A Study of Gene Expression in Respiring *Saccharomyces cerevisiae* during Cold Acclimation using Microarray Analysis

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Introduction

Saccharomyces cerevisiae is a complex unicellular eukaryote organism that contains 6,400 genes. *S. cerevisiae* is an excellent model organism because its genome has been sequenced and yeast microarrays are available for studying gene expression.

A yeast microarray is a glass slide with about 6,400 DNA genes printed on it. To use a microarray, RNA is isolated from the cells and made into complimentary DNA (cDNA), which binds to homologous sequences on the array. The control sample is labeled with a green fluorescent dye and the experimental sample is labeled with a red fluorescent dye. The microarray is next scanned to determine the ratio of red-to-green signal.

The first study performed using microarrays examined gene expression during the diauxic shift from anaerobic to aerobic metabolism in *S. cerevisiae* (DeRisi, 1997). We based our experimental methods on this land mark study.

The purpose of this study was to examine changes in gene expression in response to cold temperature in yeast. The results from this study will tell us how organisms adapt to cold temperature. Changes in gene expression in yeast have not been extensively studied at temperatures below 15º C. We examined changes in gene expression in yeast grown in galactose (YPGAL) at 10ºC using microarray analysis. Yeast grown in YPGAL use the metabolic process of oxidative phosphorylation, which means they respire, to produce ATP for energy. We hypothesized that respiring yeast cells would experience more oxidative stress during cold acclimation compared to fermenting cells.

Materials & Methods

Grow Yeast at 28ºC & 10ºC in YPGAL

Harvest warm samples and harvest the cold samples at 30 minutes, 2 hours, and 8 hours and flash freeze at -80ºC

Isolate RNA from the cells using Ambion Ribo-Pure kit

Check quality of RNA using Nano-drop and quality using agarose electrophoresis

Synthesize cDNA adding capture sequence to warm samples that will bind green dye and capture sequence to cold samples that recognize red dye using Genisphere kit.

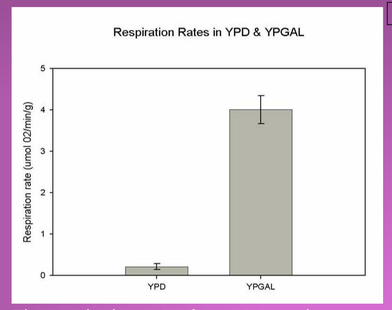
Hybridize the array with the cDNA at 50ºC overnight

Wash and hybridize the miroarray with red and green dyes and incubate at 4 hours at 50ºC

Wash microarray and send to Davidson College for scanning

Data analysis of the red & green intensities using Magic Tool

Results



The respiration rate of yeast grown in YPGAL was 4.005 umol/ min/ g as compared to YPD which was 0.22 umol/ min/ g.

YPGAL 8	YPGAL 2	YPGAL 30

Here is the RNA separated by agarose electrophoresis. The top bright band is 28 s and the one below is 18 s. The RNA was intact.

