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Research Article

Yeast require an Intact Tryptophan Biosynthesis Pathway and Exogenous Tryptophan for Resistance to Sodium Dodecyl Sulfate

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Sodium Dodecyl Sulfate, or SDS, is an anionic detergent with widespread use in industrial and household cleaning products, scientific laboratories, and personal care products such as toothpaste and shampoo. The potential toxicity of SDS has been well-characterized in whole organism studies and its potential effects on the environment continue to be studied. Herein, we undertake a chemical-genetic screen to explore whether low concentrations of SDS have any discernible effects at the cellular level. Our screen of the homozygous diploid yeast deletion collection identified numerous gene deletions that confer sensitivity to SDS. Subsequent bioinformatics and biological analyses reveal that yeast unable to synthesize tryptophan are especially sensitive to the presence of SDS. Interestingly, even wild-type yeast with an intact tryptophan biosynthetic pathway exhibit growth defects in the presence of SDS on media lacking tryptophan. Altogether, we have shown that low levels of SDS, primarily through effects on tryptophan availability, impact the basic cell biology of a eukaryotic cell.

Keywords: *Saccharomyces cerevisiae*; genome-wide chemical-genetic screen; SDS

Introduction

The anionic detergent sodium dodecyl sulfate (SDS; $C_{12}H_{25}C_6H_4SO_3Na$), also known as sodium lauryl sulfate, is a commonly used synthetic surfactant in industry, as well as in household cleansers and personal hygiene products including shampoo, toothpaste, and cosmetics. SDS is also extensively used in scientific laboratories, primarily to disrupt cellular or intracellular membranes, or as a protein denaturant/charge neutralizer in analytical applications such as SDS-PAGE (Polyacrylamide Gel Electrophoresis). The specific mechanism by which SDS denatures the tertiary structure of proteins is still being actively investigated (Bhuyan, 2009). Due to its widespread human use, animal studies on the effects of SDS exposure have long been conducted and studies of anionic surfactant toxicology in the environment continue to be published (Final safety report, 1983**;** Scott and Jones, 2000; Wibbertmann *et al*., 2011). Such studies have revealed that while SDS is a potent eye, skin, and respiratory tract irritant, these effects are generally temporary and alleviated by extensive rinsing and/or fresh air. Additionally, the amounts of SDS consumers are generally exposed to are orders of magnitude lower than amounts that cause adverse effects in animal studies (Wibbertmann *et al*., 2011). Of note, however, safety studies look broadly at toxicity by examining whole organ or system effects (visible skin irritation/dermatitis upon contact; nausea or vomiting upon ingestion, etc.).

In order to investigate whether very low concentrations of SDS have specific effects on the general biological processes of a cell, we utilized the model single-celled eukaryotic organism *Saccharomyces cerevisiae*, also known as the budding yeast. Yeast deletion collections exist, which permit the genome-wide analysis of growth under a given condition, for example, in the presence of a drug (Winzeler *et al*., 1999; Giaever *et al*., 2002; McClellan *et al*., 2007). In this study, we conducted a chemical-genetic screen examining the ability of ~5,800 homozygous diploid yeast deletion strains to grow in the absence and presence of a low concentration (0.015%) of SDS. Our initial growth experiments, coupled with extensive re-testing and bioinformatics analyses, reveal for the first time that SDS strongly affects the viability of yeast that are unable to synthesize their own tryptophan. Further, our results suggest that an intact tryptophan biosynthesis pathway, in the absence of exogenous tryptophan, is inadequate to permit growth in the presence of SDS. Thus, it appears that SDS has specific effects on cell biological processes beyond its known abilities to disrupt cellular membranes and denature proteins.

Results

Genome-wide Screen for Nonessential Yeast Genes Required for Resistance to SDS

The entire collection of BY4743 background homozygous diploid yeast deletion strains (~5800 strains; Winzeler *et al.*, 1999) was tested for growth on rich media (YPD; Yeast extract, Peptone, Dextrose) lacking or containing 0.015% SDS. This concentration of SDS was chosen based on research demonstrating that 0.01% SDS slows the growth of wild-type (WT) yeast in liquid culture (Sirisattha *et al.*, 2004). All sets of plates were examined by at least two different individuals; deletion strains that exhibited poor growth on SDS (for example, see Figure 1) by consensus of all individuals that examined a given set of plates, were included in the initial candidate deletion strain list of 156 strains (Table 1).

