

Metabolic pathways are differently affected in *saccharomyces cerevisiae* thiol peroxidase mutants during redox stress with hydrogen peroxide

Amy L. Olex¹, Brian M. Westwood², Leslie B. Poole³, Jacquelyn S. Fetrow^{1,4}

¹Department of Computer Science, Wake Forest University, Winston-Salem, NC 27109, USA

²Department of Molecular Genetics and Genomics, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA

³Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC, 27157, USA

⁴Department of Physics, Wake Forest University, Winston-Salem, NC 27109, USA

Background

Thiol peroxidases have been conserved throughout evolution and are found in almost every known organism from bacteria to human. These proteins play a key role in maintaining redox homeostasis and have been implicated in other roles such as cell signaling and sensing hydrogen peroxide and passing that signal along to transcription factors. To gain a better understanding of the role each plays in redox regulation on a global level, Fomenko, Gladyshev and colleagues [1] performed a series of microarray experiments where different combinations of the 8 thiol peroxidases [3 glutathione peroxidase homologues (Gpx) and 5 peroxiredoxins (Prx)] present in yeast were knocked out, including one mutant where all 8 peroxidases were removed. Surprisingly, all the mutants, including 8-delta, were viable and could withstand redox stresses; however, they were unable to activate or repress transcriptional events in response to hydrogen peroxide treatments, which was most evident in the 8-delta mutant. In this work network analysis is used to gain a better understanding of the biological networks whose gene expression is affected by these mutations.

Methods

Microarray data (provided by [1]) was processed for input into the Cytoscape plug-in jActiveModules. Active sub-networks for select mutants were identified using all yeast interactions found in KEGG (Kyoto Encyclopedia of Genes and Genomes [<http://www.genome.jp/kegg/>]) as the background network (including protein-protein, metabolic, and gene expression interactions). Nodes in each sub-network were input into DAVID (Database for Annotation, Visualization and Integrated Discovery [<http://david.abcc.ncifcrf.gov/>]) to identify which KEGG pathways were present.

Results

206 genes appeared in one or more of the active sub-networks. Only 7 genes were present in sub-networks of all strains, including a known oxidative stress-induced aldose reductase (GRE3), 4 putative aryl-alcohol dehydrogenases (AAD3, AAD6, AAD10 and AAD14), a mitochondrial aldehyde dehydrogenase (ALD4) and a xylulokinase (XKS1). All were up-regulated on average by 6- to 12-fold in all strains except for 8-delta with a 1.5-fold average up-regulation and -5Prx-delta with a 3-fold average up-regulation.

Many metabolic pathways were affected by the knockouts; the pathway types affected were dependent on which peroxidase was knocked-out. This result suggests that different thiol peroxidases may have a significant and specific impact on the regulation of metabolic pathways during oxidative stress.

Surprisingly, the Gpx3-delta active sub-network was similar to the Gpx1-delta and Gpx2-delta sub-networks. Gpx3 is known to sense hydrogen peroxide and pass that signal along to transcription factors; thus, it was expected that this sub-network would differ from the other Gpx mutants. Additionally, our results found that amino acid metabolism, biosynthesis and degradation pathways were active in wild type cells, but few mutant strains contained these same pathways.

Conclusions

The results of this work indicate that thiol peroxidases, along with playing a key role in maintaining redox homeostasis, may also play a significant role in the regulation of metabolic pathways in yeast, thus illuminating the global role that thiol peroxidases play in oxidative stress.

References

1. Fomenko DE, Koc A, Agisheva N, Jacobsen M, Kaya A, Malinouski M, Rutherford JC, Siu KL, Jin DY, Winge DR, Gladyshev VN: **Thiol peroxidases mediate specific genome-wide regulation of gene expression in response to hydrogen peroxide.** *Proc Natl Acad Sci U S A* 2011, **108**:2729-2734.

