Whole-genome Transcriptional Responses to Hypoxia in Respiration-proficient and Respiration-deficient Yeasts: Implication of the Mitochondrial Respiratory Chain in Oxygen-regulated Gene Expression

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Cells sense, respond, and adapt to a low oxygen environment called hypoxia, which is widely involved in a variety of human diseases. Adaptation to low oxygen concentrations includes gene expression changes by inducing hypoxic genes and reducing aerobic genes. Recently, the mitochondrial respiratory chain has been implicated in the control of these oxygen-regulated genes when cells experience hypoxia. In order to obtain an insight into the effect of the mitochondrial respiratory chain on cellular response to hypoxia, we here examined whole genome transcript signatures of respiration-proficient and respiration-deficient budding yeasts exposed to hypoxia using DNA microarrays. By comparing whole transcriptomes to hypoxia in respiration-proficient and respiration-deficient yeasts, we found that there are several classes of oxygen-regulated genes. Some of them require the mitochondrial respiratory chain for their expression under hypoxia while others do not. We found that the majority of hypoxic genes and aerobic genes need the mitochondrial respiratory chain for their expression under hypoxia. However, we also found that there are some hypoxic and aerobic genes whose expression under hypoxia is independent of the mitochondrial respiratory chain. These results indicate a key involvement of the mitochondrial respiratory chain in oxygen-regulated gene expression and multiple mechanisms for controlling oxygen-regulated gene expression. In addition, we provided gene ontology analyses and computational promoter analyses for hypoxic genes identified in the study. Together with differentially regulated genes under hypoxia, these post-analysis data will be useful resources for understanding the biology of response to hypoxia.

Key words: Aerobic genes, hypoxia, hypoxic genes, mitochondria, respiration

Introduction

Cells are capable of responding to an exposure to a low oxygen environment, i.e., hypoxia [6]. This response to hypoxia includes immediate changes in cellular energy metabolism as a short-term adaptation as well as the activation of gene expression pathways as a long-term adaptation, which help the cell cope with an insufficient concentration of oxygen [6, 15]. Upon exposure to hypoxia, these gene expression pathways function to up-regulate a large number of hypoxic genes that are induced under low oxygen environments, and down-regulate a large number of aerobic genes that are expressed optimally in normoxic environments. Thus, the changes in expression of these oxygen-regulated genes (i.e., both hypoxic and aerobic genes) are crucial for cellular adaptation to a prolonged hypoxic environment.

Recent microarray studies have demonstrated that a substantial number (up to 15%) of the entire genes in yeast are oxygen-regulated [3, 23]. Expression of these oxygen-regulated genes is known to be mediated through the control of a handful of transcription factors. For example, Rox1p represses transcription of hypoxic genes under normoxic conditions [18] while Mga2p activates transcription of hypoxic genes in response to hypoxia [17]. And, Mot3p represses transcription of hypoxic genes [19] whereas Hap1p activates transcription of aerobic genes, including ROXI, under aerobic conditions [25]. So far, much of the work on these yeast transcription factors has focused on the effects of oxygen...
and heme levels on the activity of Hap1p, which is known to regulate most aerobic genes in response to oxygen levels. The activity of Hap1p is controlled by the level of an intracellular heme pool. Under conditions of low oxygen, heme and Rox1p levels drop [31] and Rox1p-regulated hypoxic genes are de-repressed, allowing for their expression under hypoxic conditions. Moreover, Hap1p actively represses transcription of ROX1 in oxygen limiting conditions [10].

In addition to transcription factors mentioned above, several recent studies have implicated the mitochondrial respiratory chain in the induction of hypoxic nuclear genes in both yeast [21, 26] and mammals [9]. Initially, it was proposed that the mitochondrial respiratory chain participates in the expression of oxygen-regulated genes under hypoxia in the budding yeast, Saccharomyces cerevisiae. By comparing whole genome responses to hypoxia and that the mitochondrial-generated ROS function in hypoxic signaling by stabilizing HIF-1, an important regulator of hypoxic genes [5, 14]. However, it is not clear from these studies if ROS are sufficient for HIF-1 stability. Regardless of exact mechanisms, it seems that the mitochondrial respiratory chain plays a key role in expression of oxygen-regulated genes under hypoxia.

In the present study, we investigated a role for the mitochondrial respiratory chain in oxygen-regulated gene expression at whole genome levels using DNA microarrays in the budding yeast, S. pombe. By comparing whole genome responses to hypoxia in respiration-proficient and respiration-deficient yeasts, we found that there are several classes of oxygen-regulated genes. Some of them require the mitochondrial respiratory chain for their expression under hypoxia while others do not. These results suggest that the mitochondrial respiratory chain participates in expression of oxygen-regulated genes under hypoxia in multiple ways.