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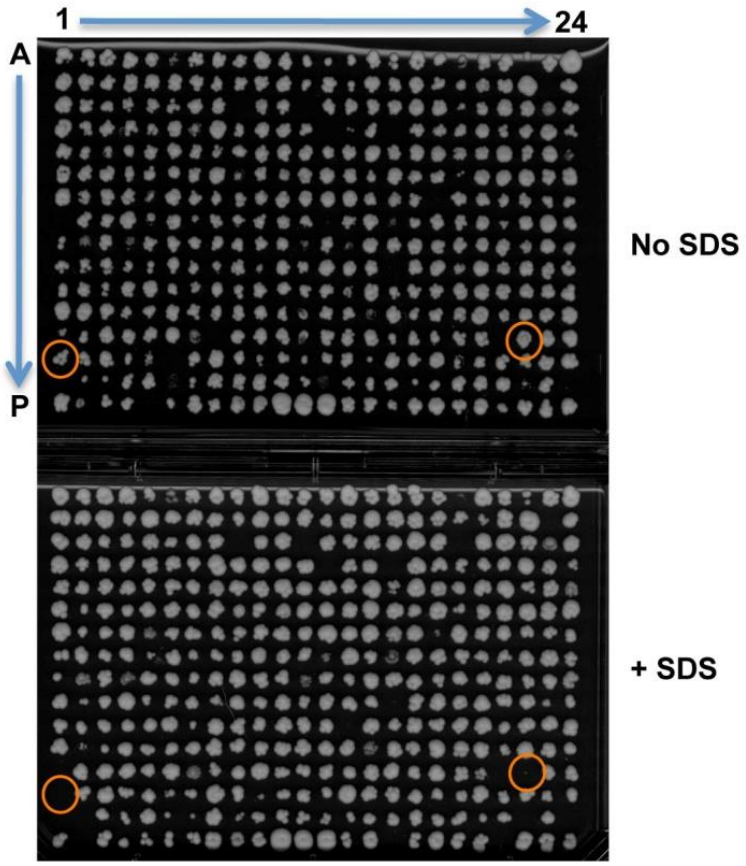

Figure 1: Example of 384-well plate spotting and growth comparison. Homozygous yeast deletion strains spotted onto rich media (YPD) without (top; No SDS) or containing (bottom; + SDS) 0.015% SDS. Orange circles indicate strains that grew in the absence, but not presence, of SDS (384-well plate coordinates M22 and N1, in this example).

