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Construction and application of a protein and genetic interaction network (yeast interactome)

Gregory R. Stuart[1], William C. Copeland[1,*] and Micheline K. Strand[1,2]

[1]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences and [2]Life Sciences Division, U.S. Army Research Office, Research Triangle Park, NC 27709, USA

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ABSTRACT

Cytoscape is a bioinformatic data analysis and visualization platform that is well-suited to the analysis of gene expression data. To facilitate the analysis of yeast microarray data using Cytoscape, we constructed an interaction network (interactome) using the curated interaction data available from the *Saccharomyces* Genome Database (www.yeastgenome.org) and the database of yeast transcription factors at YEASTRACT (www.yeastract.com). These data were formatted and imported into Cytoscape using semi-automated methods, including Linux-based scripts, that simplified the process while minimizing the introduction of processing errors. The methods described for the construction of this yeast interactome are generally applicable to the construction of any interactome. Using Cytoscape, we illustrate the use of this interactome through the analysis of expression data from a recent yeast diauxic shift experiment. We also report and briefly describe the complex associations among transcription factors that result in the regulation of thousands of genes through coordinated changes in expression of dozens of transcription factors. These cells are thus able to sensitively regulate cellular metabolism in response to changes in genetic or environmental conditions through relatively small changes in the expression of large numbers of genes, affecting the entire yeast metabolome.

INTRODUCTION

Cytoscape (www.cytoscape.org) is an open source bioinformatics software platform originally intended for, but not limited to, the analysis of molecular interaction data associated with changes in gene expression and other data (1). Cytoscape's core distribution provides a basic set of features for data integration and visualization, with additional features available as plugins. Additionally, the visual display properties are highly customizable, including the use of annotation files that allow additional information to be visually represented in a more meaningful manner (Figure 1).

Several years ago, we determined that disruption of the *POS5* gene in *Saccharomyces cerevisiae* results in a 50-fold increase in the reversion of a frameshift deletion in mtDNA and demonstrated that *POS5* encodes a NAD(H) kinase, the sole source of $NADP^+$ and NADPH in the mitochondrion of *S. cerevisiae* (2). In a recent follow-up study, we used a yeast microarray to evaluate the changes in gene expression in *S. cerevisiae* due to genetic and environmental factors associated with oxidative stress (3). To facilitate those analyses, we created a high-quality yeast interaction network (interactome) suitable for use in Cytoscape, illustrated through the analysis of data from a recent diauxic shift experiment in wild-type yeast cells that serves as an in-house reference source of yeast expression data. Analyses of these data additionally revealed that transcription factors and their target genes form highly complex, interconnected networks affecting all aspects of cellular metabolism in *S. cerevisiae*.

MATERIALS AND METHODS

Strain

Saccharomyces cerevisiae strain YPH925 (*ade2-101 cyh2 his3-Δ200 kar1-Δ15 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52*) (4) was employed for this work, and for convenience is referred to as being 'wild-type'.

Bioinformatic platforms

Microarray fluorescence data (3) were imported into Rosetta Resolver (Rosetta Biosoftware, Seattle, WA, USA) for the estimation of random error by application of an error model that calculated the confidence limits (*P*-values) for the expression values. The Agilent GeneSpring Analysis Platform (Agilent Technologies, Palo Alto, CA, USA) was used for LOWESS data normalization. To aid the visualization and the analysis of the

*To whom correspondence should be addressed. Tel: +1 919 541 4792; Fax: +1 919 541 7613; Email: copelan1@niehs.nih.gov

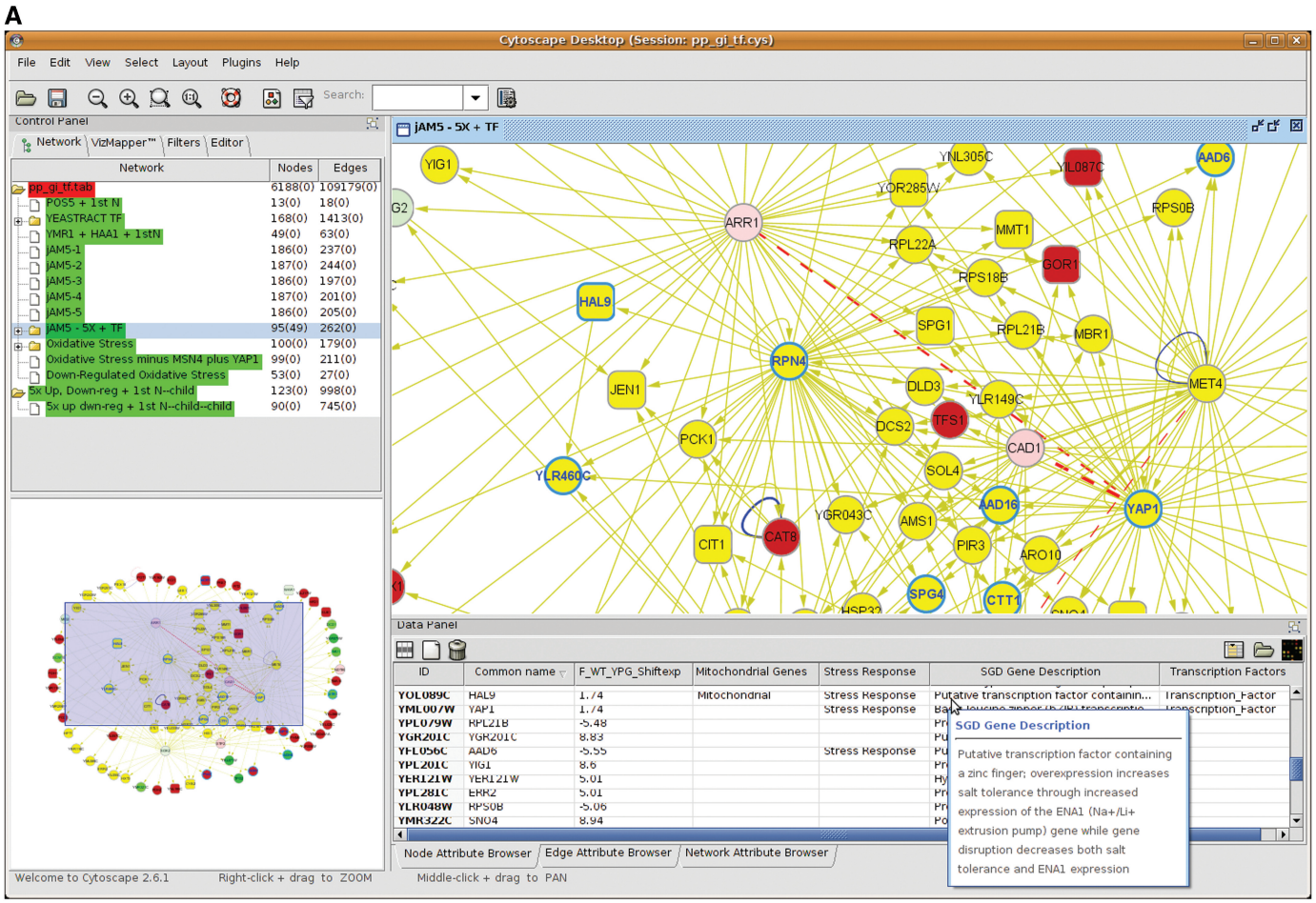

Figure 1. Selected illustration from the yeast interactome, using a screen capture from a Cytoscape 'session' (Cytoscape sessions can be saved, preserving the work for future use). (**A**) This panel illustrates part of the subnetwork identified by the jActiveModules plugin, as described in the text and summarized in Table 1. The main, upper right frame displays a close-up view of some of the genes present in this subnetwork, while the lower left frame shows an overview of the entire subnetwork, with the part shown in the main frame indicated by the shaded region. The upper left frame contains a list of the subnetworks arising from various analyses within Cytoscape, including the numbers of nodes and edges, and the number of these currently selected, that are highlighted in yellow in the displayed subnetworks. In this example, genes directly associated with (i.e. regulated by) transcription factor Rpn4 are selected and shown in yellow (these selections are made from the Select menu). The data panel at the lower right of the image displays information associated with each of these nodes, imported from various user-defined annotation files, including (as shown) common gene names, the expression values, genes associated with the mitochondrion and the response to stress, descriptions of the genes from SGD and genes encoding transcription factors. In this example, transcription factor Hal9 is a mitochondrial stress response gene that was up-regulated 1.7-fold in

expression data, a *S. cerevisiae* protein–protein and protein–DNA interactome was constructed as described in the following section. The microarray expression data were then mapped onto this interactome using Cytoscape.