Materials and Methods

Yeast strains, media, and growth conditions

The following S. cerevisiae strains were used in this study: JM43 (MATα, his4Δ58, trp1Δ289, lex2Δ2-3, 112, ura3Δ52 [pρ]) [24] and JM43Δρ (MATα, his4Δ58, trp1Δ289, lex2Δ2-3, 112, ura3Δ52 [pρ]) [29]. JM43 and JM43Δρ are iso-chromosomal. Yeast cells were grown in SSG-TEA, a semisynthetic galactose medium, supplemented with Tween 80, ergosterol, silicon antifoam, and amino acids and uracil, as described [7]. Pre-cultures were grown to steady-state on a controlled environment incubator shaker (New Brunswick Scientific, Enfield, USA) at 200 rpm and 30°C and kept in logarithmic growth phase for at least 10 generations. Logarithmic phase pre-cultures were used to inoculate main cultures, which were grown under normoxia or anoxia in a New Brunswick BIOFLO 3,000 fermentor, as described [7]. Steady-state normoxic and anoxic cultures were collected by centrifugation and immediately used or frozen at -80°C prior to further analyses. Anoxic cultures were exposed to anoxia for 24 hr.

RNA isolation and DNA microarray analysis

Total RNA from JM43 and JM43Δρ was isolated by the phenol-chloroform method [13], further purified with a RNeasy midi kit (Qiagen, Venlo, Netherlands) according to the manufacturer’s instructions, and subjected to DNA microarray experiments. cDNAs were synthesized from 5 µg of total RNA by using the Superscript Choice kit (Invitrogen, Waltham, USA) with a T7-(dT)24 primer incorporating a T7 RNA polymerase promoter. cRNAs were generated and biotin-labeled by T7-dependent in vitro transcription (Enzo Biochemical, New York, USA). Labeled cRNAs were fragmented by incubation at 94°C for 35 min in 40 mM Tris acetate, pH 8.1, 100 mM potassium acetate, and 30 mM magnesium acetate. Fifteen micrograms of fragmented cRNAs were hybridized for 16 hr at 45°C to Yeast Genome 2.0 Array (Affymetrix, Santa Clara, USA). This yeast genomic chip contains probe sets for two yeast species (both S. cerevisiae and S. pombe). Probe sets for S. pombe were excluded and only probe sets for S. cerevisiae were considered in later analysis. The chips were washed, stained with streptavidin-phycocyanin in a fluidics station and scanned at 3-µm resolution in the Affymetrix Genechip System confocal scanner (Agilent Technologies, Massy, France). For each yeast strain JM43 and JM43Δρ, three independent RNA isolations and microarray experiments including both normoxic and anoxic conditions, were performed.

Microarray data preparation and analysis

All data processing and analysis were performed using R (http://www.r-project.org). Raw data from array scans were normalized using the Robust Multi-chip Average method [16] through the RMA package of Bioconductor [2].
After normalization, the data were filtered using two criteria; (1) Affymetrix mRNA detection calls were used to exclude all Probe Set IDs with an ‘Absent’ call in all samples. (2) Transcripts that demonstrate little variation across all arrays were removed. This is performed by comparing the variance of the log-intensities for each gene with the median of all variance for the entire array. Those genes not significantly more variable then the median are filtered out. After filtering, we performed a two-tailed t-test and corrected the p-value with False Discovery Rate (FDR) procedure [4]. At FDR of 0.05, the analysis produced the final lists of 1127 and 740 differently expressed genes in anaerobic conditions relative to matching aerobic conditions in JM43 and JM43ρ0, respectively. These differentially expressed genes were clustered using hierarchical clustering approach [2] through the R packages ‘heatmap’ and ‘gradient.rect’. Co-regulated genes were further grouped with respect to existing annotations (e.g., Gene Ontology) [1] using FunSpec (a web-based tool available online at http://funspec.med.utoronto.ca) [27]. Transcription regulatory associations between transcription factors and co-regulated genes were explored using the YEASTRACT database (www.yeastract.com) [28]. This database provides tools analyzing transcriptional regulatory networks in yeast based on experimental evidence underlying these regulatory associations, which is spread throughout hundreds of published articles.

Results and Discussion

Involvement of the mitochondrial respiratory chain in global gene expression in response to hypoxia