Table 1: Candidate SDS-Sensitive Yeast Deletion Strains Subjected to Validation

ORF	*GENE*	*ORF*	*GENE*	*ORF*	*GENE*	*ORF*	*GENE*
YAL047C	**SPC72**	**YER086W**	**ILV1**	YJL214W	HXT8	YNL252C	MRPL17
YBL005W	PDR3	**YER090W**	**TRP2**	YJR063W	RPA12	YNL255C	GIS2
YBL006C	LDB7	YER111C	SWI4	**YJR102C**	**VPS25**	YNL275W	BOR1
YBL085W	BOI1	YFL013W-A	Unknown	YJR105W	ADO1	**YNL280C**	**ERG24**
YBR009C	HHF1	YFL025C	BST1	YKL017C	HCS1	YNL314W	DAL82
YBR068C	BAP2	YFL041W	FET5	**YKL211C**	**TRP3**	YNL334C	SNO2
YBR127C	VMA2	YFR018C	YFR018C	**YKR001C**	**VPS1**	YNR007C	ATG3
YBR134W	YBR134W	YGL012W	ERG4	YKR054C	DYN1	**YNR052C**	**POP2**
YBR176W	ECM31	**YGL026C**	**TRP5**	YLR025W	SNF7	YOL009C	MDM12
YBR222C	PCS60	YGL070C	RPB9	**YLR148W**	**PEP3**	YOL012C	HTZ1
YBR240C	THI2	**YGL071W**	**AFT1**	YLR235C	YLR235C	**YOL025W**	**LAG2**
YBR267W	REI1	**YGL084C**	**GUP1**	**YLR242C**	**ARV1**	**YOL086C**	**ADH1**
YBR289W	SNF5	YGL148W	ARO2	YLR244C	MAP1	YOR037W	CYC2
YCL025C	AGP1	**YGL167C**	**PMR1**	**YLR268W**	**SEC22**	YOR080W	DIA2
YCR020W-B	**HTL1**	YGL206C	CHC1	**YLR304C**	**ACO1**	YOR094W	ARF3
YCR053W	THR4	YGL211W	NCS6	YLR320W	MMS22	**YOR144C**	**ELG1**
YCR076C	FUB1	YGL223C	COG1	YLR330W	CHS5	YOR187W	TUF1
YCR094W	**CDC50**	YGR078C	PAC10	YLR338W	YLR338W	**YOR200W**	**YOR200W**
YCR107W	AAD3	YGR092W	DBF2	YLR369W	SSQ1	YOR205C	GEP3
YDL006W	**PTC1**	YGR152C	RSR1	**YLR417W**	**VPS36**	YOR237W	HES1
YDL106C	PHO2	YGR162W	TIF4631	YML009C	MRPL39	**YOR252W**	**TMA16**
YDR007W	**TRP1**	**YGR167W**	**CLC1**	YML012W	ERV25	YOR263C	YOR263C
YDR027C	**VPS54**	YGR174C	CBP4	YML013C-A	Unknown	YPL032C	SVL3
YDR115W	YDR115W	YGR183C	QCR9	YML095C-A	Unknown	**YPL045W**	**VPS16**
YDR162C	**NBP2**	YGR184C	UBR1	**YML097C**	**VPS9**	YPL055C	LGE1
YDR178W	SDH4	YGR234W	YHB1	YML110C	COQ5	YPL071C	YPL071C
YDR194C	**MSS116**	YGR236C	SPG1	YMR011W	HXT2	YPL096W	PNG1
YDR195W	**REF2**	YGR252W	GCN5	YMR016C	SOK2	YPL103C	FMP30
YDR207C	**UME6**	**YGR262C**	**BUD32**	**YMR021C**	**MAC1**	YPL111W	CAR1
YDR226W	**ADK1**	YHL030W	ECM29	**YMR077C**	**VPS20**	YPL135W	ISU1
YDR270W	CCC2	YHL031C	GOS1	YMR091C	NPL6	**YPL215W**	**CBP3**
YDR320C	SWA2	**YHR026W**	**VMA16**	YMR145C	NDE1	YPL248C	GAL4
YDR326C	YSP2	YHR028C	DAP2	YMR190C	SGS1	**YPR049C**	**ATG11**
YDR347W	MRP1	YHR111W	UBA4	YMR207C	HFA1	YPR065W	ROX1
YDR354W	**TRP4**	YHR129C	ARP1	YMR226C	YMR226C	YPR066W	UBA3
YDR442W	YDR442W	YHR167W	THP2	YMR244W	YMR244W	**YPR072W**	**NOT5**
YER047C	SAP1	YIL154C	IMP2'	**YNL133C**	**FYV6**	YPR201W	ARR3
YER074W	RPS24a	**YJL028W**	**YJL028W**	YNL246W	VPS75	YNL252C	MRPL17
YER083C	GET2	YJL193W	YJL193W	**YNL250W**	**RAD50**	YNL255C	GIS2

Of the ~5,800 deletion strains screened for growth on media containing 0.015% SDS, the 156 listed above were initially scored as deficient for growth in the presence of SDS. All 156 strains were subsequently individually retested by serial dilution growth assays. Those highlighted in bold above represent the 46 deletion strains ultimately confirmed to have SDS-induced growth defects (herein the data set).

Each candidate strain was then individually tested versus wild-type (WT) BY4743 yeast for growth on YPD lacking or containing SDS. Representative examples of deletion strain re-testing by serial dilution are shown in Figure 2. As expected from previous work (Sirisattha *et al*., 2004), some inhibition of WT growth is observed on plates containing 0.015% SDS. While many re-tested deletion strains did not exhibit a significant loss of viability on SDS (for example, Figure 2, strains Δ*swi4*, Δ*rpa12*, Δ*mrpl39*), nearly 30% of re-tested strains were confirmed to exhibit little to no growth in the presence of SDS (for example, Figure 2, strains Δ*pop2*, Δ*spc72*, Δ*trp3*). Overall, 46 strains comprise our final data set of confirmed SDS-sensitive deletion strains (Table 1, bold).