Genes associated with the highest scoring subnetworks were identified using the Cytoscape jActiveModules plugin. Active Modules are connected subnetworks within the interaction network whose genes show significant coordinated changes in mRNA expression over particular experimental conditions (1). The algorithm iteratively reduces network complexity by pinpointing regions whose states are perturbed by the conditions of interest, while removing false-positive interactions and interactions not involved in the perturbation response. Genes present in each of the five top-scoring networks in wild-type cells shifted to growth in glycerol were identified using the jActiveModules algorithm. Since many of the genes in each of these five subnetworks (186–187 genes each) were present in two or more of these subnetworks, for simplicity these groups of genes were combined, resulting in a single jActiveModules gene list.

Construction of a high-quality yeast interactome

The interactome described in this article was constructed in April 2008 using semi-automated methods to format the interaction data from the *Saccharomyces* Genome Database (SGD: www.yeastgenome.org) and the transcription factor data from YEASTRACT (5) (yeastract.com) into a form suitable for use with Cytoscape, as described in detail in the Supplementary Material (Supplementary file: Constructing a Yeast Interactome). Briefly, the interactions.tab file downloaded from SGD was processed by deleting unnecessary text (e.g. 'Bait' and 'Hit') and columns and reclassifying the various interaction types as either 'pi' (physical interactions, e.g. protein–protein) or 'gi' (genetic interactions, e.g. synthetic lethality). This step dramatically simplified the visual display of the interaction types (edges) between the various nodes (genes; proteins), while allowing us to assign 'weights' to each of the edges, reflecting the numbers of interactions documented between nodes. These interaction weights provided a measure of the number of times that two genes were found to interact with one another in a specific manner, from among the data curated at SGD from various sources.

Next, an interaction file containing a list of *S. cerevisiae* transcription factors and their documented target genes, RegulationTwoColumnTable_Documented_2008410_1839_1043605408.tsv, was downloaded from YEASTRACT. In preparation for use in Cytoscape, the yeast common gene names provided by YEASTRACT were converted to their systematic names, as found at SGD (e.g. POS5 was converted to YPL188W). Next, all letters in the gene names appearing in lowercase were converted to uppercase, again a requirement for Cytoscape. This list of genes was then processed through the 'Batch Download' tool at SGD to identify 'rogue' genes (e.g. MAL63 is not present in the in the systematic sequence of the SGD reference strain S288c; or, two or more genes sharing the same common name). Next, a column of interactions weights (all equal to 1) was appended to the transcription factor interaction file, for compatibility with the weighted SGD interaction data.

The SGD and YEASTRACT plain-text, tab-delimited interaction files were then concatenated as a single file (Supplementary file: pp_gi_tf.tab) and imported into Cytoscape using the import tool located under the File menu. At this time various annotation files, including gene-expression data and lists of stress response and mitochondrial genes, were also imported into Cytoscape. Lastly, the visual display properties of the nodes and edges were defined using the Cytoscape VizMapper tool.

The computer used for this work employed an Intel Pentium 4 CPU operating at 3.0 GHz, 1.5 GB of RAM, and the Microsoft Windows XP Professional Version 2002 Service Pack 2 operating system. To facilitate the steps summarized above associated with manipulating and formatting the raw interaction data files, simple perl and awk scripts were employed using Cygwin (http://www.cygwin.com/), a Linux-like environment for Windows [GNU bash shell, version 3.2.33(18)-release (i686-pc-cygwin]. On Macintosh and Linux-based operating systems, the awk and perl programming languages can be implemented directly in a command shell. Cytoscape is available for any major computer platform, including the Windows, Macintosh and Linux operating systems. All of the tools and source data described in this article are freely available from the indicated sources, while the yeast interactome described in this article is provided as Supplementary Material (Supplementary file: pp_gi_tf.cys) a Cytoscape session file with 6,188 nodes (genes/proteins) and 109,179 edges (interactions), that also includes the sample microarray expression data from our yeast diauxic shift experiment, plus the VizMapper visual display settings. For use with older versions of Cytoscape (or for use with other platforms), data files separately containing the diauxic shift expression data, the interactome (Supplemental file: pp_gi_tf.tab), node annotation files (lists of genes associated with the mitochondrion, the response to stress or transcription factors; common gene names; SGD gene descriptions), as well as the VizMapper visual display properties

wild-type diauxic-shifted cells. Also illustrated is a a box containing the SGD gene description, that automatically pops up when the cursor is placed on top of a gene description, here showing the complete SGD gene description for Hal9. Mitochondrial-associated nodes are shown as rectangles, and nodes associated with the response to stress are drawn with emphasized, blue borders with the gene names also displayed in blue text. Node–node interactions (edges) are color-coded with blue edges indicating protein–protein interactions, gold edges indicating transcription factors with the arrow pointing from the transcription factor toward the regulated gene, and broken red edges indicating genetic (rather than physical) interactions, e.g. synthetic lethality—the loss of viability when both alleles are inactivated. Nodes in which the blue edge loops back on itself indicate self-regulated genes. The thickness of the edges represent weights, i.e. heavier edges indicate more (multiple) interactions, of the indicated type, between two nodes, as described in the Materials and Methods section. (**B**) The 'Gradient Editor for Node Color' editor from the Cytoscape VizMapper tool. In each of the Cytoscape displays in this article, up-regulated genes (nodes) are colored red, while down-regulated genes are colored green, with the extent of shading proportional to the level of expression as indicated in an expanded view in the image in the lower part of this panel.

