNA processing of *S. cerevisiae* cells.

Genes involved in aromatic amino acid biosynthesis and SDS tolerance

We have identified mutants for five genes involved in the synthesis of aromatic amino acids, *ARO1*, *ARO2*, *ARO7*, *TRP1* and *TRP5* that were sensitive to 0.03% SDS (Table 1; Fig. 1). Previously, Aro1 catalyzes steps 2 through 6 in the biosynthesis of chorismate, which is a precursor to aromatic amino acids [25]; Aro2 catalyzes the conversion of 5-enolpyruvylshikimate 3-phosphate (EPSP) to form chorismate; and Aro7 catalyzes the conversion of chorismate to prephenate to initiate the tyrosine/phenylalanine-specific branch of aromatic amino acid biosynthesis [44-46]. Trp1 and Trp5 involved in the synthesis of tryptophan, where Trp1 catalyzes the third step in tryptophan biosynthesis and Trp5 catalyzes the last step of tryptophan biosynthesis [47, 48]. These results confirmed the previous findings that tryptophan exhibited protection from membrane disruptions and thus conferred resistance to SDS stress [49].

Oxidative stress is involved in SDS tolerance

Since SDS had been confirmed to induce the oxidative stress response [2], we next measured the intracellular ROS levels of the 108 SDS-sensitive mutants under 0.015% SDS treatment. In the wild-type BY4743 cells, the intracellular ROS level was significantly increased under SDS stress (Fig. 2 and Additional file 3: Figure S2). Of these 108 SDS-sensitive mutants, 85 mutants accumulated significantly higher intracellular ROS levels under SDS stress compared with wild-type cells (Additional file 3: Figure S2), while the rest 23 mutants accumulated similar or lower intracellular ROS levels when treated with SDS compared with wild type cells (Additional file 3: Figure S2). Interestingly, six mutants for *ARG82*, *TRP5*, *GRR1*, *MSH1*, *LAS21*, and *YNL296W*, accumulated lower intracellular ROS levels when treated with 0.015% SDS than without SDS (Additional file 3: Figure S2). It suggested that these six genes might not be directly involved in the regulation of intracellular ROS levels under SDS stress.

The relative ROS levels in 11 mutants for *PRS3*, *TRP1*, *NEM1*, *EAF1*, *IKI3*, *CBP3*, *VPS20*, *VPS36*, *VPS63*, *VPS25*, and *TUS1* were all higher than that of wild-type cells (Fig. 2), indicating that these 11 genes were all important for dealing with the oxidative damage generated by SDS stress. To further confirm these results, we constructed the 11 plasmids expressing the above 11 genes in pRS316 plasmid, respectively, and then transformed them into the corresponding mutants. The growth defect of SDS-treatment mutant cells could be suppressed by introducing the expression plasmid back into the corresponding mutants (Fig.

3A), and their intracellular ROS levels were also recovered to that of the wild-type cells (Fig. 3B). Taken together, these results indicate that yeast cells lacking any of the above 11 genes are sensitive to SDS stress, leading to increased intracellular ROS levels.

SDS generates oxidative stress by regulating the expression of genes involved in redox homeostasis

It was reported that many of the oxidative stress scavenging genes could be induced by SDS stress in a DNA microarray analysis [2]. To investigate whether the deletion of genes PRS3, TRP1, NEM1, EAF1, IKI3, CBP3, VPS20, VPS36, VPS63, VPS25, and TUS1 influence the expression of genes coding for the antioxidant defenses, we tested the expression of GSH1 (glutamylcysteine synthetase), SOD1 (cooper/zinc superoxide dismutase), CTT1 (cytosolic catalase T), GPX2 (2-Cys peroxiredoxin), TRR1 (thioredoxin reductase) and TRX2 (thioredoxin 2) by quantitative real-time PCR analyses. In the wild-type cells, the expression levels of GSH1, SOD1, CTT1 and GPX2 were significantly up-regulated after treatment with SDS (Fig. 4), while no significant difference in the expression levels of TRR1 or TRX 2 were observed when treated with or without SDS (Additional file 4: Figure S3). Interestingly, both of the expression levels of SOD1 and CTT1 were reduced in the 11 mutants compared with wild type cells (Fig. 4B and 4C). In addition, the expression levels of GSH1 and GPX2 were also reduced in these mutants except the mutants for NEM1 and VPS25, or EAF1, respectively (Fig. 4A and 4D). Overall, our results demonstrate that the decreased expression of GSH1, SOD1, CTT1 and GPX2 might be responsible for the high intracellular ROS levels accumulated in these mutants.

Discussion

The main goals of this study were to identify the genes involved in SDS tolerance, and to investigate the primary mechanism that SDS induces oxidative stress in yeast cells.

SDS is considered as a generally recognized safe ingredient for food and hygiene products. However, safety concern arises as oral ulcer or skin irritation was reported to be caused by products containing SDS in recent studies [50, 51]. S. cerevisiae, a budding yeast used in brewing beer and baking, is a single-celled eukaryote used extensively in labory due to the fact that its genome has been sequenced and its genetics are easily manipulated. Here, we used S. cerevisiae to examiner the genome-wide SDS stress on eukaryotes and identified 108 SDS-sensitive mutants from the yeast nonessential gene deletion library, representing 2.3% of the screened 4757 gene deletion mutants. Previous study reported that 295 ORFs were up-regulated and 118 ORFs were down-regulated after SDS treatment , and the functional classifications of these genes were involved in a number of major cellular processes, including metabolism, protein sorting, transcription, cellular transport and biogenesis, DNA and protein synthesis, cellular communication / signal transduction and ionic homeostasis, etc [2]. Interestingly, The 108 SDS-sensitive genes encoded proteins that are also involved in many of these cellular processes.

A significant aspect of SDS toxicity may be related to its effect on biological membranes that SDS may penetrate the membrane through pores in the yeast cell wall and destroy the membrane [52]. For example, SDS is used as a perturbing agent to cell wall integrity, and through MPT5 and SSD1 signaling pathway SDS can result in sensitivity to changes in external osmolarity, defect budding, and cell lysis [53]. Our results support this by showing that the largest functional category (28) of these identified SDS-sensitive genes are of cellular transport, transport facilities and transport routes. Beside the stress on cell wall, SDS also introduced stresses on intracellular sorting and delivery of soluble vacuolar proteins, because there are 11 SDS-sensitive genes have been identified for VPS pathway alone in our study. The absence of these genes might reduce the supply of cell wall and/or cell membrane components, leading to cell membrane damage or defects in cell wall structure in the SDS stress.