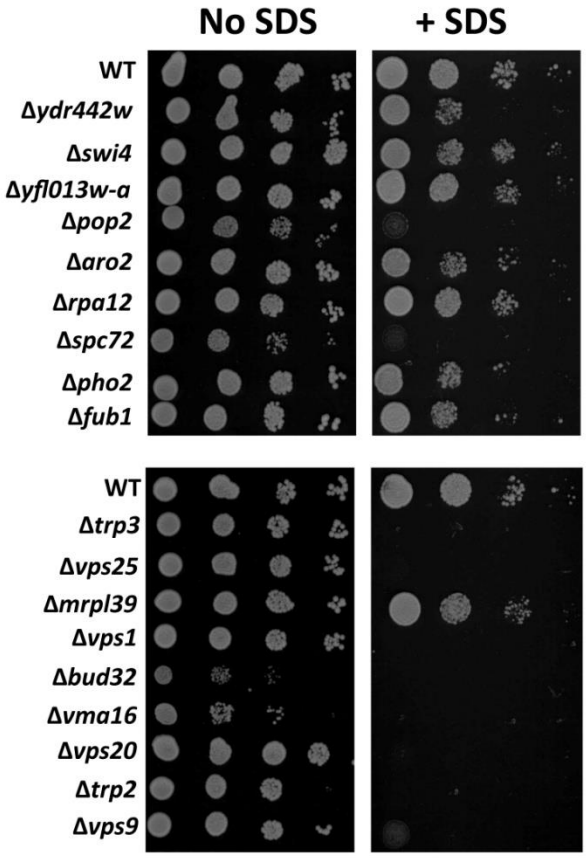

Figure 2: Candidate strain re-testing. Representative serial dilutions on YPD plates lacking (No SDS) or containing (+ SDS) 0.015% SDS. Spots, from left to right, are ten-fold serial dilutions starting from cultures diluted to an OD_{600} of 0.01 (0.01, 0.001, 0.0001, 0.00001).

Bioinformatic Analyses Suggest Tryptophan Biosynthesis is Important for SDS Tolerance

In order to identify any specific cell biological processes in yeast affected by growth in the presence of SDS, we utilized FunSpec (http://funspec.med.utoronto.ca/; Robinson *et al*., 2002) to determine any statistically significant Gene Ontology (GO; Ashburner *et al*., 2000) classifications amongst our data set genes. Of the top five enriched GO Biological Process categories, four of them relate to amino acid biosynthesis, specifically, to the tryptophan biosynthetic pathway (Table 2; see also Figure 4). Of note, the GO classification for protein targeting to the vacuole is also statistically significant within our data set. The FunSpec analysis also identified that 24 of our 46 data set deletion strains are classified as slow growing (Δ*spc72*, Δ*htl1*, Δ*vps54*, Δ*mss116*, Δ*ref2*, Δ*ume6*, Δ*adk1*, Δ*aft1*, Δ*gup1*, Δ*pmr1*, Δ*clc1*, Δ*bud32*, Δ*vma16*, Δ*pep3*, Δ*arv1*, Δ*sec22*, Δ*aco1*, Δ*fyv6*, Δ*rad50*, Δ*erg24*, Δ*pop2*, Δ*adh1*, Δ*vps16*, Δ*not5*; hereafter collectively referred to as "slow growers"). As strains lacking those genes have inherent fitness defects (Giaever *et al*., 2002), it is possible that their growth will be further impeded by numerous types of drug treatments and may not be specific to SDS (see Discussion). To account for this possibility, we re-ran the FunSpec analysis with a truncated data set list excluding slow growers. Importantly, this did not change the top five enriched GO Biological Processes and, in fact, increased the statistical significance ascribed to the four categories associated with amino acid biosynthesis (Table 2, p-value column versus p-value (slow growers removed) column).

Table 2: Top Five Statistically Significant Gene Ontology Classifications

Enriched GO Biological Process Categories	**Data Set Genes in Category**	**p-value**	**p-value** (slow growers removed)	**Data Set Genes/ Total Genes** (in Category)
tryptophan biosynthetic process [GO:0000162]	TRP1 TRP4 TRP2 TRP5 TRP3	1.313e-11	2.531e-13	5/5
aromatic amino acid family biosynthetic process [GO:0009073]	TRP1 TRP4 TRP2 TRP5 TRP3	1.002e-08	1.967e-10	5/12
tryptophan metabolic process [GO:0006568]	TRP1 TRP5 TRP3	3.165e-07	3.211e-08	3/3
protein targeting to vacuole [GO:0006623]	VPS25 VPS1 VPS9 VPS9 VPS16	8.16e-06	8.634e-06	5/41
cellular amino acid biosynthetic process [GO:0008652]	TRP1 TRP4 ILV1 TRP2 TRP5 TRP3	5.313e-05	5.643e-07	6/98

The final data set of 46 deletion strains was analyzed by FunSpec (http://funspec.med.utoronto.ca/; Robinson *et al*., 2002) to identify any statistically enriched Gene Ontology (GO) Biological Process classifications. The five most significantly enriched GO Biological Processes are displayed, as well as the data set genes ascribed to each category, relevant p-values, and the fraction of total genes assigned to each category that occur in our data set.