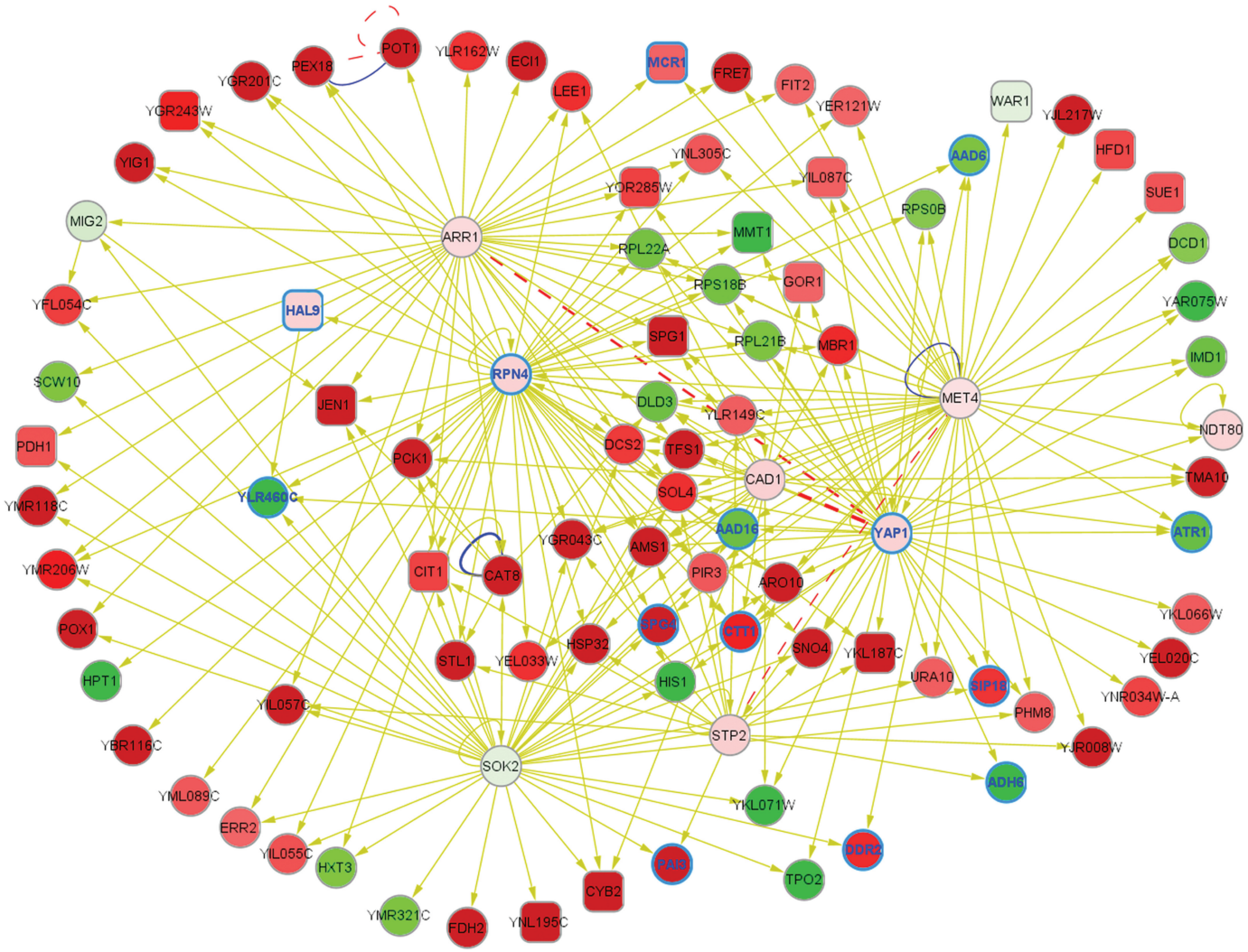

Figure 2. A schematic showing the 95 genes present among the five top-scoring Cytoscape jActiveModules subnetworks from YPG-shifted cells with changes in expression ≥5-fold, plus the associated transcription factors, displayed using Cytoscape, and also shown at lower magnification in the lower left frame in Figure 1A. For convenience, these genes are also summarized in Table 1. For a description of the visual display elements (node colors, etc.), please refer to the Figure 1 legend.

(vizmap.props) file, and lastly an Excel look-up table that can be used to convert common yeast names to their systematic gene name are included in the supplementary interactome files: (Supplementary interactome files: Mitochondrial_Gene_Names.txt; Stress_Response_Genes.txt; Transcription_Factor_Gene_List.txt; Common_Gene_Names.txt; SGD_Gene_Descriptions.txt; vizmap.props; Common_to_Systematic_Name_Lookup_Table.xls; WT_YPG_Shift_Expression_Data.pvals).

RESULTS

Changes in expression occurring during a glycerol-induced diauxic shift

In a related study (3), we examined and compared changes in gene expression in cells containing a deletion of the *POS5* gene using Cytoscape (1), a bioinformatic data analysis and visualization tool. Here, we describe the construction of the robust yeast interactome used in those analyses. To better describe the application and versatility of this interactome, we describe the results from a parallel study from our laboratory that examined gene expression in a wild-type yeast strain grown to the mid-logarithmic phase of growth, then shifted to growth in glycerol for 2 h.

Similar to previous reports (6–12), we observed profound changes in gene expression following the switch from a fermentable carbon source (glucose) to the nonfermentable carbon source (glycerol), as summarized in Figure 2 and Table 1. Specifically, 3777 of the 6256 genes on the Agilent yeast chip (60.4%) were found to be differentially expressed at a significance level of $<10^{-4}$ (at this level of significance, ~0.6 false-positives were expected). To reduce this list of genes to a more meaningful and manageable dataset, we used the Cytoscape jActiveModules plugin to identify genes showing coordinated, significant changes in expression.

Table 1. Expression levels of genes in glycerol-shifted wild-type cells identified by the Cytoscape jActiveModules, with expression values ≥5-fold up- or down-regulated, plus their associated transcription factors

Systematic name	Common name	Fold-change[a]	*Saccharomyces* Genome Database gene description (abbreviated)
YIL160C	POT1	74.67	3-ketoacyl-CoA thiolase; cleaves 3-ketoacyl-CoA into acyl-CoA and acetyl-CoA during beta-oxidation of fatty acids
YPL276W	FDH2	62.63	NAD(+)-dependent formate dehydrogenase; may protect cells from exogenous formate
YKL217W	JEN1	57.30	Lactate transporter (uptake of lactate, pyruvate); derepressed by Cat8p under nonfermentative growth conditions
YMR107W	SPG4	52.99	Required for survival at high temperature during stationary phase; not required for growth on nonfermentable carbon sources
YIL057C	YIL057C	50.37	Hypothetical protein
YGR236C	SPG1	38.51	Required for survival at high temperature during stationary phase; not required for growth on nonfermentable carbon sources
YKR097W	PCK1	36.18	Phosphoenolpyruvate carboxykinase; gluconeogenesis; repressed by glucose; regulated by Mcm1p and Cat8p
YKL187C	YKL187C	36.13	Putative protein of unknown function; detectable in highly purified mitochondria
YGL205W	POX1	24.28	Fatty-acyl coenzyme A oxidase, involved in the fatty acid beta-oxidation pathway; localized to the peroxisomal matrix
YDR536W	STL1	21.12	Plasma membrane glycerol proton symporter; subject to glucose-induced inactivation; transiently induced by osmotic shock
YML054C	CYB2	19.79	Cytochrome b2; mitochondrial intermembrane space; required for lactate utilization; repressed by glucose
YMR280C	CAT8	17.69	Transcriptional activator; derepresses a variety of genes under non-fermentative growth conditions, active after diauxic shift
YGR043C	NQM1	16.42	Putative protein of unknown function; transcription is repressed by Mot1p and induced during diauxic shift
YBR116C	YBR116C	14.90	Hypothetical protein
YHR160C	PEX18	14.68	Part of a two-member peroxin family (Pex18p and Pex21p)
YMR174C	PAI3	13.67	Cytoplasmic proteinase A inhibitor, dependent on Pbs2p and Hog1p protein kinases for osmotic induction
YMR118C	YMR118C	13.42	Protein of unknown function with similarity to succinate dehydrogenase cytochrome b subunit; nonessential gene
YNL195C	YNL195C	13.34	Hypothetical protein
YLR327C	TMA10	12.62	Protein of unknown function that associates with ribosomes
YLR178C	TFS1	10.10	Carboxypeptidase Y inhibitor; phosphatidylethanolamine-binding protein involved in protein kinase A signaling pathway
YDR380W	ARO10	9.62	Phenylpyruvate decarboxylase (decarboxylation of phenylpyruvate to phenylacetaldehyde); first specific step of Ehrlich pathway
YJL217W	YJL217W	9.59	Cytoplasmic protein of unknown function; induced by copper sensing transcription factor Mac1p during copper deficiency
YEL020C	YEL020C	9.37	Hypothetical protein with low sequence identity to Pdc1p
YLR284C	ECI1	9.04	Peroxisomal delta3,delta2-enoyl-CoA isomerase; essential for the beta-oxidation of unsaturated fatty acids, oleate-induced
YMR322C	SNO4	8.94	Possible chaperone and cysteine protease; similar to Hsp31p, Hsp32p, and Hsp33p; possible role in pyridoxine metabolism
YGR201C	YGR201C	8.83	Putative protein of unknown function
YPL201C	YIG1	8.60	Protein that interacts with glycerol 3-phosphatase and plays a role in anaerobic glycerol production
YOL152W	FRE7	8.60	Putative ferric reductase with similarity to Fre2p; expression induced by low copper levels
YJR008W	YJR008W	8.27	Putative protein of unknown function; expression induced by mild heat-stress on a nonfermentable carbon source.
YGL156W	AMS1	8.05	Vacuolar alpha mannosidase, involved in free oligosaccharide (fOS) degradation
YPL280W	HSP32	7.95	Possible chaperone and cysteine protease; similar to Hsp31p, Hsp33p and Sno4p
YMR206W	YMR206W	7.39	Putative protein of unknown function; YMR206W is not an essential gene
YGR088W	CTT1	7.18	Cytosolic catalase T, has a role in protection from oxidative damage by hydrogen peroxide
YGR243W	FMP43	7.02	The authentic, nontagged protein was localized to mitochondria
YKL093W	MBR1	6.70	Involved in mitochondrial function and stress response; overexpression suppresses hap2, hap3, and hap4 defects
YOL052C-A	DDR2	6.65	Multistress response protein; activated by xenobiotic agents and environmental or physiological stresses
YPL054W	LEE1	6.54	Zinc-finger protein of unknown function
YGR248W	SOL4	6.47	6-phosphogluconolactonase with similarity to Sol3p
YEL033W	YEL033W	6.42	Predicted to have metabolic role based on analysis of gene networks
YLR162W	YLR162W	6.38	Putative protein of unknown function; overexpression confers resistance to the antimicrobial peptide MiAMP1
YMR175W	SIP18	6.33	Protein of unknown function whose expression is induced by osmotic stress
YOR173W	DCS2	6.20	Non-essential protein; regulated by Msn2p, Msn4p; accumulates under glucose limitation, similar to Dcs1p
YFL054C	YFL054C	6.06	Putative channel-like protein; similar to Fps1p; mediates passive diffusion of glycerol in the presence of ethanol