The expression of about 65 genes involved in the carbon metabolism were induced by SDS stress, including genes related to amino acid metabolism, C-compound and carbohydrate metabolism, lipid, fatty acid, and isoprenoid metabolism, vitamin, cofactor, and prosthetic group metabolism, and nucleotide metabolism [2, 54]. Mutants for a large group of 17 genes involved in metabolism is revealed to be sensitive to SDS stress in the present study, including six genes related to amino acids metabolism

(*ARO1*, *ARO2*, *ARO7*, *TRP1*, *TRP5*, *PRS3* and *THR4*), four genes related to lipid and fatty acid metabolism (*ARG82*, *ELO3*, *IPK1*, and *ERG3*), four genes involved in nucleotide metabolism (*GRR1*, *REG1*, *ELM1* and *AFT1*), and two genes associated with carbohydrate metabolism (*ROM2* and *NRK1*). In a previous study, it has been showed that the cell membrane damage trigged by SDS stress was independent of Cell Wall Integrity signaling pathway, and the biosynthesis of tryptophan and tyrosine played an important role in the SDS-induced plasma membrane stress response [49]. It is might explain why the six mutants for *ARO1*, *ARO2*, *ARO7*, *TRP1*, , *PRS3* and *TRP5*, involved in the synthesis of aromatic amino acids and tryptophan, were sensitive to SDS toxicity. In addition, lipid and fatty acid metabolism has significant role in maintaining the structures of cell membrane and cell wall, nucleotide metabolism is related to the processes of DNA synthesis, cell division and DNA repair, while carbohydrate metabolism is associated to cell growth and many other cellular activities. Moreover, it has been previously reported that, cell wall defects led to cells sensitive to SDS stress for a weakened cell wall allows it to penetrate more easily [8]. Therefore, it is not surprising that deletion mutants for the other 11 genes involved in the above metabolism functions are sensitive to SDS stress.

Another concern with SDS toxicity has been its carcinogenicity; no evidence shows SDS-related tumorigenicity or carcinogenicity in early official review. SDS was extensively tested for genetic toxicity. Tests with SDS in bacterial or in mammalian systems (in vitro and in vivo) show no indication of genotoxicity with or without metabolic activation [55]. Published reports suggest that SDS has low acute mammalian toxicity and no known chronic effects. However, we have identified 15 SDS-sensitive genes of *S. cerevisiae* are involved in cell cycle and DNA processing in our study, though further investigation is required to clarify the significance.

Finally, we examined the intracellular ROS levels under SDS stress. Increased ROS level may result in significant damage to cell structures and constant high ROS level is known as oxidative stress. Most significantly, ROS is considered to damage DNA or RNA of cells. Under the SDS treatment, we observed most mutants (85/108) increased the intracellular ROS levels comparing with the wild-types, consistent with their being-affected growth. We pick up 11 mutants for *PRS3*, *TRP1*, *NEM1*, *EAF1*, *IKI3*, *CBP3*, *VPS20*, *VPS36*, *VPS63*, *VPS25*, and *TUS1*, which accumulated higher relative ROS levels than that of wild-type cells under SDS treatment (Fig. 2), to investigate the mechanism of oxidative damage induced by SDS stress. We have shown that the expression of some antioxidant defenses genes were down-regulated by SDS stress in these mutants. It suggests that some of the SDS-sensitive genes might be involved in maintaining the redox balance under SDS treatment. However, some mutants reduced its ROS production, the genes involved in these mutants may be related the detoxification of SDS by yeast cells. Another interesting result of our study is that six mutants for *ARG82*, *TRP5*, *GRR1*, *MSH1*, *LAS21*, and *YNL296W*, accumulated lower intracellular ROS levels under 0.015% SDS treatment when compared with no SDS treatment. They could also play a role in detoxification of SDS by yeast cells, but further investigations are needed.

Conclusions

In summary, 108 yeast single-gene deletion mutants that are sensitive to 0.03% SDS have been identified by screening the yeast diploid nonessential gene deletion library. We demonstrated that the intracellular ROS levels in 85 of these mutants were significantly higher than that of the wild-type cells under SDS stress. SDS can generate oxidative damage and up-regulate several antioxidant defenses genes, leading to cells sensitive to SDS stress. Our current findings would provide a basis to understand molecular mechanisms underlying the detoxification of SDS by yeast cells.

Methods

Strains, media and culture conditions

All *S. cerevisiae* strains were derived from the S288C genetic background. Yeast cells were grown at 30°C in YPD medium (1% yeast extract, 2% peptone, 2% glucose). SD-URR media (0.17% (w/v) yeast nitrogen base, 2% (w/v) glucose, 0.5% ammonia sulfate, adding 1/10 mL amino acid mixture without uracil) was used to culture yeast cells for plasmid selection. Solid media were produced by adding 2% (w/v) agar when necessary. SDS was purchased from Sangon Biotech (Shanghai, China), and Dihydroethidium was purchased from Sigma (Beijing, China).

Genome-wide screen for SDS-sensitive mutations

A collection of homozygous diploid deletion mutants for 4,757 non-essential genes were purchased from Invitrogen Inc. [http://clones.invitrogen.com/] and frozen at -80 °C in 96-well microtitre plates in liquid YPD medium containing 15% glycerol. A primary screen for SDS-sensitive mutations in *S. cerevisiae* was preformed by transferring the deletion mutant library to fresh liquid YPD medium and cultured at 30°C in new 96-well microtitre plates. Then 20 μL of each mutant strains were transferred to 180 μl fresh liquid YPD medium with or without 0.015% SDS, respectively, and incubated the cells at 30°C for about 6 to 12 hours. The growth rates of each mutant in YPD medium with and without 0.015% SDS were measured at OD600nm to determine the SDS-sensitivity in the primary screen. The SDS sensitive strains showed reduced growth and was defined as mutants with a relative OD600nm reduced by more than 30% in liquid YPD medium containing supplemented SDS but not in liquid YPD medium without supplemented SDS as compared to that of the wild-type.

Phenotypic analysis by spot dilution growth assays

Mutants that appeared sensitive were retested by plating dilutions of the cells on the same plates. Namely, individual deletion mutants were grown in liquid YPD at 30°C overnight and serially diluted by 10 times with ddH$_2$O. Each dilution of 3 μL was spotted onto YPD plates with or without 0.03% SDS and were incubated at 30°C for 2-3 days.