Genes induced ≥ 3- fold at 30 minutes for Protein

ORF	Gene	Biological Process	Molecular Function
YLR088W	GAA1	attachment of GPI anchor to protein	GPI-anchor transamidase activity
YOR234C	RPL33B	protein biosynthesis	structural constituent of ribosome
YBR173C	UMP1	protein catabolism	chaperone activity*
YHR166C	CDC23	ubiquitin-dependent protein catabolism*	protein binding activity*
YDL159W	STE7	protein amino acid phosphorylation*	MAP kinase kinase activity
YHR205W	SCH9	protein amino acid phosphorylation*	protein serine/threonine kinase activity
YMR194W	RPL36A	protein biosynthesis	structural constituent of ribosome*
YMR297W	PRC1	vacuolar protein catabolism	carboxypeptidase C activity
YML026C	RPS18B	protein biosynthesis	structural constituent of ribosome
YOR061W	CKA2	protein amino acid phosphorylation*	protein kinase CK2 activity
YMR089C	YTA12	protein complex assembly*	ATPase activity*
YNL315C	ATP11	protein complex assembly	chaperone activity
YML078W	CPR3	protein folding	peptidyl-prolyl cis-trans isomerase activity
YBL076C	ILS1	protein biosynthesis	isoleucine-tRNA ligase activity
YHR091C	MSR1	protein biosynthesis*	arginine-tRNA ligase activity
YER003C	PMI40	protein amino acid glycosylation*	mannose-6-phosphate isomerase activity
YGR214W	RPS0A	protein biosynthesis*	structural constituent of ribosome
YOR357C	GRD19	protein localization	protein binding activity
YDR116C	MRPL1	protein biosynthesis*	structural constituent of ribosome
YDL190C	UFD2	ubiquitin-dependent protein catabolism*	ubiquitin conjugating enzyme activity
YBR181C	RPS6B	protein biosynthesis	structural constituent of ribosome
YMR286W	MRPL33	protein biosynthesis	structural constituent of ribosome
YNL185C	MRPL19	protein biosynthesis	structural constituent of ribosome
YPL079W	RPL21B	protein biosynthesis	structural constituent of ribosome
YOR124C	UBP2	protein deubiquitination	ubiquitin-specific protease activity
YGL095C	VPS45	protein complex assembly*	chaperone activity
YNL064C	YDJ1	protein-mitochondrial targeting	chaperone regulator activity*
YNL297C	MON2	protein-vacuolar targeting*	molecular_function unknown
YOR312C	RPL20B	protein biosynthesis	structural constituent of ribosome
YML041C	VPS71	protein-vacuolar targeting	molecular_function unknown
YNL298W	CLA4	protein amino acid phosphorylation*	protein serine/threonine kinase activity
YLR248W	RCK2	protein amino acid phosphorylation*	protein serine/threonine kinase activity
YLR048W	RPS0B	protein biosynthesis*	structural constituent of ribosome
YPR166C	MRP2	protein biosynthesis	structural constituent of ribosome
YDL229W	SSB1	protein biosynthesis	chaperone activity*
YPL106C	SSE1	protein folding	co-chaperone activity
YPL140C	MKK2	protein amino acid phosphorylation*	MAP kinase kinase activity
YDL122W	UBP1	protein deubiquitination	ubiquitin-specific protease activity
YHL015W	RPS20	protein biosynthesis	structural constituent of ribosome
YKL122C	SRP21	protein-ER targeting	protein signal sequence binding activity
YJR123W	RPS5	protein biosynthesis	structural constituent of ribosome
YIL147C	SLN1	protein amino acid phosphorylation*	osmosensor activity*
YLR106C	MDN1	protein complex assembly	ATPase activity
YBR181C	RPS6B	protein biosynthesis	structural constituent of ribosome
YLR333C	RPS25B	protein biosynthesis	structural constituent of ribosome
YOR117W	RPT5	ubiquitin-dependent protein catabolism	ATPase activity*
YPL084W	BRO1	ubiquitin-dependent protein catabolism*	molecular_function unknown
YLR406C	RPL31B	protein biosynthesis	structural constituent of ribosome
YBR181C	RPS6B	protein biosynthesis	structural constituent of ribosome
YOR150W	MRPL23	protein biosynthesis*	structural constituent of ribosome

Genes involved in protein biosynthesis and the ubiquitin pathway were up regulated.

Genes repressed ≥ 3-fold at 30 minutes for Stress

ORF	Name	Biological Process	Molecular Function
YBR023C	CHS3	response to osmotic stress*	chitin synthase activity
YJR032W	CPR7	response to stress	chaperone activity*
YHR043C	DOG2	response to stress*	2-deoxyglucose-6-phosphatase activity
YBL075C	SSA3	response to stress*	heat shock protein activity
YLL039C	UBI4	response to stress*	protein tagging activity*
YLL039C	UBI4	response to stress*	protein tagging activity*
YDL166C	FAP7	response to oxidative stress	molecular_function unknown
YLL039C	UBI4	response to stress*	protein tagging activity*
YLL039C	UBI4	response to stress*	protein tagging activity*
YLL026W	HSP104	response to stress*	heat shock protein activity*
YGR209C	TRX2	response to oxidative stress*	thiol-disulfide exchange intermediate activity
YBR244W	GPX2	response to oxidative stress	glutathione peroxidase activity
YLL039C	UBI4	response to stress*	protein tagging activity*
YLL026W	HSP104	response to stress*	heat shock protein activity*

Classification of genes induced ≥ 3- fold in response to cold temperature

		30 min		2hr		8hr	
	All	up	%	up	%	up	%
Biogenesis	229	18	8%	15	7%	12	5.2%
Cell Cycle	50	1	2%	2	4%	0	0.0%
Chaperone	84	8	10%	5	6%	4	4.8%
DNA Repair	53	3	6%	2	4%	3	5.7%
DNA Replication	42	2	5%	4	10%	3	7.1%
Folding	41	2	5%	4	10%	5	12.2%
Heat Shock	24	3	13%	3	13%	6	25.0%
Metabolism	206	18	9%	11	5%	5	2.4%
Modification	37	3	8%	4	11%	1	2.7%
Oxidative Phosphorylation	3	1	33%	1	33%	0	0.0%
Oxidative Stress	28	3	11%	3	11%	5	17.9%
Protein	770	50	6%	42	5%	43	5.6%
Respiration	48	6	13%	3	6%	0	0.0%
Signal	56	3	5%	5	9%	2	3.6%
Stress	142	14	10%	13	9%	14	9.9%
Transcription	289	24	8%	23	8%	23	8.0%
Translation	65	6	9%	6	9%	3	4.6%
Transport	396	30	8%	32	8%	10	2.5%
Unknown	2258	155	7%	136	6%	100	4.4%