(continued)

Table 1. Continued

Systematic name	Common name	Fold-change[a]	*Saccharomyces* Genome Database gene description (abbreviated)
YOR285W	YOR285W	5.98	Protein of unknown function, localized to the mitochondrial outer membrane
YNR001C	CIT1	5.93	Citrate synthase (condensation of acetyl coenzyme A and oxaloacetate to citrate); rate-limiting TCA cycle enzyme
YNR034W-A	YNR034W-A	5.91	Hypothetical protein
YMR110C	HFD1	5.78	Putative fatty aldehyde dehydrogenase, located in the mitochondrial outer membrane and also in lipid particles
YPR002W	PDH1	5.55	Mitochondrial protein that participates in respiration, induced by diauxic shift
YIL055C	YIL055C	5.53	Hypothetical protein
YPR151C	SUE1	5.45	Mitochondrial protein required for degradation of unstable forms of cytochrome c
YNL305C	YNL305C	5.38	Hypothetical protein
YKL163W	PIR3	5.35	Cell wall protein required for cell wall stability; expression is regulated by cell cycle and the cell integrity pathway
YML089C	YML089C	5.34	Hypothetical protein
YER037W	PHM8	5.27	Protein of unknown function, expression is induced by low phosphate levels and by inactivation of Pho85p
YKL066W	YKL066W	5.26	Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species;
YLR149C	YLR149C	5.20	Putative protein of unknown function; YLR149C is not an essential gene
YMR271C	URA10	5.17	One of two isozymes that catalyze the fifth enzymatic step in the de novo biosynthesis of pyrimidines
YIL087C	YIL087C	5.15	Hypothetical protein
YKL150W	MCR1	5.04	Mitochondrial NADH-cytochrome b5 reductase, involved in ergosterol biosynthesis
YNL274C	YNL274C	5.03	Putative hydroxyisocaproate dehydrogenase
YER121W	YER121W	5.01	Hypothetical protein
YOR382W	FIT2	5.01	Cell wall mannoprotein involved in the retention of siderophore-iron in the cell wall
YPL281C	ERR2	5.01	Protein of unknown function, has similarity to enolases
YHR006W	STP2	1.90	Transcription factor that activates transcription of amino acid permease genes
YDR423C	CAD1	1.80	Transcriptional activator involved in stress responses, iron metabolism, drug resistance and protein stabilization
YML007W	YAP1	1.74	Transcription factor required for oxidative stress tolerance; mediates pleiotropic drug and metal resistance
YOL089C	HAL9	1.74	Putative transcription factor; salt tolerance through increased expression of the ENA1 ($Na+/Li+$ extrusion pump) gene
YDL020C	RPN4	1.73	Transcription factor that stimulates proteasome gene expression; regulated by various stress responses
YHR124W	NDT80	1.69	Meiosis-specific transcription factor required for full meiotic recombination; activates sporulation genes
YPR199C	ARR1	1.50	Transcriptional activator required for transcription of genes involved in resistance to arsenic compounds
YNL103W	MET4	1.21	Transcriptional activator responsible for the regulation of the sulfur amino acid pathway
YMR016C	SOK2	−1.19	Regulatory role in the cyclic AMP (cAMP)-dependent protein kinase (PKA) signal transduction pathway
YML076C	WAR1	−1.22	Transcription factor; induces transcription of PDR12 (acid transporter) and FUN34 (putative ammonia transporter)
YGL209W	MIG2	−1.81	Protein involved in repression, along with Mig1p, of SUC2 (invertase) expression by high levels of glucose
YHR144C	DCD1	−5.00	Deoxycytidine monophosphate (dCMP) deaminase required for dCTP and dTTP synthesis
YLR048W	RPS0B	−5.06	Protein component of the small (40S) ribosomal subunit; required for maturation of 18S rRNA
YDR345C	HXT3	−5.31	Low affinity glucose transporter; expression is induced in low or high glucose conditions
YMR305C	SCW10	−5.34	Cell wall protein with similarity to glucanases; may play a role in conjugation during mating
YMR321C	YMR321C	−5.47	Hypothetical protein
YPL079W	RPL21B	−5.48	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl21Ap
YFL056C	AAD6	−5.55	Putative aryl-alcohol dehydrogenase; involved in the oxidative stress response
YLR061W	RPL22A	−6.02	Protein component of the large (60S) ribosomal subunit, has similarity to Rpl22Bp
YML026C	RPS18B	−6.03	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps18Ap
YEL071W	DLD3	−6.48	D-lactate dehydrogenase; retrograde regulon (genes stimulated by damage to mitochondria...)
YAR073W	IMD1	−6.60	Nonfunctional protein with homology to IMP dehydrogenase; probable pseudogene, located close to the telomere
YFL057C	AAD16	−6.74	Putative aryl-alcohol dehydrogenase; mutational analysis has not yet revealed a physiological role
YML116W	ATR1	−7.41	Multidrug efflux pump; required for resistance to aminotriazole and 4-nitroquinoline-N-oxide
YMR318C	ADH6	−7.67	NADPH-dependent cinnamyl alcohol dehydrogenase; possible role in fusel alcohol synthesis or aldehyde tolerance
YLR460C	YLR460C	−8.32	Hypothetical protein

(continued)

Table 1. Continued

Systematic name	Common name	Fold-change[a]	*Saccharomyces* Genome Database gene description (abbreviated)
YER055C	HIS1	−8.99	ATP phosphoribosyltransferase, a hexameric enzyme, catalyzes the first step in histidine biosynthesis
YDR399W	HPT1	−9.53	Dimeric hypoxanthine-guanine phosphoribosyltransferase, catalyzes the formation of both IMP and GMP
YMR177W	MMT1	−10.16	Putative metal transporter involved in mitochondrial iron accumulation; closely related to Mmt2p
YKL071W	YKL071W	−14.01	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm
YAR075W	YAR075W	−19.31	Nonfunctional protein with homology IMP dehydrogenase
YGR138C	TPO2	−19.36	Polyamine transport protein specific for spermine; localizes to the plasma membrane; regulated by Haa1p

[a]Each of these genes were significantly differentially expressed with a *P*-value of $\leq 10^{-4}$, determined using Rosetta Resolver (see Materials and Methods section).

The five top-scoring Active Modules subnetworks (jAM5-1 through jAM5-5) each contained 186 or 187 genes, as indicated in Figure 1. Since these subnetworks partially overlapped, these genes were combined into a single list which was further simplified by selecting genes that were greater than 5-fold up- or down-regulated, plus their associated transcription factors (Table 1).