In order to obtain insights into roles for the mitochondrial respiratory chain in the global gene expression responding to hypoxia in yeast, we have used two S. cerevisiae strains; the respiration-proficient wild-type JM43 and its respiration-deficient derivative, JM43ρ0. JM43ρ0 is iso-chromosomal to JM43 but lacks a mtDNA and a functional mitochondrial respiratory chain, resulting in a complete loss of respiration. We cultured these strains first aerobically and then exposed them to anoxia for 24 hr. Aerobic and anaerobic cells were harvested by centrifugation. Subsequently, total RNA was isolated, purified, and subjected to Yeast Genome 2.0 Array from Affymetrix. All experiments were triplicated. For each strain, we compared its aerobic transcriptomes with matching anaerobic transcriptomes. These comparisons revealed differentially expressed genes in JM43 or JM43ρ0, in response to oxygen availability. As shown in Fig. 1A, at the false discovery rate of 0.05, exposure of yeast cells to anoxia for 24 hr led to change in expression levels of 1127 genes in JM43 and of 740 genes in JM43ρ0. These differentially expressed genes in anaerobic cultures relative to aerobic cultures are oxygen-regulated and include both up-regulated and down-regulated genes. They correspond to an overall change in approximately 17% and 11% of the whole yeast transcriptome in JM43 and JM43ρ0, respectively. From Fig. 1A, 233 genes are affected in both JM43 and JM43ρ0, indicating that their regulation is not dependent on respiration. However, the gene expression change of 894 genes in JM43

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Fig. 1. Venn diagrams representing statistically significant genes that are differentially expressed in response to anoxia for 24 hr. (A) A Venn diagram showing all of differentially expressed genes which include both induced and repressed genes in aerobic JM43 and JM43ρ0 cultures. (B) A Venn diagram illustrating hypoxia-induced genes (hypoxic genes), which are up-regulated at least 1.5-fold in aerobic cultures relative to normoxic cultures. These hypoxic genes are classified into Categories I-III, which are indicated in parentheses. (C) A Venn diagram illustrating down-regulated genes (aerobic genes) that are reduced at least 1.5-fold in anoxic cultures relative to normoxic cultures. These aerobic genes are classified into Categories IV-VI, which are indicated in parentheses.
and that of 507 genes in JM43\(\rho\) are unique to cell's ability to respire, indicating that respiring cells respond differently to lack of oxygen from non-respiring cells.

These oxygen-regulated genes were further selected by modes of expression (i.e., induced or reduced) and by degrees of change in expression (i.e., >1.5-fold up or down) under hypoxia (Fig. 1B and C). Fig. 1B shows up-regulated genes in response to lack of oxygen. These up-regulated genes are referred to hypoxic genes because their expression is induced under hypoxia. These hypoxic genes comprise three categories (Categories I-III). First, Category I genes are up-regulated exclusively in JM43. Second, Category II genes are increased in both JM43 and JM43\(\rho\). Third, Category III genes are induced only in JM43\(\rho\). Category I genes requires the mitochondrial respiratory chain for their induced expression upon hypoxia while Categories II and III genes do not. As shown in Fig. 2B, we revealed 467 yeast hypoxic genes of which 248 genes are Category I, 109 genes are Category II, and 110 genes are Category III. Therefore, the induction of 248 hypoxic genes (Category I) in JM43 is associated with respiration-proficiency. However, the up-regulation of 109 hypoxic genes (Category II) in both JM43 and JM43\(\rho\), and that of 110 genes (Category III) in JM43\(\rho\) do not require respiration. Gene names and their expression levels corresponding to Categories I-III are shown in Fig. 2-Fig. 4.

Fig. 1C represents down-regulated genes in response to anoxia. These down-regulated genes are referred to aerobic genes because their optimal expression is reduced under hypoxia. Similarly to hypoxic genes in Fig. 1B, these aerobic genes comprise three categories (Categories IV-VI). First, Category IV genes are down-regulated exclusively in JM43. Second, Category V genes are decreased in both JM43 and JM43\(\rho\). Third, Category VI genes are reduced only in JM43\(\rho\). Category IV genes requires the mitochondrial respiratory chain for their reduced expression upon hypoxia while Categories V and VI genes do not. From Fig. 1C, we found 678 yeast aerobic genes of which 275 genes are Category IV, 103 genes are Category V, and 300 genes are Category VI. Thus, the down-regulation of 275 aerobic genes (Category IV) in JM43 is dependent on cell's ability to respire. But the reduced expression of 103 aerobic genes (Category V) in both JM43 and JM43\(\rho\), and that of 300 in JM43\(\rho\) (Category VI) do not require respiration. Gene names and expression levels for Categories IV-VI are shown in Fig. 5-Fig. 7.

Overall, from above results, a large number of genes in yeast genome are needed for the adaptation from aerobic to anaerobic environments. Among them, some hypoxic and/or aerobic genes are dependent on respiration for their change in expression but others are not. In addition, from the finding that many aerobic genes are down-regulated in response to hypoxia, it is apparent that both up-regulation of hypoxic genes and down-regulation of aerobic genes are required to maximal cellular adaptation to hypoxia. This notion is particularly important for hypoxic/aerobic gene pairs. Because these gene pairs encode interchangeable proteins with different functional properties, the failure to switch off one gene while the other is switched on would result in competition between hypoxic and aerobic isoforms, preventing maximal adaptation to hypoxia. Here, we were able to confirm that the expression of hypoxic/aerobic gene pairs is tightly regulated by oxygen levels. For examples, hypoxic genes COX5b, CYC7, HMG2, and ANB1 are up-regulated in response to hypoxia as their aerobic counterparts COX5a, CYC1, HMG1, and HYP2 (TIF51a) respectively, are simultaneously down-regulated.