We next analyzed our data set with regard to established connections (published genetic and/or physical interactions) between the genes therein. The resulting interaction network (Figure 3) contains 27 well-connected nodes (including 11 slow growers), six nodes with only one connection (five of the six are slow growers), and 13 loner nodes (seven of which are slow growers). The most well-connected and high-confidence module of the interaction network consists of the five genes involved in tryptophan biosynthesis, *TRP1*, *TRP2*, *TRP3*, *TRP4*, and *TRP5* (Figure 4).

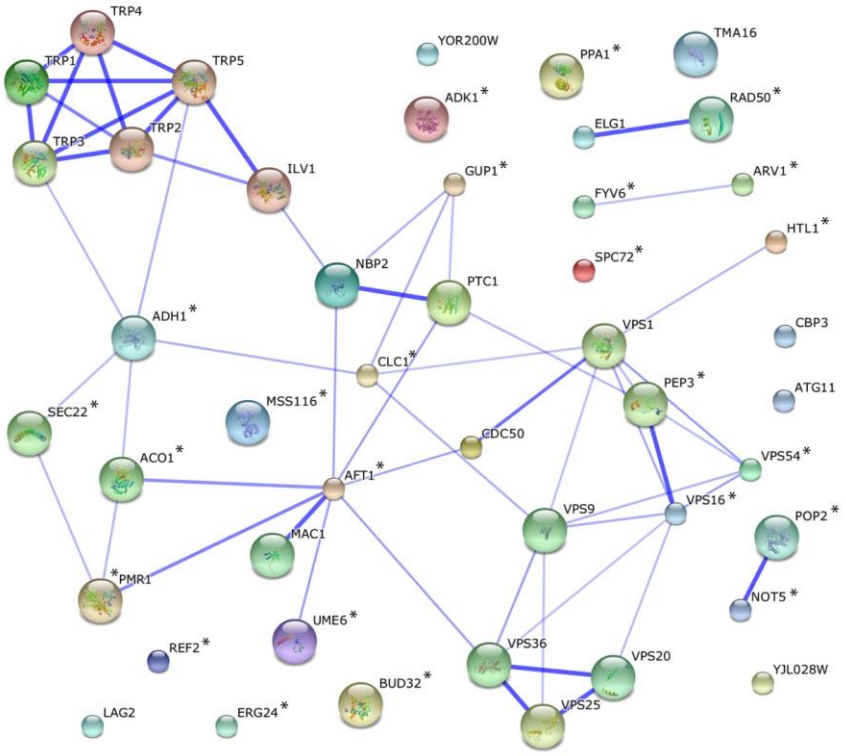

Figure 3: STRING v9.1 (Franceschini *et al*., 2013; http://string-db.org/) generated protein-protein interaction network for final data set. The degree of confidence for a given interaction is indicated by the thickness of the edge connecting two nodes (the thicker the edge, the more established the interaction). Inherently slow growing strains in the data set are indicated with an asterisk (*).

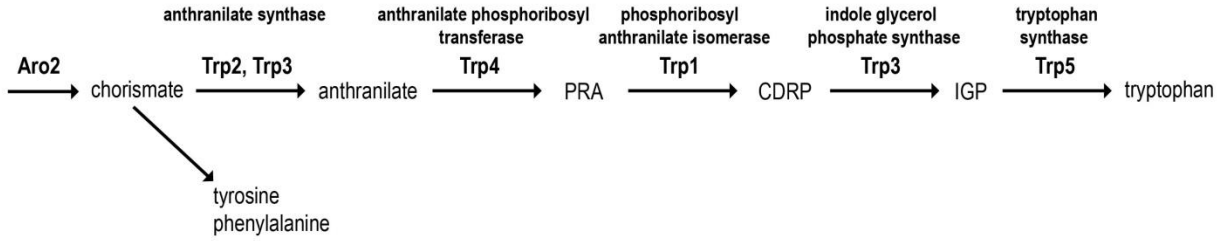

Figure 4: Outline of the yeast tryptophan biosynthesis pathway. Enzymes encoded by *TRP* genes are printed in bold above the relevant protein-encoding gene name(s). Abbreviations used: PRA = N-(5'-phosphoribosyl)-anthranilate, CDRP = 1-(o-carboxyphenylamino)-1'-deoxyribulose-5'-phosphate, IGP = indole glycerol phosphate.