Examining these genes, we noted the up-regulation of genes associated with mitochondrial function, gluconeogenesis, the TCA cycle, the β-oxidation of fatty acids, transport (including the uptake of amino acids), cell wall stability, copper and iron utilization (both required as prosthetic groups in the cytochromes in the electron transport chain) and glycerol and lactate utilization. Conversely, we observed down-regulation of genes associated with the accumulation of iron in the mitochondrion (required for heme and cytochrome biosyntheses), ribosomal subunit biosynthesis, and cellular growth—a response to glucose starvation.

The increased mitochondrial activity associated with oxidative phosphorylation, required for respiratory growth on nonfermentable carbon sources including ethanol and glycerol, summarized by Maris *et al.* (8), is accompanied by increased production of reactive oxygen species. In response, we found that numerous genes associated with the response to oxidative stress were up-regulated in our diauxic-shifted cells (Figure 3; Table 2), most notably *CTA1* (catalase A, present in the peroxisomal and mitochondrial matrices; 40.2-fold up-regulated), *HSP12* (plasma membrane heat-shock protein;18.4-fold), *CTT1* (cytosolic catalase T; 7.2-fold), *PRX1* (mitochondrial peroxiredoxin; 5.5-fold), *MCR1* (mitochondrial NADH-cytochrome *b5* reductase; 5.0-fold) and *GPX1* (phospholipid hydroperoxide glutathione peroxidase; 4.6-fold). The superoxide dismutases encoded by *SOD1* (cytosol; mitochondrial intermembrane space) and *SOD2* (mitochondrial matrix) were modestly up-regulated (2.1 and 1.9-fold, respectively), indicating that the burden of the response to increased reactive oxygen species in this strain under these conditions is shared by the other antioxidant defense mechanisms (Cta1, Ctt1, etc.). Interestingly, many genes associated with the response to oxidative stress were down-regulated (Figure 3; Table 2), most notably *GPX2* (cytoplasmic phospholipid hydroperoxide glutathione peroxidase; 5.7-fold), *TRR1* (cytoplasmic thioredoxin reductase; 4.0-fold) and *GSH1* (glutathione biosynthesis; 3.0-fold).

The numbers of genes differentially expressed in these cells, the modest changes in expression of *SOD1* and *SOD2*, and the down-regulation of oxidative stress-related genes *GPX2*, *TRR1* and *GSH1* suggests that the regulation of gene expression in these cells is rather complex. Examining our yeast interactome, it is readily apparent that the regulation of gene expression in yeast is extraordinarily complex, with most genes simultaneously regulated by two or more transcription factors, as indicated in Figures 1–3. Additionally, the 168 transcription factors downloaded from YEASTRACT interact among one another in an extraordinarily complex way while directly regulating the expression of at least 5902 target genes (our yeast interactome: data not shown).

DISCUSSION

Our laboratory has a long-standing interest in exploring mitochondrial function and maintenance, including the stability and replication of the mitochondrial genome, and mutations and naturally occurring nucleotide polymorphisms associated with mitochondrial disease (13–15). Among the tools that we employ for these studies is the model organism, *S. cerevisiae* (2,3,13,16).

Energy demands in the facultative anaerobe *S. cerevisiae* are met under different physiological states when cells are grown on fermentable carbon sources such as glucose versus non-fermentable carbon sources such as ethanol or glycerol (8,17,18). Fermentation and glycolysis supply the cell with energy through the breakdown of glucose and other simple fermentable sugars; however, when the glucose concentration drops below ~0.2%, the cells stop growing for a few hours as they undergo the diauxic shift, accompanied by transcriptional and translational changes including the mitochondrial biosynthesis. The cells then resume slower growth for a few generations by oxidative phosphorylation using the ethanol, glycerol and

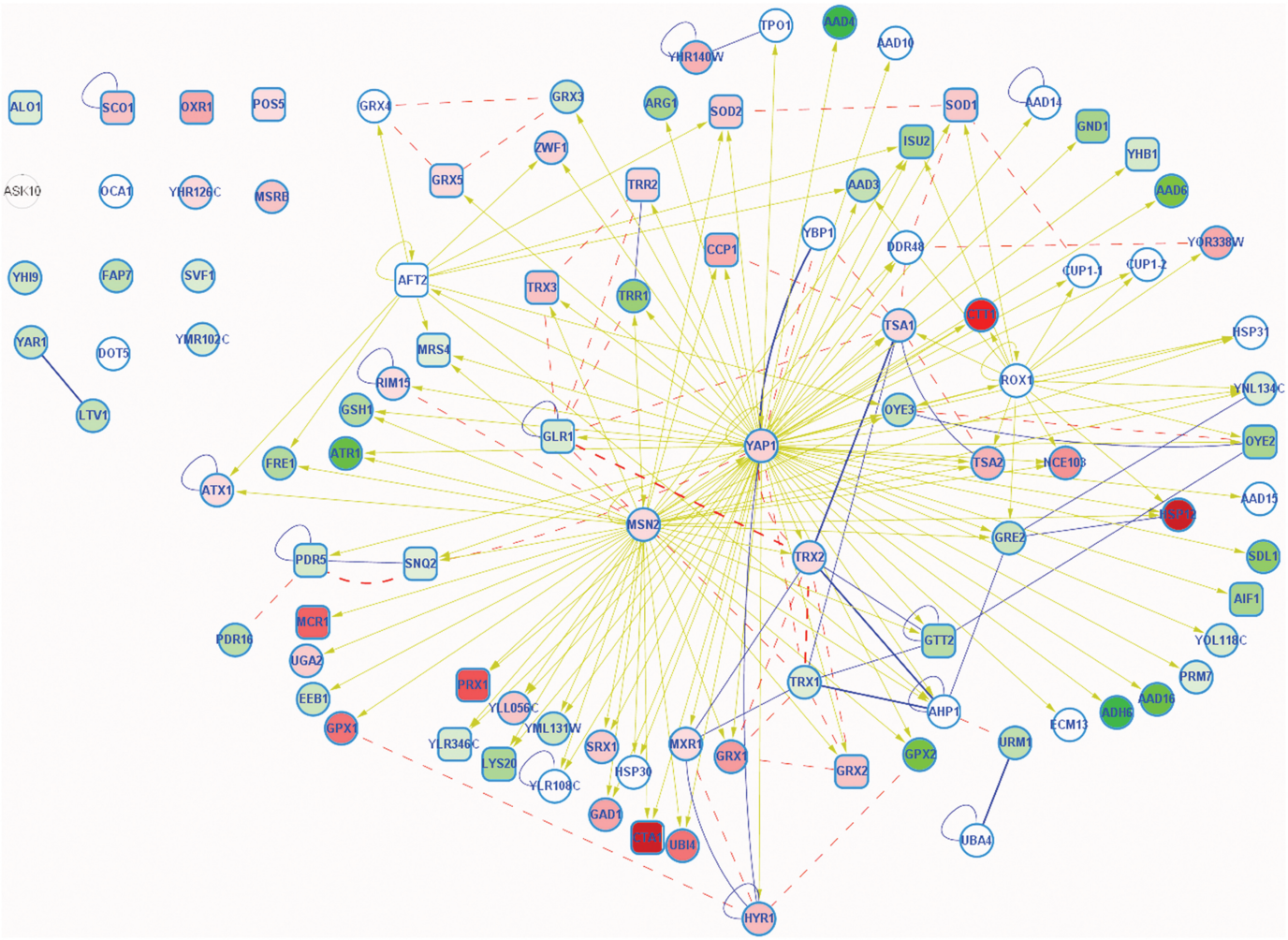

Figure 3. A schematic displaying genes associated with the response to oxidative stress, in cells shifted to growth on glycerol. For clarity, interactions associated with transcription factor Msn4—that virtually mirror those from Msn2—were removed from this figure. For convenience, these genes are also summarized in Table.