**Gene ontology (GO) biological processes enriched in hypoxic genes and aerobic genes (Categories I-VI) relative to the whole yeast genome**

Table 1 lists GO biological processes overrepresented in 248 hypoxic genes (Category I) which require respiration. Notably, twenty-two genes involved in autophagy are induced in response to hypoxia, indicating that autophagy is an important cell survival response to hypoxia. This notion is also supported by studies with mammalian cell cultures [30], in which mitochondrial autophagy is a hypoxia-induced adaptive response required for preventing increased levels of ROS and cell death. Another interesting finding is that several GO processes (e.g., DNA damage responses) mediated by MEC1 are enriched in Category I hypoxic genes. MEC1 encodes a yeast homolog of the mammalian tumor suppressor ATM kinase and ensures genome integrity. Thus, in respiring cells the up-regulation of MEC1 may provide protective responses to hypoxia which can induce ROS-mediated DNA damages. GO biological processes involved in the syntheses of cell wall and sterol/ergosterol lipid are enriched in 109 hypoxic genes (Category II) induced in both JM43 and JM43\(\rho\), indicating these processes are common responses to hypoxia regardless of cell's ability to respire. Sterol lipid biosynthesis process is overrepresented in 110 hypoxic genes (Category III) induced only in JM43\(\rho\),
Fig. 2. Category I hypoxic genes. In a left margin, a microarray heat map shows Category I hypoxic genes that are up-regulated in anoxic JM43 cultures but not in anoxic JM43<sup>ρ</sup> cultures relative to their matching normoxic cultures. Red indicates an induced gene expression and green indicates a reduced gene expression according to the scale bar. Three sections (A to C) of the heat map are enlarged and gene names are represented. Each gene is represented by a single row of colored boxes. Three independent microarrays for each strain and oxygen availability are represented by three consecutively adjacent columns.
Fig. 3. Category II hypoxic genes. In a left margin, a microarray heat map shows Category II hypoxic genes that are up-regulated in both anoxic JM43 cultures and anoxic JM43\(^{-}\) cultures relative to their matching normoxic cultures. Red indicates an induced gene expression and green indicates a reduced gene expression according to the scale bar. Two sections (A and B) of the heat map are enlarged and gene names are represented. Each gene is represented by a single row of colored boxes. Three independent microarrays for each strain and oxygen availability are represented by three consecutively adjacent columns.
Fig. 4. Category III hypoxic genes. In a left margin, a microarray heat map shows Category III hypoxic genes that are up-regulated in anoxic JM43ρ₂ cultures but not in anoxic JM43 cultures relative to their matching normoxic cultures. Red indicates an induced gene expression and green indicates a reduced gene expression according to the scale bar. Two sections (A and B) of the heat map are enlarged and gene names are represented. Each gene is represented by a single row of colored boxes. Three independent microarrays for each strain and oxygen availability are represented by three consecutively adjacent columns.
Fig. 5. Category IV aerobic genes. In a left margin, a microarray heat map shows Category IV aerobic genes that are down-regulated in anoxic JM43 cultures but not in anoxic JM43ρ cultures relative to their matching normoxic cultures. Red indicates an induced gene expression and green indicates a reduced gene expression according to the scale bar. Four sections (A to D) of the heat map are enlarged and gene names are represented. Each gene is represented by a single row of colored boxes. Three independent microarrays for each strain and oxygen availability are represented by three consecutively adjacent columns.
Fig. 6. Category V aerobic genes. In a left margin, a microarray heat map shows Category V aerobic genes that are down-regulated in both anoxic JM43 cultures and anoxic JM43ρ cultures relative to their matching normoxic cultures. Red indicates an induced gene expression and green indicates a reduced gene expression according to the scale bar. Two sections (A to B) of the heat map are enlarged and gene names are represented. Each gene is represented by a single row of colored boxes. Three independent microarrays for each strain and oxygen availability are represented by three consecutively adjacent columns.

Further supporting that the hypoxia-induced up-regulation of sterol/ergosterol lipid metabolism is independent of respiration (data not shown).