The Effects of SDS on Tryptophan Biosynthesis and Uptake

The results thus far support that yeast without the ability to make tryptophan are unable to grow in the presence of SDS. As an important control, we next tested whether restoring tryptophan biosynthesis to a deficient strain rescues growth in the presence of SDS. Indeed, Δ*trp1* yeast transformed with a plasmid harboring a functional *TRP1* gene (pESC TRP1, Agilent Technologies) grew well on YPD both lacking and containing SDS (Figure 5, bottom row). We also tested whether *ARO2*, a gene important for the synthesis of the tryptophan, tyrosine, and phenylalanine precursor chorismate (see Figure 4), was critical for SDS tolerance. Figure 5 shows that Δ*aro2* yeast are viable both in the absence and presence of SDS, suggesting that the problem lies specifically with tryptophan synthesis and not with the general production of aromatic amino acids. Next, we explored whether it is possible that yeast lacking components of the tryptophan biosynthesis pathway cannot grow in the presence of SDS due to problems with tryptophan uptake from the media. Yeast lacking either *TAT1* or *TAT2*, which encode a low-affinity tryptophan permease and high-affinity tryptophan permease, respectively (Schmidt *et al*., 1994), grew in the absence and presence of SDS (Figure 5). This result suggests that yeast with an intact tryptophan biosynthesis pathway are viable on SDS either due to synthesis of their own tryptophan, or due to adequate uptake of tryptophan from the media. To distinguish between these possibilities, we next examined the ability of WT and Δ*trp* yeast to grow on YPD or synthetic media lacking tryptophan (-TRP), in the absence and presence of SDS. As expected, all strains tested grew robustly on YPD lacking SDS (Figure 6, left panel) and strains defective for tryptophan biosynthesis (Δ*trp1*, Δ*trp2*, Δ*trp3*, Δ*trp4*, Δ*trp5*) were unable to grow on YPD containing SDS, or on –TRP media lacking or containing SDS (Figure 6). Surprisingly, however, while WT yeast grew well on –TRP media lacking SDS, WT yeast grew poorly on –TRP media containing SDS, despite having an intact tryptophan biosynthetic pathway. This suggests that, at least in the presence of SDS, TRP+ yeast obtain the tryptophan that they require from the media, not via tryptophan biosynthesis.

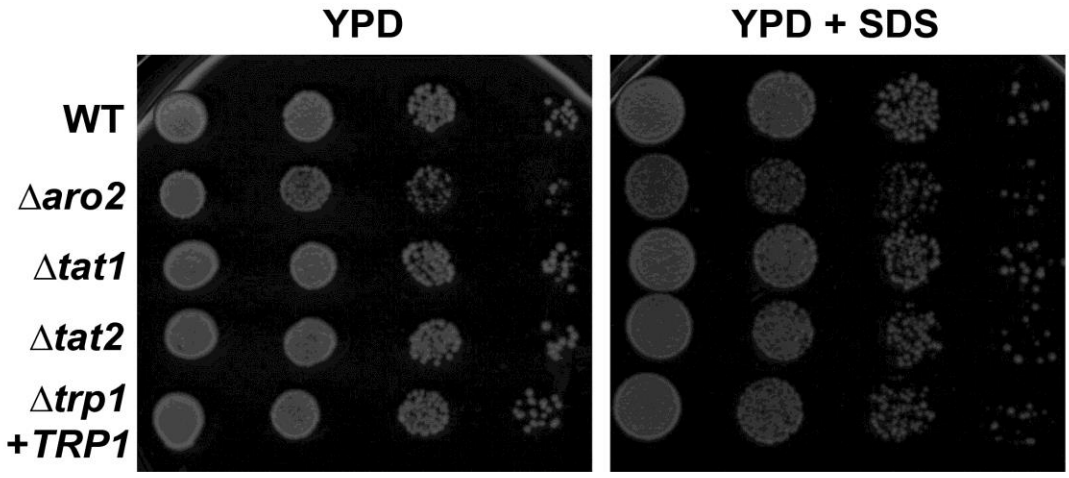

Figure 5: Yeast lacking *ARO2*, *TAT1*, or *TAT2* grow on YPD containing SDS. Representative serial dilutions on YPD plates lacking or containing 0.015% SDS. Spots, from left to right, are ten-fold serial dilutions starting from cultures diluted to an OD_{600} of 0.01 (0.01, 0.001, 0.0001, 0.00001).