To further confirm the SDS sensitivity of mutants for *PRS3*, *TRP1*, *NEM1*, *EAF1*, *IKI3*, *CBP3*, *VPS20*, *VPS36*, *VPS63*, *VPS25*, and *TUS1* genes, we introduced the pRS316 vector and pRS316 vector expressing the corresponding genes back into mutants for *PRS3*, *TRP1*, *NEM1*, *EAF1*, *IKI3*, *CBP3*, *VPS20*, *VPS36*, *VPS63*, *VPS25*, and *TUS1*. The sensitivity to SDS of the transformants was examined on YPD and YPD+0.03% SDS plates using the above serial dilution assay method.

DNA manipulations

To express the *TRP1* gene in the plasmid pRS316, the DNA fragment which contains the promoter, ORF and terminator region, was first amplified with primers TRP1-F and TRP1-R (Additional file 1: Table S1), and were cloned into the *Bam*HI and *Hin*dⅢ sites of pRS316 to yield pRS316-*TRP1*. The other plasmids of pRS316-*IKI3*, pRS316-*PRS3*, pRS316-*CBP3*, pRS316-*NEM1*, pRS316-*VPS36*, pRS316-*VPS25*, pRS316-*VPS63*, pRS316-*VPS20*, pRS316-*TUS1* and pRS316-*EAF1* were all constructed by the same method described above. All the inserts were confirmed by DNA sequencing.

Oxidative stress assay for SDS-sensitive mutants

To determine the cellular oxidative stress of the SDS-sensitive mutants, we tested the intracellular ROS level by the dihydroethidium as previously described [56]. Briefly, overnight cell cultures were inoculated in YPD to an optical density OD$_{600}$=0.1, grown to middle log phase, and split into two aliquots. They were grown with or without 0.015% SDS for an additional 2 hours. Then about 5×10^6 cells were harvested by centrifugation and resuspended in 250 μl PBS with 2.5 μg/ml DHE, and incubated in the dark for 30 min. The relative fluorescence units (RFU) were tested by a fluorescence reader (Synergy™ H4, BioTek).

RNA extraction and quantitative PCR analysis

For extracting the total RNA, the mutants yeast cells were first grown to an OD600nm of 0.6-0.8, and then they were grown in the presence or absence of 0.015% SDS for an additional 1 hour. Cells were collected and the total RNA was extracted by hot phenol method. The genomic DNA was first removed from the total RNA with RNase-free DNase I. The first-strand cDNA synthesis was

performed using the Primer Script RT reagent kit (Cwbiotech, China) according to the manufacturer's instructions. The expression mRNA levels of *TRR1*, *TRX2*, *GSH1*, *SOD1*, *CTT1*, *GPX2* and *PGK1* were detected by quantitative PCR (qPCR) with primer pairs TRR1-F/TRR1-R, TRX2-F/TRX2-R, GSH1-F/GSH1-R, SOD1-F/SOD1-R, CTT1-F/CTT1-R, GPX21-F/GPX2-R, and PGK1-RT-F/ PGK1-RT-R (Additional file 1: Table S1), respectively, where the *PGK1* gene was used as an internal control. qPCR reactions containing SYBR Premix Ex Taq were performed in a Thermo Scientific CFX96 instrument. Each reaction was carried out in triplicate, and the expression levels of each were calculated using the −ΔΔCt method [57].

Declarations

Additional files

Additional file 1: Table S1. Primers used in this study

Additional file 2: Figure S1. Genotype confirmation of the 108 gene deletion mutants by PCR. Cells of the 108 gene mutants were grown overnight in YPD medium at 30°C and then collected for DNA extraction. PCR was performed with genomic DNA of each of these mutants with the primer located at the upstream of its open reading frame and the reverse primer KanMX4-R from the internal sequence of the KanMX4. PCR products were separated on 1% agarose gel, and sizes of the DNA marker were indicated on the left or right of the gel.

Additional file 3: Figure S2. Intracellular ROS levels of 108 SDS-sensitive gene mutants in response to SDS stress. a: Metabolism; b: Cell cycle and DNA Processing; c: Transcription; d: Protein with Binding Function or Cofactor Requirement (structural or catalytic); e: Cellular Transport, Transport Facilities and Transport Routes; f: Biogenesis of cellular components; g: Cellular communication/signal transduction mechanism; h: Unclassified Proteins. Log-phase cells were grown with or without 0.015% SDS for two hours before they were collected for measurement of intracellular ROS levels stained by the dihydroethidium. The intracellular ROS levels of these SDS-sensitive mutants were listed according to their categories in comparion to that of wild type cell BY4743. The value is the average of three independent assays for each strain.

Additional file 4: Figure S3. The expression of *TRR1* and *TRX2* under SDS stress. (A-B) WT and the indicated 11 mutants were treated to SDS medium for 1 h. The expression of the indicated genes was tested by qRT-PCR. The value is the average of three independent assays for each strain.

Abbreviations

SDS, sodium dodecyl sulfate; ROS, reavtive oxygen species; VPS, vacuolar protein sorting; VMA, vacuolar membrane ATPase; qPCR: quantitative PCR; RFU: relative fluorescence units

Acknowledgements

We thank the Test Platform for Large Instruments and Equipment of School of Biotechnology and the State Key Laboratory of Food Science and Technology for the technical supports.

Funding

This work was supported by the Natural Science Foundation of Jiangsu Province (BK20181345) and the Open Foundation of Jiangsu Key Laboratory of Industrial Biotechnology (KLIB-KF201807). None of the funders had any role in designing and/or conducting of the study; collection,

management, analysis and interpretation of the data; and preparation, review or approval of the manuscript.

Authors' contributions

ZYY designed the experiment and revised the manuscript. CCL and CZF performed the experiment. CCL and YPB wrote and revised the manuscript. We confirm that the final version manuscript has been read and approved by all named authors.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

[1]National Engineering Laboratory for Cereal Fermentation Technology (NELCF), Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu 214122, China. [2]Jiangsu Provincial Research Center for Bioactive Product Processing Technology, Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu 214122, China. [3]College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu 225009, China.

References

1. Wang Y, Zhang Y, Li X, Sun M, Wei Z, Wang Y, Gao A, Chen D, Zhao X, Feng X. Exploring the effects of different types of surfactants on zebrafish embryos and larvae. Sci Rep 2015; 5:10107.

2. Sirisattha S, Momose Y, Kitagawa E, Iwahashi H. Toxicity of anionic detergents determined by *Saccharomyces cerevisiae* microarray analysis. Water Res 2004; 38(1):61-70.