Table 2. Expression levels of genes associated with the response to oxidative stress in cells shifted to growth on glycerol

Systematic name	Common name	Fold-change[a]	*Saccharomyces* Genome Database gene description (abbreviated)
YDR256C	CTA1	40.22	Catalase A, breaks down hydrogen peroxide in the peroxisome formed during fatty acid beta-oxidation
YFL014W	HSP12	18.36	Plasma membrane protein; induced by heat shock, oxidative stress, glucose depletion
YGR088W	CTT1	7.18	Cytosolic catalase T, has a role in protection from oxidative damage by hydrogen peroxide
YBL064C	PRX1	5.47	Mitochondrial peroxiredoxin; induced during respiratory growth and under conditions of oxidative stress
YKL150W	MCR1	5.04	Mitochondrial NADH-cytochrome b5 reductase, involved in ergosterol biosynthesis
YKL026C	GPX1	4.57	Phospholipid glutathione peroxidase; induced by glucose starvation; protection from oxidative stress
YLL039C	UBI4	4.50	Ubiquitin; marks proteins for selective degradation; essential for the cellular stress response
YNL036W	NCE103	3.62	Carbonic anhydrase; poorly transcribed under aerobic conditions
YCL035C	GRX1	3.38	Hydroperoxide and superoxide-radical responsive oxidoreductase; protection from oxidative damage
YMR250W	GAD1	3.11	Glutamate decarboxylase (glutamate to gamma-aminobutyric acid); response to oxidative stress
YPL196W	OXR1	2.97	Protein of unknown function required for resistance to oxidative damage
YOR338W	YOR338W	2.94	Hypothetical protein
YKR066C	CCP1	2.89	Mitochondrial cytochrome-c peroxidase; degrades reactive oxygen species; response to oxidative stress
YHR140W	YHR140W	2.71	Putative integral membrane protein of unknown function

(continued)

Table 2. Continued

Systematic name	Common name	Fold-change[a]	*Saccharomyces* Genome Database gene description (abbreviated)
YDR453C	TSA2	2.61	Inducible cytoplasmic thioredoxin peroxidase; removal of reactive oxygen, nitrogen and sulfur species
YIR037W	HYR1	2.43	Thiol peroxidase; senses intracellular hydroperoxide levels, transduces a redox signal to Yap1p
YCR083W	TRX3	2.24	Mitochondrial thioredoxin; maintains cellular redox homeostasis with Trr2p and Glr1p
YBR037C	SCO1	2.20	Mitochondrial inner membrane copper-binding protein required for cyt c oxidase activity and respiration
YCL033C	MSRB	2.19	Putative protein-methionine-R-oxide reductase; involved in response to oxidative stress
YDR513W	GRX2	2.18	Cytoplasmic glutaredoxin; involved in maintaining redox state of target proteins; induced by stress
YLL056C	YLL056C	2.11	Putative protein of unknown function; activated along with genes involved in pleiotropic drug resistance
YJR104C	SOD1	2.06	Copper, Zinc-containing superoxide dismutase
YKL086W	SRX1	2.02	Sulfiredoxin, contributes to oxidative stress resistance by reducing peroxiredoxins Tsa1p and Ahp1p
YBR006W	UGA2	1.95	Succinate semialdehyde dehydrogenase; utilization of gamma-aminobutyrate as a nitrogen source
YHR008C	SOD2	1.93	Manganese-containing superoxide dismutase; protects cells against oxygen toxicity
YNL241C	ZWF1	1.75	Glucose-6-phosphate dehydrogenase (pentose phosphate pathway); adaption to oxidative stress
YML007W	YAP1	1.74	Transcription factor required for oxidative stress tolerance; mediates pleiotropic drug, metal resistance
YHR106W	TRR2	1.63	Mitochondrial thioredoxin reductase; oxidative stress protection; with Glr1p maintains Trx3p redox status
YHR126C	YHR126C	1.47	Hypothetical protein
YML028W	TSA1	1.46	Ubiquitous housekeeping thioredoxin peroxidase, reduces reactive oxygen, nitrogen and sulfur species
YMR037C	MSN2	1.46	Transcriptional activator related to Msn4p; activated in stress conditions
YPL059W	GRX5	1.42	Mitochondrial hydroperoxide, superoxide-radical responsive oxidoreductase; iron-sulfur center synthesis
YGR209C	TRX2	1.39	Cytoplasmic thioredoxin isoenzyme; protects cells against both oxidative and reductive stress
YNL259C	ATX1	1.35	Cytosolic copper metallochaperone; copper eventually inserted into Fet3p (high-affinity iron uptake)
YPL188W	POS5	1.34	Mitochondrial NAD(H) kinase; required for the response to oxidative stress
YER042W	MXR1	1.33	Reverses oxidation of methionine residues; involved in repair and resistance to oxidative stress
YFL033C	RIM15	1.28	Glucose-repressible protein kinase; signal transduction during cell proliferation in response to nutrients
YBL043W	ECM13	1.21	Nonessential protein of unknown function
YER174C	GRX4	1.19	Hydroperoxide, superoxide-radical responsive oxidoreductase; protection from oxidative damage
YBR216C	YBP1	1.17	Oxidation of transcription factor Yap1p, resulting in nuclear localization of Yap1p in response to stress
YNL331C	AAD14	1.15	Putative aryl-alcohol dehydrogenase; mutational analysis has not yet revealed a physiological role
YDR533C	HSP31	1.12	Possible chaperone and cysteine protease with similarity to Hsp32p, Hsp33p, and Sno4p
YPR065W	ROX1	1.11	Heme-dependent repressor of hypoxic genes
YGR097W	ASK10	1.11	Component of the RNA polymerase II holoenzyme, phosphorylated in response to oxidative stress
YLR108C	YLR108C	1.03	Protein of unknown function; green fluorescent-fusion protein localizes to the nucleus; non-essential
YNL099C	OCA1	1.03	Putative protein tyrosine phosphatase; induces cell cycle arrest in response to oxidative DNA damage
YLL028W	TPO1	−1.04	Polyamine transporter; catalyzes uptake of polyamines at alkaline pH and excretion at acidic pH
YIL010W	DOT5	−1.08	Nuclear thiol peroxidase; functions as an alkyl-hydroperoxide reductase during post-diauxic growth
YCR021C	HSP30	−1.12	Plasma membrane stress response protein; negatively regulates H(+)-ATPase Pma1p
YOL165C	AAD15	−1.15	Putative aryl-alcohol dehydrogenase; mutational analysis has not yet revealed a physiological role
YHR111W	UBA4	−1.16	Urmylates thioredoxin peroxidase Ahp1p, suggesting a role of urmylation in oxidative stress response
YPL202C	AFT2	−1.16	Iron-regulated transcriptional activator, required for iron homeostasis and resistance to oxidative stress
YLR109W	AHP1	−1.21	Thiol-specific peroxiredoxin, reduces hydroperoxides to protect against oxidative damage
YJR155W	AAD10	−1.22	Putative aryl-alcohol dehydrogenase; mutational analysis has not yet revealed a physiological role
YHR053C	CUP1-1	−1.23	Metallothionein, binds copper and mediates resistance to high concentrations of copper and cadmium

(continued)