As shown in Table 2, several GO biological processes are enriched in 275 aerobic genes (Category IV) which are down-regulated exclusively in JM43. Not surprisingly, reduced genes function in the processes requiring oxygen (e.g., respiration and oxidation). Also, genes involved in translation and ribosome biogenesis are down-regulated in response to anoxia. These results are consistent with the notion that hypoxia induces rapid and dramatic changes in cellular metabolism, in part through inhibition of target of rapamycin (TOR) kinase complex 1 (TORC1) activity which regulates protein synthesis. Therefore, under hypoxic conditions yeast cells rapidly initiate a variety of adaptive mechanisms that reduce energy expenditure through inhibition of energy-intensive processes including protein translation. The reduced expression of genes involved in oxidation and translation is also over-represented in 103 aerobic genes (Category V) relative to the whole genome. This finding suggests that down-regulation of oxidation and translation processes is a common response to lack of oxygen in both respi-
Fig. 7. Category VI aerobic genes. In a left margin, a microarray heat map shows Category VI aerobic genes that are down-regulated in anoxic JM430 cultures but not in anoxic JM43 cultures relative to their matching normoxic cultures. Red indicates an induced gene expression and green indicates a reduced gene expression according to the scale bar. Four sections (A to D) of the heat map are enlarged and gene names are represented. Each gene is represented by a single row of colored boxes. Three independent microarrays for each strain and oxygen availability are represented by three consecutively adjacent columns.
Table 1. Overrepresentation of Gene Ontology (GO) Biological Process in 248 Category I hypoxic genes that are up-regulated in JM43 but not in JM43\textsuperscript{0} in response to hypoxia in this study.

| GO biological process and gene | Number of gene | \(p\)-value |
|-------------------------------|----------------|------------|
| ascospore wall assembly [GO:0030476] | 10 54 | 2.6E-05 |
| autophagy [GO:0006914] | 22 242 | 9.9E-05 |
| nucleobase, nucleoside, nucleotide and nucleic acid metabolic process [GO:0006139] | 6 24 | 2.0E-04 |
| regulation of Rho protein signal transduction [GO:0035023] | 3 6 | 9.6E-04 |
| meiosis [GO:0007126] | 15 165 | 1.3E-03 |
| meiotic recombination [GO:0007131] | 7 53 | 3.4E-03 |
| transmembrane transport [GO:0055085] | 5 30 | 4.7E-03 |
| carbohydrate metabolic process [GO:0005975] | 12 136 | 4.9E-03 |
| ascospore formation [GO:0030477] | 8 75 | 6.8E-03 |
| meiotic DNA double-strand break formation [GO:0042138] | 3 11 | 6.9E-03 |
| glycine metabolic process [GO:0006544] | 2 4 | 8.0E-03 |
| positive regulation of glycolysis [GO:0045821] | 2 4 | 8.0E-03 |
| NADH oxidation [GO:0006116] | 3 12 | 9.0E-03 |
| sexual reproduction [GO:0019953] | 4 23 | 9.8E-03 |

GO Biological Process number is shown in brackets and Category I hypoxic genes in a given GO Biological Process are listed. ‘A’ denotes number of genes from Category I hypoxic genes in a given GO Biological Process while ‘B’ indicates number of genes in the Process from the total 6603 genes in GO data base. The GO Biological Process enrichment \(p\)-value was calculated by hypergeometric distribution analysis.
Table 2. Overrepresentation of Gene Ontology (GO) Biological Process in 275 Category IV aerobic genes that are down-regulated in JM43 but not in JM43\(^a\) in response to hypoxia in this study