3. Bondi CA, Marks JL, Wroblewski LB, Raatikainen HS, Lenox SR, Gebhardt KE. Human and environmental toxicity of Sodium Lauryl Sulfate (SLS): evidence for safe use in household cleaning products. Environ Health Insights 2015; 9:27-32.

4. Messina CM, Faggio C, Laudicella VA, Sanfilippo M, Trischitta F, Santulli A. Effect of sodium dodecyl sulfate (SDS) on stress response in the *Mediterranean mussel* (*Mytilus Galloprovincialis*): regulatory volume decrease (Rvd) and modulation of biochemical markers related to oxidative stress. Aquat Toxicol 2014; 157:94-100.

5. Bindu PC, Babu P. Surfactant-induced lipid peroxidation in a tropical euryhaline teleost *Oreochromis mossambicus* (Tilapia) adapted to fresh water. Indian J Exp Biol 2001; 39(11):1118-22.

6. Igual JC, Johnson AL, Johnston LH. Coordinated regulation of gene expression by the cell cycle transcription factor Swi4 and the protein kinase C MAP kinase pathway for yeast cell integrity. EMBO J 1996; 15(18):5001-13.

7. Levin DE. Regulation of cell wall biogenesis in *Saccharomyces cerevisiae:* the cell wall integrity signaling pathway. Genetics 2011; 189(4):1145-75.

8. Levin DE. Cell wall integrity signaling in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 2005; 69(2):262-91.

9. Morimoto Y, Tani M. Synthesis of mannosylinositol phosphorylceramides is involved in maintenance of cell integrity of yeast *Saccharomyces cerevisiae*. Mol Microbiol 2015; 95(4):706-22.

10. Gubbens J, Slijper M, de Kruijff B, de Kroon AIPM. Protein complexes in bacterial and yeast mitochondrial membranes differ in their sensitivity towards dissociation by SDS. Biochim Biophys Acta 2008; 1784(12):2012-8.

11. Madeo F, Frohlich E, Ligr M, Grey M, Sigrist SJ, Wolf DH, Frohlich KU. Oxygen stress: a regulator of apoptosis in yeast. J Cell Biol 1999; 145(4):757-67.

12. Fleury C, Mignotte B, Vayssiere JL. Mitochondrial reactive oxygen species in cell death signaling. Biochimie 2002; 84(2-3):131-41.

13. Gao Q, Liou LC, Ren Q, Bao X, Zhang Z. Salt stress causes cell wall damage in yeast cells lacking mitochondrial DNA. Microb Cell 2014; 1(3):94-9.

14. Luo C, Cao CL, Jiang LH. The endosomal sorting complex required for transport (ESCRT) is required for the sensitivity of yeast cells to nickel ions in *Saccharomyces cerevisiae*. Fems Yeast Res 2016; 16(3).

15. Bowers K, Stevens TH. Protein transport from the late Golgi to the vacuole in the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 2005; 1744(3):438-54.

16. Kane PM. The where, when, and how of organelle acidification by the yeast vacuolar H+-ATPase. Microbiol Mol Biol Rev 2006; 70(1):177-91.

17. Nishi T, Forgac M. The vacuolar (H+)-ATPases--nature's most versatile proton pumps. Nat Rev Mol Cell Biol 2002; 3(2):94-103.

18. Graham LA, Powell B, Stevens TH. Composition and assembly of the yeast vacuolar H(+)-ATPase complex. J Exp Biol 2000; 203(Pt 1):61-70.

19. Milgrom E, Diab H, Middleton F, Kane PM. Loss of vacuolar proton-translocating ATPase activity in yeast results in chronic oxidative stress. J Biol Chem 2007; 282(10):7125-36.

20. Ho MN, Hill KJ, Lindorfer MA, Stevens TH. Isolation of vacuolar membrane H(+)-ATPase-deficient yeast mutants; the *VMA5* and *VMA4* genes are essential for assembly and activity of the vacuolar H(+)-ATPase. J Biol Chem 1993; 268(1):221-7.

21. Keenan Curtis K, Kane PM. Novel vacuolar H+-ATPase complexes resulting from overproduction of Vma5p and Vma13p. J Biol Chem 2002; 277(4):2716-24.

22. Szczypka MS, Zhu Z, Silar P, Thiele DJ. *Saccharomyces cerevisiae* mutants altered in vacuole function are defective in copper detoxification and iron-responsive gene transcription. Yeast 1997; 13(15):1423-35.

23. Graham LA, Stevens TH. Assembly of the yeast vacuolar proton-translocating ATPase. J Bioenerg Biomembr 1999; 31(1):39-47.

24. Schweiggert J, Stevermann L, Panigada D, Kammerer D, Liakopoulos D. Regulation of a spindle positioning factor at kinetochores by SUMO-targeted ubiquitin ligases. Dev Cell 2016; 36(4):415-27.

25. van de Pasch LA, Miles AJ, Nijenhuis W, Brabers NA, van Leenen D, Lijnzaad P, Brown MK, Ouellet J, Barral Y, Kops GJ *et al*. Centromere binding and a conserved role in chromosome stability for SUMO-dependent ubiquitin ligases. PLoS One 2013; 8(6):e65628.

26. Schweiggert J, Stevermann L, Panigada D, Liakopoulos D. Regulation of a spindle positioning factor at kinetochores by SUMO-targeted ubiquitin ligases. Mol Biol Cell 2015; 26.

27. Mookerjee SA, Lyon HD, Sia EA. Analysis of the functional domains of the mismatch repair homologue Msh1p and its role in mitochondrial genome maintenance. Curr Genet 2005; 47(2):84-99.

28. Page N, Gerard-Vincent M, Menard P, Beaulieu M, Azuma M, Dijkgraaf GJ, Li H, Marcoux J, Nguyen T, Dowse T *et al*. A *Saccharomyces cerevisiae* genome-wide mutant screen for altered sensitivity to K1 killer toxin. Genetics 2003; 163(3):875-94.

29. Trujillo KM, Roh DH, Chen L, Van Komen S, Tomkinson A, Sung P. Yeast xrs2 binds DNA and helps target rad50 and mre11 to DNA ends. J Biol Chem 2003; 278(49):48957-64.

30. Bungard D, Reed M, Winter E. RSC1 and RSC2 are required for expression of mid-late sporulation-specific genes in *Saccharomyces cerevisiae*. Eukaryot Cell 2004; 3(4):910-8.