Discrete Correlate Summation and Disparate Molecular Interaction Profiles in Yeast Peroxidase Knock-Out Response to H₂O₂

Brian M. Westwood¹, Amy L. Olex², James L. Norris², Leslie B. Poole³, Jacquelyn S. Fetrow^{2,4}

¹Department of Molecular Genetics and Genomics, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA

²Department of Computer Science, Wake Forest University, Winston-Salem, NC 27109, USA

³Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC, 27157, USA

⁴Department of Physics, Wake Forest University, Winston-Salem, NC 27109, USA

Background

Given differential gene expression data across divergent mutant strain arrays of two enzyme subgroups, it would be logical to segregate by protein group ablation (PGA). Discrete correlate summation (DCS) was utilized to examine the differential effects of a hydrogen peroxide stressor on discrete and total yeast knock outs for glutathione peroxidase (Gpx) and peroxiredoxin (Prx), both groups pivoting off the wild-type strain [1]. While the half-life of the total Gpx knock out is intermediate to that of wild type and the transient total Prx knock out, the distribution of passage number of the various mutant strains can be separated into two groups independent of Gpx/Prx state. Based on half-viability: totalPrx<<<nPrx<<Gpx3=Tsa1<totalGpx<mPrx<<<Gpx1<Gpx2<<Ahp1=WT<<<Tsa2 (p<0.0005, two tailed t-test, n=5,6). DCS was also employed for the boundary between robust and gracile cultures. The aim of this study was finding the characteristic response of the transcriptome, from the perspective of PGA versus strain viability (SV).

Method

DCS is a method used to score variables that can be classified into two groups [2]. It is a composite score of a gene's mean group change and overall interaction difference relative to all others tested. Transcripts were included in this analysis only if values for all conditions passed microarray quality control, and were present in the KEGG (Kyoto Encyclopedia of Genes and Genomes [<http://www.genome.jp/kegg/>]) network. Randomly sorted edges were sampled for comparison (p<0.001, two tailed t-test, n=8372). Edges sorted on average DCS score, grouped by biologic process yielded a distinctive topology (p<1E-85, two tailed t-test, n=8372). Identified transcripts were subjected to functional annotation in DAVID (Database for Annotation, Visualization and Integrated Discovery [<http://david.abcc.ncifcrf.gov/>]).

Results

Application of DCS to the individual and complete gene knockouts of Gpx (3) and Prx (5) identified 92 transcripts based on PGA and 43 on SV, with a 13 gene overlap (Arg1p, Aah1p, Ade17p, Pgm2p, Cat2p, Cdd1p, Mae1p, Arg3p, Nma2p, Ole1p, Cta1p, Spb1p and Cds1p). Function annotation analysis of the 92 PGA transcripts identified the following functions: pyrimidine metabolism, steroid biosynthesis, purine metabolism, RNA polymerase and terpenoid backbone biosynthesis. Ergosterol biosynthesis, gluconeogenesis and transcription from Pol I/III promoters were major biologic process categories for this set. Interestingly, terpenoids feed into the steroid pathway, which result in the vitamin D2 precursor ergosterol. Analysis of the 43 SV transcripts identified starch and sucrose metabolism, butanoate metabolism and fructose and mannose metabolism. Stress response was the key biologic process for this arm of the study. No functional annotations were statistically significant for the common genes. Transcripts identified with PGA of either the Gpx or the Prx genes tend toward transcriptional control mechanisms, while SV associated transcripts track with metabolic necessities.

References

1. Fomenko DE, Koc A, Agisheva N, Jacobsen M, Kaya A, Malinouski M, Rutherford JC, Siu KL, Jin DY, Winge DR, Gladyshev VN: **Thiol peroxidases mediate specific genome-wide regulation of gene expression in response to hydrogen peroxide.** *Proc Natl Acad Sci USA* 2011, **108**:2729-2734.
2. Westwood, B; Chappell, M. **Application of correlate summation to data clustering in the estrogen- and salt-sensitive female mRen2.Lewis rat.** *TMBIO(ACM)* 2006,21-26.