Classification of genes repressed ≤ 3- fold in response to cold temperature

	30 min						
	All	down	%	down	%	down	%
Biogenesis	229	4	1.7%	11	4.8%	13	5.7%
Cell Cycle	50	0	0.0%	0	0.0%	3	6.0%
Chaperone	84	0	0.0%	3	3.6%	1	1.2%
DNA Repair	53	1	1.9%	3	5.7%	5	9.4%
DNA Replication	42	2	4.8%	2	4.8%	0	0.0%
Folding	41	1	2.4%	3	7.3%	2	4.9%
Heat Shock	24	0	0.0%	1	4.2%	0	0.0%
Metabolism	206	4	1.9%	7	3.4%	5	2.4%
Modification	37	0	0.0%	1	2.7%	0	0.0%
Oxidative Phosphorylation	3	0	0.0%	0	0.0%	1	33.3%
Oxidative Stress	28	4	14.3%	2	7.1%	2	7.1%
Protein	770	22	2.9%	27	3.5%	43	5.6%
Respiration	48	0	0.0%	3	6.3%	2	4.2%
Signal	56	0	0.0%	3	5.4%	1	1.8%
Stress	142	4	2.8%	5	3.5%	8	5.6%
Transcription	289	4	1.4%	12	4.2%	13	4.5%
Translation	65	1	1.5%	5	7.7%	3	4.6%
Transport	396	5	1.3%	10	2.5%	38	9.6%
Unknown	2258	39	1.7%	88	3.9%	182	8.1%

A 3- fold change in gene expression was considered a significant difference. We focused our analysis on the expression of genes that were repressed or induced at the 30 minute time point.

Overall there are more genes induced than repressed.

Most genes that change in expression are involved in metabolism, stress response, transcription, transport, and protein metabolism.

Genes induced ≥ 3- fold at 30 minutes for Metabolism

ORF	Name	Biological Process	Molecular Function
YMR272C	SCS7	fatty acid metabolism	oxidoreductase activity
YFR053C	HXK1	fructose metabolism	hexokinase activity
YNL045W		lipid metabolism*	aminopeptidase activity*
YDR173C	ARG82	arginine metabolism*	inositol/phosphatidylinositol kinase activity
YHR044C	DOG1	glucose metabolism	2-deoxyglucose-6-phosphatase activity
YOR236W	DFR1	folic acid and derivative metabolism	dihydrofolate reductase activity
YHR046C	INM1	myo-inositol metabolism	myo-inositol-1(or 4)-monophosphatase activity
YOL001W	PHO80	regulation of phosphate metabolism	cyclin-dependent protein kinase, regulator activity
YPL273W	SAM4	sulfur amino acid metabolism	homocysteine S-methyltransferase activity
YKR091W	SRL3	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	molecular_function unknown
YDR204W	COQ4	ubiquinone metabolism	molecular_function unknown
YJR139C	HOM6	methionine metabolism*	homoserine dehydrogenase activity
YER024W	YAT2	alcohol metabolism*	carnitine O-acetyltransferase activity
YKR080W	MTD1	one-carbon compound metabolism*	methylenetetrahydrofolate dehydrogenase (NAD+) activity
YDR502C	SAM2	methionine metabolism	methionine adenosyltransferase activity
YKL038W	RGT1	glucose metabolism	DNA binding activity*
YDL109C		lipid metabolism	lipase activity
YMR170C	ALD2	aldehyde metabolism*	aldehyde dehydrogenase activity
YOR065W	CYT1	oxidative phosphorylation, ubiquinone to cytochrome c*	electron transporter, transferring electrons within CoQH2-cytochrome c reductase complex activity

Metabolic genes involved in amino acid biosynthesis, fatty acid synthesis, and oxidative phosphorylation genes were up regulated.

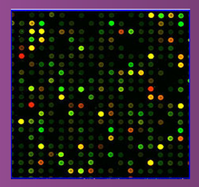
In response to stress, chaperones were up regulated to enhance protein folding. Also, genes involved in the ubiquitin pathway, which degrades proteins were up regulated.

Discussion

Our studies show that in response to cold temperature, the expression of genes involved in protein biosynthesis and the ubiquitin pathway are up regulated at 30 minutes in yeast. This suggests that low temperature has a denaturing effect on proteins. Ubiquitin is a small protein that gets attached to damaged proteins and targets them for degradation. Genes involved in amino acid biosynthesis may be up regulated to replace damaged proteins. In addition, the expression of chaperones was induced. Chaperones assist in protein folding.

After 8 hours at 10ºC, many of the stress response genes and genes involved in protein biosynthesis were no longer up regulated and some were repressed. This suggests that the cell has adapted to the cold.

In this study, we were surprised that cells grown in YPGAL did not appear to experience more oxidative stress than cells grown in YPD. We expected that because cells grown in YPGAL have more mitochondria for oxidative phosphorylation, than cells grown in YPD, they would experience more oxidative stress in the cold. Instead, the genes that were induced or repressed in response to cold temperature were actually quite similar between cells grown in YPD and YPGAL. One exception was that cells grown in YPGAL had more mitochondrial genes up regulated at 30 minutes.



This is an example of a scanned microarray. The red dots mean the gene was induced in response to cold temperature, the green dots mean the gene was repressed, and yellow means there was no changes.

Literature Cited

DeRisi, J, Vishwanath, I, Brown, P. (1997). "Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic Scale". *Science* vol. 278, pg 680-686.

Acknowledgements

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