31. Auger A, Galarneau L, Altaf M, Nourani A, Doyon Y, Utley RT, Cronier D, Allard S, Cote J. Eaf1 is the platform for NuA4 molecular assembly that evolutionarily links chromatin acetylation to ATP-dependent exchange of histone H2A variants. Mol Cell Biol 2008; 28(7):2257-70.

32. Jonsson ZO, Jha S, Wohlschlegel JA, Dutta A. Rvb1p/Rvb2p recruit Arp5p and assemble a functional Ino80 chromatin remodeling complex. Mol Cell 2004; 16(3):465-77.

33. Mayer ML, Gygi SP, Aebersold R, Hieter P. Identification of RFC (Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister chromatid cohesion in *S. cerevisiae*. Mol Cell 2001; 7(5):959-70.

34. Hanna JS, Kroll ES, Lundblad V, Spencer FA. *Saccharomyces cerevisiae CTF18* and *CTF4* are required for sister chromatid cohesion. Mol Cell Biol 2001; 21(9):3144-58.

35. Barbosa AD, Pereira C, Osorio H, Moradas-Ferreira P, Costa V. The ceramide-activated protein phosphatase Sit4p controls lifespan, mitochondrial function and cell cycle progression by regulating hexokinase 2 phosphorylation. Cell Cycle 2016; 15(12):1620-30.

36. Bhandari D, Zhang J, Menon S, Lord C, Chen S, Helm JR, Thorsen K, Corbett KD, Hay JC, Ferro-Novick S. Sit4p/PP6 regulates ER-to-Golgi traffic by controlling the dephosphorylation of COPII coat subunits. Mol Biol Cell 2013; 24(17):2727-38.

37. Bleackley MR, Young BP, Loewen CJ, MacGillivray RT. High density array screening to identify the genetic requirements for transition metal tolerance in *Saccharomyces cerevisiae*. Metallomics 2011; 3(2):195-205.

38. Huang D, Friesen H, Andrews B. Pho85, a multifunctional cyclin-dependent protein kinase in budding yeast. Mol Microbiol 2007; 66(2):303-14.

39. Outten CE, Falk RL, Culotta VC. Cellular factors required for protection from hyperoxia toxicity in Saccharomyces cerevisiae. Biochem J 2005; 388(Pt 1):93-101.

40. Cai H, Kauffman S, Naider F, Becker JM. Genomewide screen reveals a wide regulatory network for di/tripeptide utilization in *Saccharomyces cerevisiae*. Genetics 2006; 172(3):1459-76.

41. Dudley AM, Janse DM, Tanay A, Shamir R, Church GM. A global view of pleiotropy and phenotypically derived gene function in yeast. Mol Syst Biol 2005; 1:2005 0001.

42. Kim Y, Gentry MS, Harris TE, Wiley SE, Lawrence JC, Jr., Dixon JE. A conserved phosphatase cascade that regulates nuclear membrane biogenesis. Proc Natl Acad Sci U S A 2007; 104(16):6596-601.

43. Lenoir G, Williamson P, Puts CF, Holthuis JC. Cdc50p plays a vital role in the ATPase reaction cycle of the putative aminophospholipid transporter Drs2p. J Biol Chem 2009; 284(27):17956-67.

44. Henstrand JM, Schaller A, Braun M, Amrhein N, Schmid J. *Saccharomyces cerevisiae* chorismate synthase has a flavin reductase activity. Mol Microbiol 1996; 22(5):859-66.

45. Tkach JM, Yimit A, Lee AY, Riffle M, Costanzo M, Jaschob D, Hendry JA, Ou J, Moffat J, Boone C *et al*. Dissecting DNA damage response pathways by analysing protein localization and abundance changes during DNA replication stress. Nat Cell Biol 2012; 14(9):966-76.

46. Ball SG, Wickner RB, Cottarel G, Schaus M, Tirtiaux C. Molecular cloning and characterization of *ARO7-OSM2*, a single yeast gene necessary for chorismate mutase activity and growth in hypertonic medium. Mol Gen Genet 1986; 205(2):326-30.

47. Leng G, Song K. Watch out for your TRP1 marker: the effect of *TRP1* gene on the growth at high and low temperatures in budding yeast. Fems Microbiol Lett 2016; 363(10).

48. Zalkin H, Yanofsky C. Yeast gene TRP5: structure, function, regulation. J Biol Chem 1982; 257(3):1491-500.

49. Schroeder L, Ikui AE. Tryptophan confers resistance to SDS-associated cell membrane stress in *Saccharomyces cerevisiae*. PLoS One 2019; 14(3):e0199484.

50. Lippert F. An introduction to toothpaste - its purpose, history and ingredients. Monogr Oral Sci 2013; 23:1-14.

51. Marrakchi S, Maibach HI. Sodium lauryl sulfate-induced irritation in the human face: regional and age-related differences. Skin Pharmacol Physiol 2006; 19(3):177-80.

52. Levin DE, Bartlett-Heubusch E. Mutants in the *S. cerevisiae PKC1* gene display a cell cycle-specific osmotic stability defect. J Cell Biol 1992; 116(5):1221-9.

53. Kaeberlein M, Guarente L. *Saccharomyces cerevisiae MPT5* and *SSD1* function in parallel pathways to promote cell wall integrity. Genetics 2002; 160(1):83-95.

54. Nickerson KW, Aspedon A. Detergent-shock response in enteric bacteria. Mol Microbiol 1992; 6(8):957-61.

55. Kumar S, Kirha TJ, Thonger T. Toxicological effects of sodium dodecyl sulfate. J Chem Pharm Res 2014; 6(5):1488-92.

56. Buttner S, Eisenberg T, Carmona-Gutierrez D, Ruli D, Knauer H, Ruckenstuhl C, Sigrist C, Wissing S, Kollroser M, Frohlich KU *et al*. Endonuclease G regulates budding yeast life and death. Mol Cell 2007; 25(2):233-46.

57. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25(4):402-8.

Tables

Table 1 Functional categories of 108 genes whose deletion mutants are sensitive to 0.03% SDS

The number of asterisks represents SDS-sensitivity of different mutants. Mutant with five asterisks was most sensitive to SDS stress, while mutant with one asterisk was least sensitive to SDS.