| GO biological process and gene | Number of gene | p-value |
|-------------------------------|----------------|---------|
| **Cellular respiration [GO:0045333]** | | |
| EHD3 MRPL35 RML2 TIM13 MRP4 MRPL49 MRPL15 MRPL4 YML6 YMR166C MRPL44 YNR040W MDMA2 ISM1 ALD6 CAR1 COX10 RTC6 | 18 | 50 | 4.38E-13 |
| **Translation [GO:0006412]** | | |
| MRPL16 MRPL36 RIM2 MRPL32 SLM5 IMG1 MTF2 MRPL11 RSM10 MRPL1 RSM24 MRPL7 MHR1 MRPL35 MRPS28 BCS1 MRPL28 AIM8 RSM28 TIM9 RML2 RSM18 RMD9 RSM23 YGR054W MRPL13 MRPS35 RSM27 MRP4 RRF1 FYV4 MRPL6 MAM33 MRPL49 RSM26 RSM22 MRP49 MST1 MEF1 MRPL15 MRPL4 YML6 RPM2 MRPS8 YMR166C MRPL24 MRPL44 MSK1 YNL122C NAM9 MRPS18 ATP23 MRPL50 YNR040W PET123 AIM42 ISM1 MSY1 MRPS1 COX10 MRPL40 RTC6 TOM5 | 63 | 755 | 2.62E-08 |
| **Aerobic respiration [GO:0009060]** | | |
| COR1 MBA11 DLD1 MRPL1 RSM24 BCS1 RPI1 QCR6 RMD9 SUA5 COX4 COX13 SHY1 MRPS35 MAM33 PPA2 QCR2 | 17 | 88 | 8.86E-08 |
| **Methionine biosynthetic process [GO:0009086]** | | |
| MIS1 MET8 STR3 MET3 ECM17 MET14 MHT1 MET17 MET2 | 9 | 40 | 2.90E-05 |
| **Oxidation reduction [GO:0055114]** | | |
| MIS1 MET8 LDL1 RPI1 MXR1 TPA1 QCR6 OLE1 COX4 COX13 ERV1 CTT1 CBP2 COX6 ECM17 HAP4 SDH1 COX8 DUS4 HMG1 RPM2 ND11 IDH1 COX5A SPS19 ALD6 | 26 | 274 | 6.39E-05 |
| **Ribosome biogenesis [GO:0042254]** | | |
| RBG1 YBL054W HMT1 MIS1 ENP1 GFD2 PRM7 NOP6 ARX1 ATC1 IZH1 TPA1 UTP7 FTR1 CGR1 SUA5 YGR054W YGR079W SLX9 SIM1 SDA1 YGR283C YHR127W IMP3 NMD3 RIX1 YIL096C SQT1 UTP18 NUC1 UTP11 SRP40 SOFI PAM18 CWC24 FPR4 TRM9 RRB1 YNL022C AAH1 NOP13 AIM38 YOR021C RKI1 SLPI LRC5 RRS1 VTS1 YPL108W IDI1 | 50 | 724 | 0.000182 |
| **Phosphorus metabolic process [GO:0006793]** | | |
| QC6 TIM13 HAP4 AIM50 RPM2 COX5A MDMA2 | 7 | 35 | 0.000496 |
| **Sulfate assimilation [GO:0000103]** | | |
| MET8 MET3 ECM17 MET14 | 4 | 12 | 0.001118 |
| **Mitochondrial electron transport, cytochrome c to oxygen [GO:0006123]** | | |
| COX4 COX6 COX8 COX5A | 4 | 13 | 0.001562 |
| **Mitochondrial metabolic process [GO:0006555]** | | |
| MET3 MET14 SAM11 MET17 | 4 | 14 | 0.002116 |
| **Mitochondrial protein processing during import [GO:0006627]** | | |
| MAS2 OCT1 MAS1 | 3 | 7 | 0.002208 |
| **Cysteine biosynthetic process [GO:0019344]** | | |
| MET3 ECM17 MET14 MET17 | 4 | 15 | 0.002792 |
| **Mitochondron organization and biogenesis [GO:0007005]** | | |
| BCS1 DNM1 RPM2 ATP11 TIM23 MDMA3 MDMA2 | 7 | 47 | 0.003028 |
| **Cofactor metabolic process [GO:00051186]** | | |
| EHD3 HAP4 AIM38 ALD6 RTC6 | 5 | 25 | 0.003232 |

GO Biological Process number is shown in brackets and Category I hypoxic genes in a given GO Biological Process are listed. ‘A’ denotes number of genes from Category I hypoxic genes in a given GO Biological Process while ‘B’ indicates number of genes in the Process from the total 6603 genes in GO data base. The GO Biological Process enrichment p-value was calculated by hypergeometric distribution analysis.
In order to examine their roles in transcriptional network underlying the induction of hypoxic genes, we have clustered hypoxic genes identified in this study based on their regulatory associations with the known transcription factors (e.g., Rox1p, Upc2p, Mot3p, Sut1p, Mga2p, Yap1p, and Msn2p) using the YEASTRACT database (www.yeastract.com) [28]. Currently, this database contains a repository of more than 48,333 regulatory associations between transcription factors and target genes in yeast. Because the YEASTRACT transcriptional regulatory information is based on experimental evidence from published results, this bioinformatics tool may provide substantial insights into transcription regulatory network for a group of co-regulated genes. As shown in Table 3, YEASTRACT computational

Table 3. Enrichment of putative transcription factors involved in regulation of hypoxic genes identified in this study

| Category | Transcription factor | Number of genes in cluster | Number of genes in genome | p-value |
|----------|----------------------|----------------------------|---------------------------|---------|
| Category I hypoxic genes up-regulated in JM43 but not in JM43 ρ (n=248) | Sok2p | 64 | 999 | 9.1E-06 |
| | Aft1p | 56 | 708 | 5.9E-08 |
| | Rpm4p | 49 | 814 | 5.8E-04 |
| | Msn2p | 48 | 511 | 3.7E-09 |
| | Rox1p | 35 | 346 | 1.2E-07 |
| | Upc2p | 13 | 208 | 5.2E-02 |
| | Mot3p | 12 | 131 | 4.4E-03 |
| | Sut1p | 8 | 68 | 4.6E-03 |
| Category II hypoxic genes up-regulated in both JM43 and JM43 ρ (n=109) | Upc2p | 46 | 208 | < 1.0E-10 |
| | Sok2p | 44 | 999 | 1.7E-10 |
| | Rox1p | 36 | 346 | < 1.0E-10 |
| | EcM22p | 33 | 269 | < 1.0E-10 |
| | Rap1p | 30 | 1191 | 9.5E-03 |
| | Sut1p | 20 | 68 | < 1.0E-10 |
| | Mot3p | 14 | 131 | 4.0E-08 |
| | Mga2p | 4 | 27 | 1.1E-03 |
| Category III hypoxic genes up-regulated in JM43 ρ but not in JM43 (n=110) | Sok2p | 41 | 999 | 1.1E-08 |
| | Yap1p | 37 | 1551 | 1.0E-02 |
| | Rap1p | 30 | 1191 | 1.1E-02 |
| | Fdr1p | 25 | 528 | 1.6E-06 |
| | Phd1p | 24 | 450 | 3.3E-07 |
| | Fhl1p | 23 | 844 | 1.1E-02 |
| | Rox1p | 14 | 346 | 1.9E-03 |
| | Upc2p | 10 | 208 | 2.6E-03 |
| | Mot3p | 7 | 131 | 6.5E-03 |