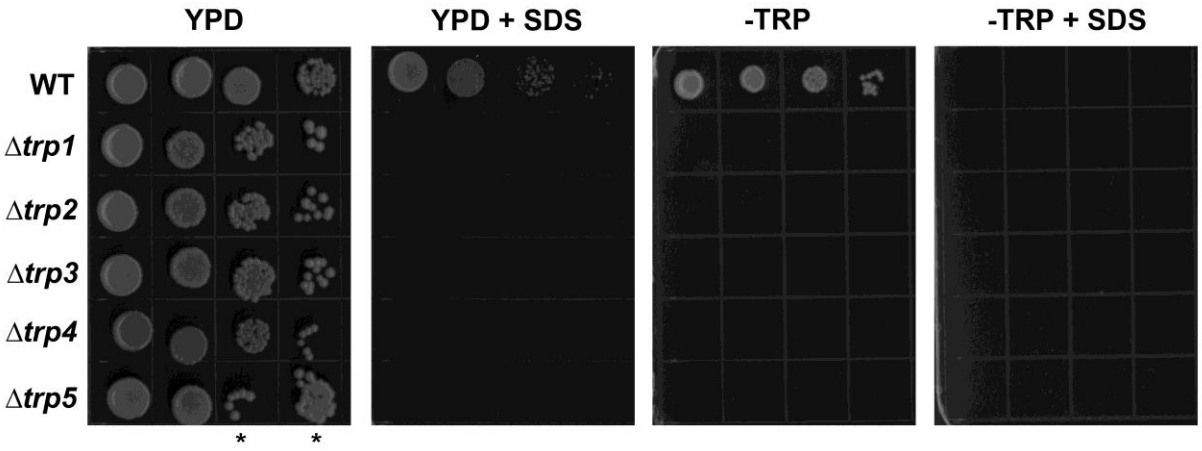

Figure 6: Representative serial dilutions on YPD and –TRP plates lacking or containing 0.015% SDS**.** Spots, from left to right, are ten-fold serial dilutions starting from cultures diluted to an OD_{600} of 0.01 (0.01, 0.001, 0.0001, 0.00001). Asterisks (*) indicate that the 0.0001 and 0.00001 dilution spots for Δ*trp5* are reversed.

Discussion

Our chemical-genetic screen of ~5,800 homozygous diploid yeast deletion strains identified 46 genes that, when deleted, compromise the ability of yeast to grow in the presence of SDS (Table 1). Bioinformatic analyses of the 46 gene data set revealed that amino acid metabolism, specifically that of tryptophan, was the primarily affected cellular process (Table 2; Figure 3). Subsequent experiments demonstrated that yeast lacking one or the other tryptophan permease (*TAT1* or *TAT2*) grew well in the presence of SDS, suggesting either that one can compensate for the other, or that it is the process of tryptophan biosynthesis itself that is directly affected by SDS. Notably, while WT yeast grow in the presence of SDS on rich media (YPD), they are unable to grow well in the presence of SDS on media lacking tryptophan (-TRP; Figure 6). Thus, we conclude that yeast that are unable to synthesize their own tryptophan, as well as yeast with intact tryptophan biosynthetic pathways but no available exogenous tryptophan, exhibit SDS-induced growth defects.

A significant number of yeast deletion strains in our final data set are known slow growers (Giaever *et al*., 2002). As mentioned briefly in the Results, yeast deletion strains known to have fitness defects are often further growth-impaired in the presence of various chemicals and, therefore, their occurrence in our data set may not be specific to an effect of SDS on growth, but rather reflect a larger issue of drug tolerance and sensitivity in these yeast. As examples, consider two slow growers from our data set: Δ*arv1* and Δ*aft1* yeast; Δ*arv1* yeast not only grow slowly in the absence of perturbation, but exhibit decreased resistance to numerous chemicals with various modes of action, including the protein synthesis inhibitor cycloheximide (Alamgir *et al*., 2010; Kapitzky *et al*., 2010), the DNA-damaging antibiotic bleomycin (Kapitsky *et al*., 2010), the antifungal nystatin (Tinkelenberg *et al*., 2008), and the HMG-CoA reductase inhibitor lovastatin (Fei W. *et al*., 2008). Yeast lacking *AFT1* also display decreased resistance to multiple chemicals, including various alcohols (ethanol, methanol, propan-1-ol; Auesukaree *et al*., 2009), the anti-malarial drug quinine (Dos Santos and Sa-Correia, 2011), and the DNA modifying drug methyl methanesulfonate (Svensson *et al*., 2011). Now, consider those same two genes in the context of our data set interaction network (Figure 3). While *ARV1* has only a single connection, and that is to another known slow grower (*FYV6*), the *AFT1* node has eight network connections and is connected by two different pathways to the primary *TRP* gene cluster. Additionally, like Δ*aft1* yeast, Δ*trp1*, Δ*trp2*, Δ*trp3*, Δ*trp4*, and Δ*trp5* yeast are hypersensitive to growth in the presence of ethanol (Yoskikawa *et al*., 2009), and yeast lacking *TRP1*, *TRP2*, or *TRP5* grow poorly in the presence of quinine (Khozoie *et al*., 2009). Altogether, this makes it tempting to speculate that *AFT1* may truly belong as part of our analysis, while the exclusion of *ARV1* is unlikely to change our findings. Overall, it seems prudent to carefully consider that some slow growing deletion strains with promiscuous chemical sensitivities are likely specifically affected by SDS, and, as such, their inclusion in our data set may be informative as far as further elucidating the specific biological effects of SDS on yeast.