Function	Genes							
Metabolism (17)	PRS3*	THR4**	ARG82****	REG1***	IPK1***	ARO1*****	TRP1*****	TRP5*****
	ELM1*****	AYT1*	ELO3*****	ARO7*****	ARO2****	NRK1****	ROM2***	ERG3****
	GRR1****							
Cell cycle and DNA Processing (15)	NEM1 ****	SIT4***	SLX5***	PHO85***	MSH1***	DCC1***	EAF1**	XRS2
	RSC2***	FYV6****	ARP5**	CTF4**	RSC1*	SLX8*	CDC50*****	
Transcription (14)	BRE1**	BDF1*****	DEP1*	SAC3****	MOT2*****	DEG1***	SFL1*	POP2****
	CTK1***	LSM1*	SRB5***	SPT4*	IKI3***	SGF29*		
Cellular Transport, Transport Facilities and Transport Routes (28)	GOS1****	PHO87**	STP22*****	VMA3*****	YPT31****	LOA1***	VMA13****	SNF8*****
	VPS36*****	VPS38****	SRN2*****	VPS63*****	VPS24****	VPS25*****	RCY1*****	VPS51****
	CHS5*****	SNF7*****	ISA1****	PIL1**	VMA5****	VPS20*****	VPS16*****	VPS1*****
	VPS33*****	VPS64**	VMA21*****	VID22****				
Protein with Binding Function or Cofactor Requirement (structural or catalytic) (10)	DIA4****	RPL35A**	NUP84**	APL2****	MAP1**	RPL13B**	CBP3**	LAS21**
	OCT1**	HIT1*						
Biogenesis of cellular components (6)	MDM10***	MRPL32*	MRPL24**	GIM5*	MDM20*****	SLG1*		
Cellular communication/signal transduction mechanism (2)	PBS2****	TUS1***						
Unclassified Proteins (16)	SRF1*	TOH1*****	YLR358C**	YNL296W***	YPR123C***	YDR149C*	YOR331C*****	YGR272C*
	YLR374C*	YKL136W*	YGR160W*	BRP1****	BUD30*	API2**	YDR008C*	YDL041W*