A web-based computational tool in the YEASTRACT database (www.yeastract.com; Teixeira et al., 2006) was used for identification of putative transcription factors involved in hypoxic genes identified in this study. Enrichment of a transcription factor in a given Category relative to the whole yeast genome was calculated by hypergeometric distribution analysis and magnitude of the enrichment is indicated by the p-value.
analysis has confirmed that Rox1p, Upc2p, and Mot3p are transcriptional regulators enriched in all Categories I-III hypoxic genes relative to the whole genome. In addition, we here identified Sok2 as a predominant transcription factor for all Categories I-III hypoxic genes. Because Sok2 has been implicated in the cyclic AMP (cAMP)-dependent protein kinase (PKA) signal transduction pathway (Ref), our results suggest a possible involvement of PKA signaling in hypoxic gene induction.

From Table 3, Rox1p, Upc2, Sok2p, Aft1p, Rpn4p, and Sut1p were identified as documented transcription factors for Category I hypoxic genes induced only in JM43. Msn2p, which is activated under stress conditions, is also suggested to be regulating Category I hypoxic genes. In contrast, Msn2p was not identified as a potential regulator for Categories II and III hypoxic genes in which are up-regulated in JM43$. This finding is further supported by the fact that JM43$ experiences lower levels of oxidative stress relative to JM43 [11]. For Category II hypoxic genes induced in both JM43 and JM43$, Upc2p and Rox1p were identified as major documented regulators and are associated with 42.2% and 33.0% of the hypoxic gene in this cluster, respectively. Furthermore, Sok2p, Ecm22p, Rap1p, and Sut1p were also identified as the putative regulators of Category II. For Category III hypoxic genes induced exclusively in JM43$, the YEASTRACT computational clustering indicated that Sok2p, Yap1p, Rap1p, Phd1p, Fhl1p, Rox1p, Upc2p, and Mot3p are mediating transcriptional regulation.

In addition, for Category I-III hypoxic genes, we have examined YEASTRACT documented targets of Rox1p, a major regulator of hypoxic genes (Table 4). Many of these genes (ERG3, ERG6, ERG7, ERG9, ERG11, ERG25, ERG26, ERG28, HES1, and HMG2) are involved in lipid, ergosterol, and sterol biosynthesis. Genes implicated in stress response and cell wall synthesis, such as DAN1, FRT2, PAU4, PAU5, PAU7, PAU8, PAU14, PAU18, TIR1, TIR2, and TIR3, were also identified as documented targets of Rox1p. Interestingly, Rox1p has implicated in the induction of several Category III hypoxic genes up-regulated only in anoxic JM43$ including COT1, CYB5, and several ERG genes. The presence of Rox1p targets in Category III hypoxic genes by YEASTRACT computational analysis suggests that Rox1p stabilization/activation under hypoxia can be independent of respiration.

In summary, the present study we have examined whole genomic responses to hypoxia in yeast using DNA microarrays. In both respiration-proficient and respiration-deficient yeast cells, we have identified transcriptional changes in many genes that cover a substantial portion of yeast whole genome. We have categorized these oxygen-regulated hypoxic and aerobic genes with respect to dependence on respiration, i.e., the mitochondrial respiratory chain, for their expression pattern under hypoxia. Each category of hypoxic and aerobic genes are listed and further analyzed for gene ontology. In addition, we provided a computational promoter analysis for hypoxic genes identified in the study. We found that the majority of hypoxic genes and aerobic genes need the mitochondrial respiratory chain for their expression under hypoxia. However, we also found that there are some hypoxic and aerobic genes whose expression under hypoxia is independent of the mitochondrial respiratory chain.