Table 2. Continued

Systematic name	Common name	Fold-change[a]	*Saccharomyces* Genome Database gene description (abbreviated)
YHR055C	CUP1-2	−1.26	Metallothionein, binds copper and mediates resistance to high concentrations of copper and cadmium
YMR173W	DDR48	−1.26	DNA damage-responsive protein, induced in response to heat-shock stress or DNA lesions
YDR011W	SNQ2	−1.29	ABC transporter protein involved in multidrug resistance and resistance to singlet oxygen species
YKR052C	MRS4	−1.31	Mitochondrial iron transporter; functions under low-iron conditions; may transport other cations
YDL039C	PRM7	−1.38	Pheromone-regulated protein, predicted to have a transmembrane segment; regulated by Gcn4p
YLR043C	TRX1	−1.40	Cytoplasmic thioredoxin isoenzyme; protects cells against both oxidative and reductive stress
YML086C	ALO1	−1.44	Catalyzes the final step of erythroascorbic acid biosynthesis, protective against oxidative stress
YNL134C	YNL134C	−1.48	Uncharacterized ORF; alcohol dehydrogenase (NADP+) activity; biological process unknown
YMR102C	YMR102C	−1.51	Protein of unknown function; activated along with genes involved in multidrug resistance
YDR346C	SVF1	−1.53	Protein with a potential role in cell survival pathways, required for the diauxic growth shift
YPL091W	GLR1	−1.58	Cytosolic and mitochondrial glutathione oxidoreductase; reduces oxidized glutathione
YLR346C	YLR346C	−1.61	Putative mitochondrial protein of unknown function; regulated by drug resistance transcription factors
YOL118C	YOL118C	−1.64	Hypothetical protein
YGR234W	YHB1	−1.75	Nitric oxide oxidoreductase; flavohemoglobin; role in the oxidative and nitrosative stress responses
YOR153W	PDR5	−1.82	Membrane ATP-binding cassette transporter; involved in transport and cellular detoxification
YDR098C	GRX3	−1.83	Hydroperoxide and superoxide-radical responsive oxidoreductase; protection from oxidative damage
YHR029C	YHI9	−1.95	Protein of unknown function possibly involved in a membrane regulation metabolic pathway
YPL239W	YAR1	−2.09	Cytoplasmic protein; proposed to link 40S ribosomal subunit biogenesis to adaption to oxidative stress
YML131W	YML131W	−2.13	Putative protein of unknown function; increased after osmotic shock; non-essential gene
YOL151W	GRE2	−2.15	NADPH-dependent methylglyoxal reductase; stress induced (osmotic, ionic, oxidative, heat, metals)
YPL095C	EEB1	−2.17	Acyltransferase; major part of short-chain fatty acid ethyl ester production during fermentation
YPL171C	OYE3	−2.38	Widely conserved NADPH oxidoreductase; may be involved in sterol metabolism
YKL143W	LTV1	−2.39	Protein required for growth at low temperature
YCR107W	AAD3	−2.40	Putative aryl-alcohol dehydrogenase; mutational analysis has not yet revealed a physiological role
YDL166C	FAP7	−2.51	Essential NTPase required for small ribosome subunit synthesis
YIL008W	URM1	−2.57	Ubiquitin-like protein; molecular function of Urm1p pathway is unknown; required for normal growth
YNL231C	PDR16	−2.71	Phosphatidylinositol transfer protein; controls levels of various lipids, may regulate lipid synthesis
YLL060C	GTT2	−2.75	Glutathione S-transferase capable of homodimerization; functional overlap with Gtt2p, Grx1p, Grx2p
YJL101C	GSH1	−2.95	Catalyzes the first step of glutathione biosynthesis; induced by oxidants, cadmium, and mercury
YLR214W	FRE1	−2.98	Ferric and cupric reductase; reduces iron and copper prior to uptake; induced by low copper, iron levels
YDL182W	LYS20	−3.20	Homocitrate synthase isozyme; catalyzes first step in the lysine biosynthesis pathway
YOR226C	ISU2	−3.29	Conserved mitochondrial matrix protein; synthesis of mitochondrial and cytosolic iron-sulfur proteins
YHR179W	OYE2	−3.39	Widely conserved NADPH oxidoreductase; may be involved in sterol metabolism
YNR074C	AIF1	−3.42	Mitochondrial cell death effector that translocates to the nucleus in response to apoptotic stimuli
YOL058W	ARG1	−3.44	Catalyzes the formation of L-argininosuccinate in the arginine biosynthesis pathway
YHR183W	GND1	−3.47	Regenerates NADPH in the pentose phosphate pathway; required for adaptation to oxidative stress
YDR353W	TRR1	−4.05	Cytoplasmic thioredoxin reductase; protects cells against both oxidative and reductive stress
YIL167W	SDL1	−4.47	Open reading frame, unlikely to produce a functional protein in S288C
YFL056C	AAD6	−5.55	Putative aryl-alcohol dehydrogenase; involved in the oxidative stress response
YBR244W	GPX2	−5.68	Glutathione peroxidase; protects cells from hydroperoxides and peroxides during oxidative stress
YFL057C	AAD16	−6.74	Putative aryl-alcohol dehydrogenase; mutational analysis has not yet revealed a physiological role
YML116W	ATR1	−7.41	Multidrug efflux pump; required for resistance to aminotriazole, 4-nitroquinoline-N-oxide
YMR318C	ADH6	−7.67	NADPH-dependent alcohol dehydrogenase; possibly involved in fusel alcohol synthesis or aldehyde tolerance
YDL243C	AAD4	−7.91	Putative aryl-alcohol dehydrogenase; involved in the oxidative stress response

[a]Each of these genes were significantly differentially expressed with a *P*-value of $\leq 10^{-4}$, determined using Rosetta Resolver (see Materials and Methods section).

other byproducts accumulated during the fermentive stage of growth, before entering the stationary phase of growth.

Oxidative phosphorylation, which is dependent on mitochondrial activity and oxygen metabolism, provides the most efficient means of energy production (19,20). However, a deleterious consequence of oxidative phosphorylation is the production of reactive oxygen species in the mitochondrion—including the superoxide anion, hydrogen peroxide (H_2O_2) and the hydroxyl radical—that must be detoxified to minimize damage to nucleic acids, proteins, carbohydrates and lipids (21). The superoxide anion radical is reduced to H_2O_2 by superoxide dismutases (SOD), and further reduced to water by the antioxidant glutathione (GSH) and the enzymatic activity of catalases and peroxidases (21,22).

Cells switched to growth dependent on mitochondrial activity—the utilization of glycerol via oxidative phosphorylation—experience elevated levels of reactive oxygen species, evidenced by the increased nuclear mutational rates in cells grown in YPG versus YPD (2), a 28-fold increase in oxidative damage to mitochondrial proteins (2), and the changes in gene expression observed in this study, facilitated using Cytoscape and our yeast interactome. The genes that were most significantly affected by the switch from growth on glucose to growth on glycerol were identified using our interactome and the Cytoscape jActiveModules plugin (Figure 2; Table 1). As shown in Figure 2, transcription factors directly associated with the regulation of these genes include the stress response transcription factors Yap1 (required for oxidative stress tolerance), Rpn4 (stimulates expression of proteasome genes), Cad1 (multiple stress responses), Arr1 (resistance to arsenic compounds), Cat8 (derepression of genes following the diauxic shift), Sok2 (signal transduction), Stp2 (external amino acid permease) and Met4 (sulfur amino acid pathway). The involvement of several of these transcription factors in regulating the cellular response to the diauxic shift (e.g. Yap1) is not surprising given the increases in oxidative phosphorylation and reactive oxygen species as a consequence of increased mitochondrial function. Transcription factor Arr1 (Yap8), normally associated with the transcription of genes involved in resistance to arsenic compounds, appeared to directly coregulate the expression of 39 of the 95 genes shown in Figure 2, including up-regulated genes associated with the β-oxidation of fatty acids, carbohydrate metabolism and the TCA cycle, and the response to diauxic shift, suggesting a substantial role for this transcription factor in diauxic-shifted cells.

The complex regulatory nature of gene expression in *S. cerevisiae* is readily apparent from the interaction data displayed in Figures 1–3. It appears that transcription factors in *S. cerevisiae* form a highly interconnected self-regulatory subnetwork, while additionally regulating at least 5734 additional genes (our interactome: data not shown), indicating that substantial redundancy exists among the regulation and utilization of metabolic pathways. These cells may thus be able to respond quickly to changes in their external (e.g. adverse growth conditions) or internal (e.g. nonlethal mutations) environment by adjusting the regulation of their cellular metabolism via modest changes in gene expression involving hundreds or thousands of genes.

The transcription factors present in our yeast interactome appear to regulate most, but not all of the genes present in this interactome. Subtracting the 168 transcription factors and their regulated genes from the yeast interactome reveals that 286 of the 6188 genes present in the interactome (4.6%) are currently not associated with any transcription factor (data not shown). These genes, ranging from 3.1-fold up-regulated (*TAM41*) to 6.2-fold down-regulated (*PAM18*), passed through the SGD GO Slim Mapper, are variously associated with unknown biological processes (58 of 286 genes; 20.3%), transport (53/286; 18.5%), transcription (16/286; 5.6%), the cell cycle (11/286 genes; 3.8%), signal transduction (10/286; 3.5%) and amino acid metabolism (3/286 genes; 1.0%). Sixty-four of these genes (64/286; 22.4%) are annotated by SGD as being associated with the mitochondrion.