Figures
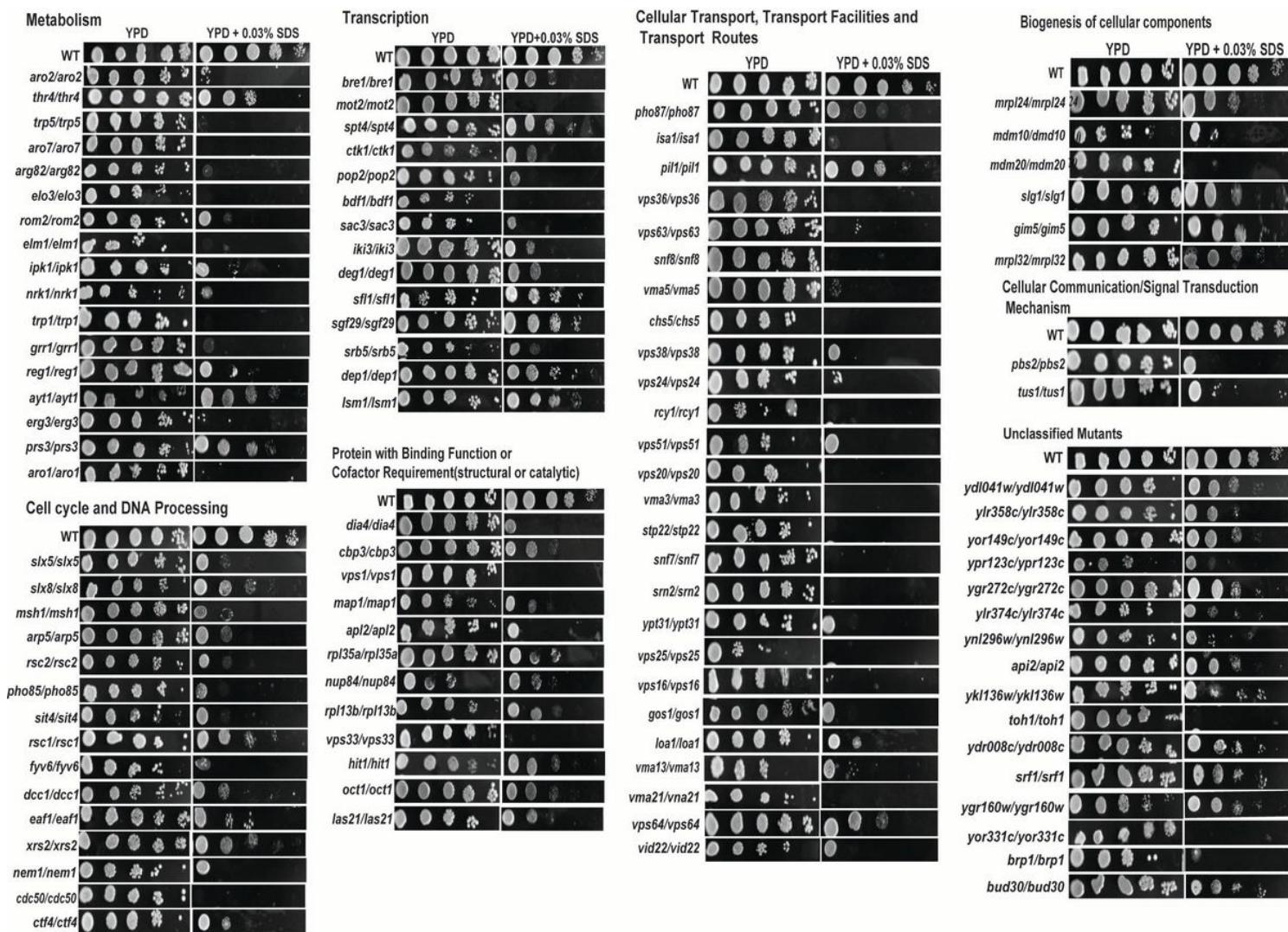
Figure 1

Phenotypes of the 108 SDS-sensitive deletion mutants of each functional category. Cells of the wild-type BY4743 and SDS-sensitive gene deletion mutants identified from the genome-scale screen were grown at 30 °C in liquid YPD overnight, serially diluted by 10 times and spotted on YPD plates with or without 0.03% SDS, respectively. Plates were incubated for 2 days at 30 °C. SDS, sodium dodecyl sulfate.
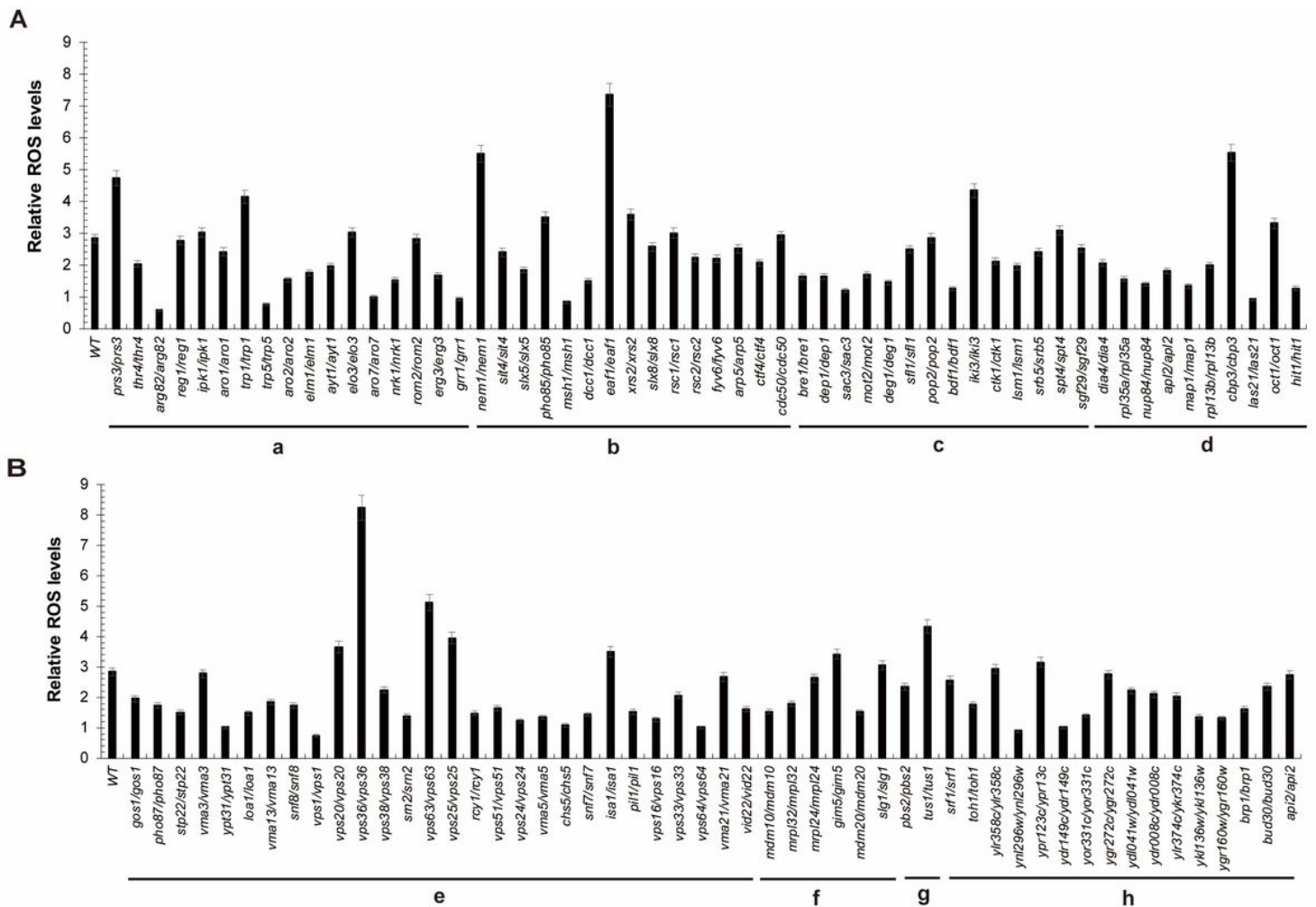
Figure 2

2 Relative ROS levels of 108 SDS-sensitive gene mutants in response to SDS stress. a: Metabolism; b: Cell cycle and DNA Processing; c: Transcription; d: Protein with Binding Function or Cofactor Requirement (structural or catalytic); e: Cellular Transport, Transport Facilities and Transport Routes; f: Biogenesis of cellular components; g: Cellular communication/signal transduction mechanism; h: Unclassified Proteins. Log-phase cells were grown with or without 0.015% SDS for two hours before they were collected for measurement of intracellular ROS levels stained by the dihydroethidium. The relative ROS levels of these SDS-sensitive mutants were listed according to their categories. Each date indicated the ratio between levels of ROS in YPD+SDS versus YPD alone. The value is the average of three independent assays for each strain.
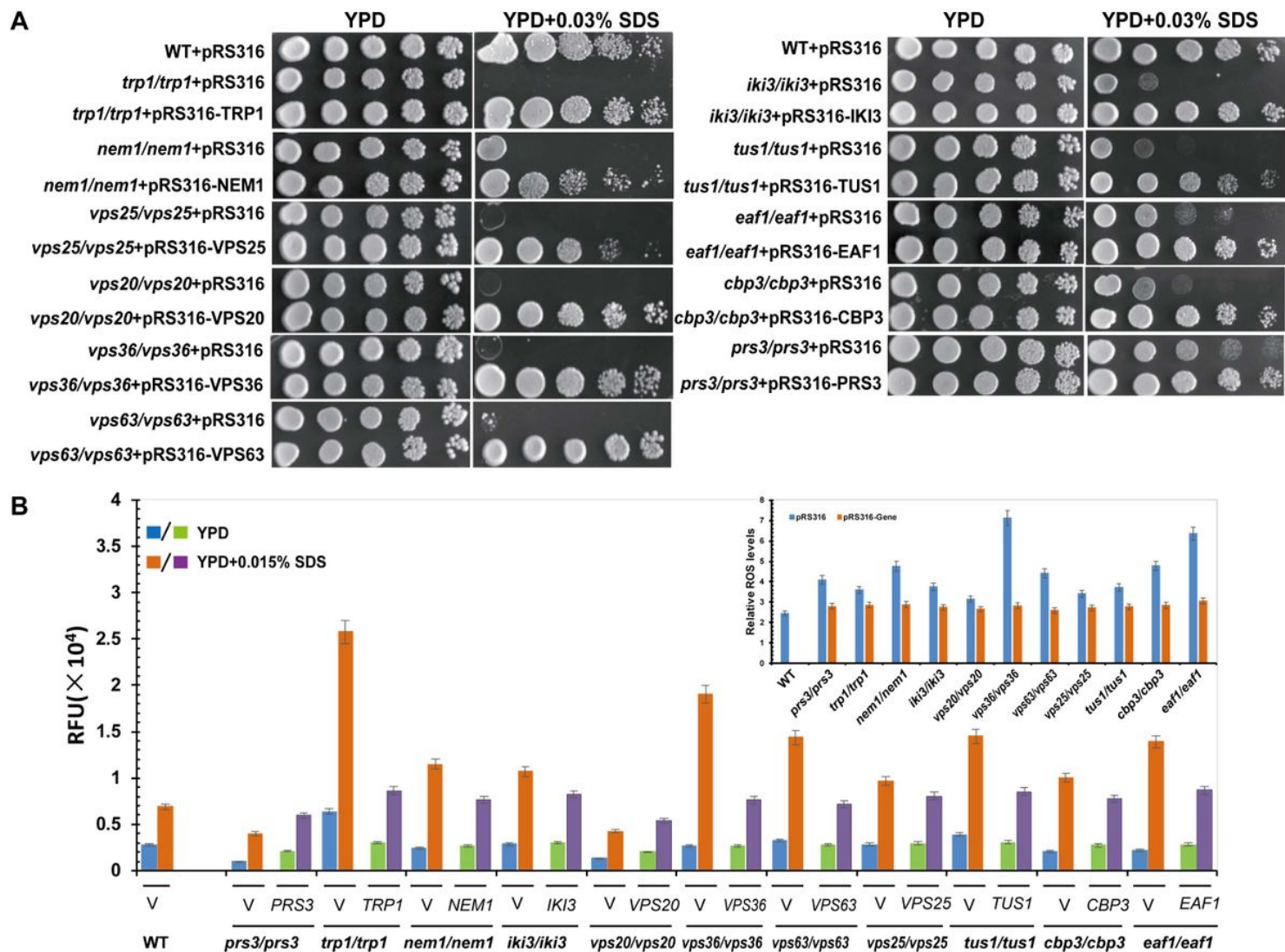
Figure 3