In addition to the specific examples of ethanol and quinine given above, tryptophan metabolism is often observed as a drug-sensitive process in yeast. In some instances, there is good evidence to support a mechanism, for example, the very strong structural similarity between tryptophan and quinine suggests competition for permease binding as a likely point of action (Khozoie *et al*., 2009). We are unable at this time to provide a concrete explanation for our observed effects of SDS on tryptophan metabolic pathways in yeast. However, it is known that the overexpression of *TAT2* or *TRP1* rescues the sensitivity of yeast to FK506, an immunosuppressive drug (Heitman *et al*., 1993; Schmidt *et al*., 1994), as well as ameliorating other conditions that impair tryptophan uptake in yeast. As our results suggest that both tryptophan uptake and, perhaps more critically, tryptophan biosynthesis, are important for resistance to SDS, similar overexpression experiments would be helpful in further clarifying the underlying mechanism(s) at work. Additionally, as there is some functional redundancy between *TAT1* and *TAT2* as tryptophan permeases, it would be interesting to test the effect of SDS on yeast lacking both genes. Since the double deletion of both *TAT1* and *TAT2* is not lethal (Schmidt *et al*., 1994), these experiments are viable next steps to extend our findings. Finally, a more complete picture of the mode of action of SDS on yeast cellular

processes may be obtained by conducting this screen on the available heterozygous yeast deletion collection. This would permit the addition to our data set of any essential genes involved by identifying genes that are haploinsufficient in the presence of SDS. This study thus represents the first step toward characterizing the mode of action of SDS at the level of the individual cell and its biological processes.

Experimental Procedures

Yeast Media Preparation and Yeast Transformation

Media were prepared and yeast transformation was conducted following standard procedures (Adams *et al*., 2007).

Solid Media Screen of Homozygous Diploid Yeast Deletion Collection

The yeast homozygous diploid collection, comprising ~5,800 deletion strains (Winzeler *et al*., 1999; Thermo Scientific) was arrayed using a Floating Pin Replicator (V&P Scientific, Inc.) onto solid YPD media (1% yeast extract, 2% peptone, 2% glucose) lacking or containing 0.015% SDS (Sigma-Aldrich). Plates were incubated at 30 °C until colonies appeared (generally 2-3 days). Plates were inspected by eye to identify deletion strains that grew poorly in the presence, but not absence, of SDS.

Bioinformatic Analyses

The final data set of 46 deletion strains was analyzed using FunSpec (Robinson *et al*., 2002; http://funspec.med.utoronto.ca/) to identify statistically enriched Gene Ontology Biological Process classifications. All available databases were selected for the query, the Bonferroni correction was not utilized, and the p-value cutoff was set to 0.001. The final data set of 46 deletion strains was also analyzed using STRING v9.1 (Franceschini *et al*., 2013; http://string-db.org/). The resulting protein-protein interaction network is shown in Figure 3.

Serial Dilution Growth Assays

WT (BY4743; Thermo Scientific) and candidate SDS-sensitive deletion strains were inoculated into 2 mL YPD and grown overnight in a shaking incubator at 30 °C. The next day, the optical density at 600 nm (OD_{600}) of each strain was determined with a GENESYS™ 20 visible spectrophotometer (Thermo Scientific). Each strain was then diluted to an OD_{600} of 0.01, 0.001, 0.0001, and 0.0001 and 5 □L of each dilution was spotted onto solid media plates. Plates were incubated at 30 °C until colonies appeared (generally 2-3 days). For experiments comparing growth on media containing or lacking tryptophan, both YPD and –TRP dropout media (2% glucose, 0.68% yeast nitrogen base lacking amino acids, and 0.2% amino acid dropout mix lacking tryptophan) were utilized.

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