There is increasing interest regarding the application of bioinformatics and systems biology to the study of organisms and their regulatory mechanisms and metabolic profiles (23–28). The data provided in this study suggest that most genes are regulated in a highly complex manner by more than one transcription factor, and that bioinformatic tools such as Cytoscape—in conjunction with a robust interactome—may provide a useful framework for additional avenues of investigation. For example, by noting the transcription factors associated with specific groups of genes that are differentially expressed, the effect of deleting these transcription factors may be determined, at least partly. Finally, by applying methods similar to those used in the construction of the interactome described in this article, additional types of interaction data—for example those associated with protein kinases and their targets—can be readily incorporated.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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REFERENCES

1. Shannon,P., Markiel,A., Ozier,O., Baliga,N.S., Wang,J.T., Ramage,D., Amin,N., Schwikowski,B. and Ideker,T. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.*, **13**, 2498–2504.
2. Strand,M.K., Stuart,G.R., Longley,M.J., Graziewicz,M.A., Dominick,O.C. and Copeland,W.C. (2003) *POS5* gene of *Saccharomyces cerevisiae* encodes a mitochondrial NADH kinase required for stability of mitochondrial DNA. *Eukaryot. Cell*, **2**, 809–820.
3. Stuart,G.A., Humble,M.M., Strand,M.K. and Copeland,W.C. (2009) Transcriptional response to mitochondrial NADH kinase deficiency in *Saccharomyces cerevisiae*. *Mitochondrion*, In press
4. Spencer,F., Hugerat,Y., Simchen,G., Hurko,O., Connelly,C. and Hieter,P. (1994) Yeast *kar1* mutants provide an effective method for YAC transfer to new hosts. *Genomics*, **22**, 118–126.
5. Teixeira,M.C., Monteiro,P., Jain,P., Tenreiro,S., Fernandes,A.R., Mira,N.P., Alenquer,M., Freitas,A.T., Oliveira,A.L. and Sá-Correia,I. (2006) The YEASTRACT database: a tool for the analysis of transcription regulatory associations in *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **34**, D446–D451.
6. Bonander,N., Ferndahl,C., Mostad,P., Wilks,M.D.B., Chang,C., Showe,L., Gustafsson,L., Larsson,C. and Bill,R.M. (2008) Transcriptome analysis of a respiratory *Saccharomyces cerevisiae* strain suggests the expression of its phenotype is glucose insensitive and predominantly controlled by Hap4, Cat8 and Mig1. *BMC Genomics*, **9**, 365.
7. DeRisi,J.L., Iyer,V.R. and Brown,P.O. (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science*, **278**, 680–686.
8. Maris,A.F., Assumpção,A.L.K., Bonatto,D., Brendel,M. and Henriques,J.A.P. (2001) Diauxic shift-induced stress resistance against hydroperoxides in *Saccharomyces cerevisiae* is not an adaptive stress response and does not depend on functional mitochondria. *Curr. Genet.*, **39**, 137–149.
9. Kuhn,K.M., DeRisi,J.L., Brown,P.O. and Sarnow,P. (2001) Global and specific translational regulation in the genomic response of *Saccharomyces cerevisiae* to a rapid transfer from a fermentable to a nonfermentable carbon source. *Mol. Cell Biol.*, **21**, 916–927.
10. Young,E.T., Dombek,K.M., Tachibana,C. and Ideker,T. (2003) Multiple pathways are co-regulated by the protein kinase Snf1 and the transcription factors Adr1 and Cat8. *J. Biol. Chem.*, **278**, 26146–26158.
11. Ohlmeier,S., Kastaniotis,A.J., Hiltunen,J.K. and Bergmann,U. (2004) The yeast mitochondrial proteome, a study of fermentative and respiratory growth. *J. Biol. Chem.*, **279**, 3956–3979.
12. Xie,X., Wilkinson,H.H., Correa,A., Lewis,Z.A., Bell-Pedersen,D. and Ebbole,D.J. (2004) Transcriptional response to glucose starvation and functional analysis of a glucose transporter of *Neurospora crassa*. *Fungal Genet. Biol.*, **41**, 1104–1119.
13. Stuart,G.R., Santos,J.H., Strand,M.K., Van Houten,B. and Copeland,W.C. (2006) Mitochondrial and nuclear DNA defects in *Saccharomyces cerevisiae* with mutations in DNA polymerase γ associated with progressive external ophthalmoplegia. *Hum. Mol. Genet.*, **15**, 363–374.
14. Graziewicz,M.A., Longley,M.J. and Copeland,W.C. (2006) DNA polymerase γ in mitochondrial DNA replication and repair. *Chem. Rev.*, **106**, 383–405.
15. Chan,S.S.L. and Copeland,W.C. (2008) DNA polymerase gamma and mitochondrial disease: Understanding the consequence of *POLG* mutations. *Biochimica et Biophysica Acta*, In press [29 October, 2008, Epub ahead of print]
16. Strand, M.K. and Copeland, W.C. (2002) Measuring mtDNA mutation rates in Saccharomyces cerevisiae using the mtArg8 assay, *Methods Mol. Biol.*, **197**, 151–157.
17. Lagunas,R. (1976) Energy metabolism of *Saccharomyces cerevisiae* discrepancy between ATP balance and known metabolic functions. *Biochim. Biophys. Acta*, **440**, 661–674.
18. Rosenfeld,E. and Beauvoit,B. (2003) Role of the non-respiratory pathways in the utilization of molecular oxygen by *Saccharomyces cerevisiae*. *Yeast*, **20**, 1115–1144.
19. Lin,S.-J., Kaeberlein,M., Andalis,A.A., Sturtz,L.A., Defossez,P.-A., Culotta,V.C., Fink,G.R. and Guarente,L. (2002) Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature*, **418**, 344–348.
20. Johnston,M. and Kim,J.-H. (2005) Glucose as a hormone: receptor-mediated glucose sensing in the yeast *Saccharomyces cerevisiae*. *Biochem. Soc. Trans.*, **33**, 247–252.
21. Jackson,M.J., Papa,S., Bolaños,J., Bruckdorfer,R., Carlsen,H., Elliott,R.M., Flier,J., Griffiths,H.R., Heales,S., Holst,B. *et al.* (2002) Antioxidants, reactive oxygen and nitrogen species, gene induction and mitochondrial function. *Mol. Aspects Med.*, **23**, 209–285.
22. Grant,C.M., Perrone,G. and Dawes,I.W. (1998) Glutathione and catalase provide overlapping defenses for protection against hydrogen peroxide in the yeast *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.*, **253**, 893–898.
23. Futcher,B. (2002) Transcriptional regulatory networks and the yeast cell cycle. *Curr. Opin. Cell Biol.*, **14**, 676–683.
24. Lee,T.I., Rinaldi,N.J., Robert,F., Odom,D.T., Bar-Joseph,Z., Gerber,G.K., Hannett,N.M., Harbison,C.T., Thompson,C.M., Simon,I. *et al.* (2002) Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science*, **298**, 799–804.
25. Begley,T.J. and Samson,L.D. (2004) Network responses to DNA damaging agents. *DNA Repair*, **3**, 1123–1132.
26. Tong,A.H.Y., Lesage,G., Bader,G.D., Ding,H., Xu,H., Xin,X., Young,J., Berriz,G.F., Brost,R.L., Chang,M. *et al.* (2004) Global mapping of the yeast genetic interaction network. *Science*, **303**, 808–813.
27. Wu,W.-S., Li,W.-H. and Chen,B.-S. (2006) Computational reconstruction of transcriptional regulatory modules of the yeast cell cycle. *BMC Bioinformatics*, **7**, 421.
28. Boone,C., Bussey,H. and Andrews,B.J. (2007) Exploring genetic interactions and networks with yeast. *Nat. Rev. Genet*, **8**, 437–449.