Introducing the conrresponding genes back into the mutants surpress their SDS-sensitive and high intracellular ROS levels. (A) Complementation of the constructed expression plasmid in the sensitivity of the corresponding mutant to 0.03% SDS. (B) Intracellular ROS levels of the 11 indicated SDS-sensitive gene mutants in response to SDS stress. Strains containing the indicated plasmid were cultured in SD-URA over night for the complementary assay. To analyze the intracellular ROS levels, strains containing the indicated plasmid were first cultured to log-phase before being shifted to YPD with 0.015% SDS for additional two hours before they were collected for measurement of intracellular ROS levels stained by the dihydroethidium. The value is the average of three independent assays for each strain.
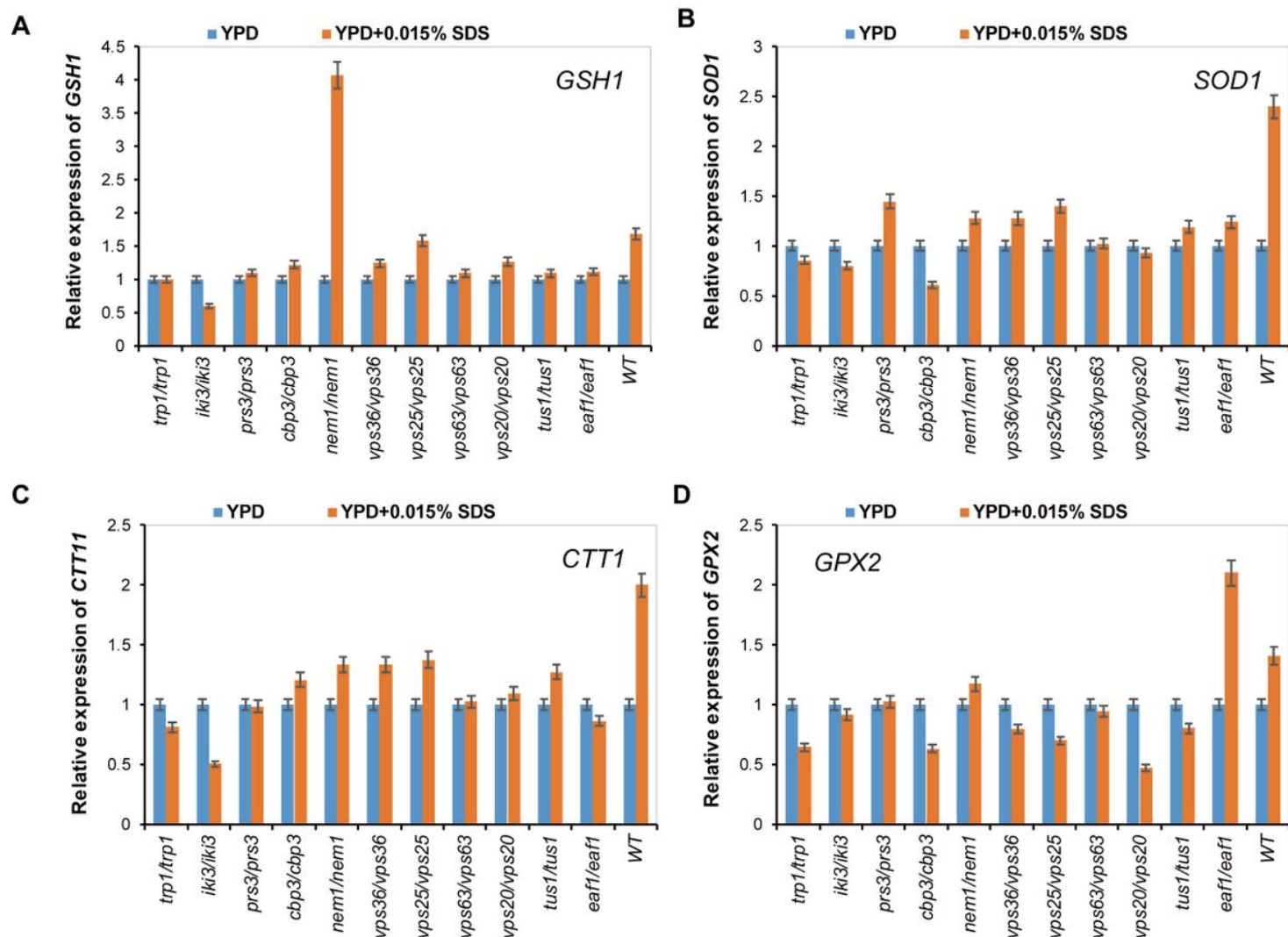
Figure 4

The expression of GSH1, SOD1, CTT1 and GPX2 genes coding for the antioxidant defenses are regulated by SDS stress. (A-D) WT and the indicated 11 mutants were treated to SDS medium for 1 h. The expression of the indicated genes was tested by qRT-PCR. The value is the average of three independent assays for each strain.