Table 4. YEASTRACT documented targets of Rox1p in hypoxic genes identified in this study

| Category | Rox1p-target gene |
|----------|-------------------|
| Category I hypoxic genes up-regulated in JM43 but not in JM43$ (n=248) | |
| AAC3 | ADH5 | ADH7 | CYC7 | DCS2 | DIP5 | EK1 | ERG28 | GDB1 | GID8 |
| GIP2 | GPG1 | GPM2 | GSY1 | HMG2 | HPF1 | HXT15 | LAC1 | MCH5 | PRM1 |
| PRM6 | SPI1 | SUT1 | USV1 | YAL068c | YFR012w | YGR066c | YGR287c | YIL169c | YIL176c |
| YMR252c | YNL200c | YNR014w | YOL083w | YPR015c | |
| Category II hypoxic genes up-regulated in both JM43 and JM43$ (n=109) | |
| ANB1 | ARE1 | COX5b | CSR1 | DAN1 | ERG26 | ERG3 | EUG1 | FET4 | FRT2 |
| GUP2 | GYP6 | HAP1 | HEM13 | HEM14 | HES1 | IRC3 | NCP1 | NRG2 | PAU4 |
| PAU5 | PAU7 | PRM4 | RNR3 | SCM4 | SPO19 | SUR2 | TIR1 | TIR2 | TIR3 |
| TSA2 | UPC2 | YEL047c | YIL169c | YL064c | YML083c | |
| Category III hypoxic genes up-regulated in JM43$ but not in JM43 (n=110) | |
| COT1 | CYB5 | ERG11 | ERG25 | ERG6 | ERG7 | ERG9 | FIT2 | FIT3 | LAP4 |
| ROX1 | VID24 | YDL038c | YGR035c | |
Together, these results indicate a key involvement of the mitochondrial respiratory chain in oxygen-regulated gene expression and multiple mechanisms for controlling oxygen-regulated gene expression.

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References

1. Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M. and Sherlock, G. 2000. Gene ontology: tool for the unification of biology. The gene ontology consortium. Nat. Genet. 25, 25-29.

2. Bates, D. M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, R., Leisch, F., Li, C., Maechler, M., Rossini, A. J., Sawitzki, G., Smith, C., Smyth, G., Tierney, L., Yang, J. Y. and Zhang, J. 2004. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5, R80.

3. Becerra, M., Lombardia-Ferreira, L. J., Hausser, N. C., Hoheisel, J. D., Tizon, B. and Cerdan, M. E. 2002. The yeast transcriptosome in aerobic and hypoxic conditions: effects of hap1, rox1, rox3, and srb10 deletions. Mol. Microbiol. 43, 545-555.

4. Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B. 57, 289-300.

5. Brunelle, J. K., Bell, E. L., Quesada, N. M., Vercauteren, K., Tiranti, V., Zeviani, M., Scarpulla, R. C. and Chandel, N. S. 2005. Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. Cell Metab. 1, 409-414.

6. Bunn, H. F. and Poyton, R. O. 1996. Oxygen sensing and molecular adaptation to hypoxia. Physiol. Rev. 76, 839-885.

7. Castello, P. R., Woo, D. K., Ball, K., Wojcik, J., Liu, L. and Poyton, R. O. 2008. Oxygen-regulated isoforms of cytochrome c oxidase have differential effects on its nitric oxide production and on hypoxic signaling. Proc. Natl. Acad. Sci. USA 105, 8203-8208.

8. Chan, K. and Roth, M. B. 2008. Anoxia-induced suspended animation in budding yeast as an experimental paradigm for studying oxygen-regulated gene expression. Eukaryot. Cell 7, 1795-1808.

9. Chandel, N. S., Mallepe, E., Goldwasser, E., Mathieu, C. E., Simon, M. C. and Schumacker, P. T. 1998. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proc. Natl. Acad. Sci. USA 95, 11715-11720.

10. Chandrel, Y., Gaisne, C., Lions, C. and Verdiere, J. 1998. The transcriptional regulator Hap1p (Cyp1p) is essential for anaerobic or heme-deficient growth of Saccharomyces cerevisiae. Genetic and molecular characterization of an extragenic suppressor that encodes a WD repeat protein. Genetics 148, 559-569.

11. Dirmeier, R., O’Brien, K. M., Engle, M., Dodd, A., Spears, E. and Poyton, R. O. 2002. Exposure of yeast cells to anoxia induces transient oxidative stress: implications for the induction of hypoxic genes. J. Biol. Chem. 277, 34773-34784.

12. Eisen, M. B., Spellman, P. T., Brown, P. O. and Botstein, D. 1998. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA 95, 14863-14868.

13. Elder, R. T., Lob, E. Y. and Davis, R. W. 1983. RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. USA 80, 2432-2436.

14. Guzy, R. D., Mack, M. M. and Schumacker, P. T. 2007. Mitochondrial complex III is required for hypoxia-induced ROS production and gene transcription in yeast. Antioxid. Redox Signal. 9, 1317-1328.

15. Hochachka, P. W., Buck, L. T., Doll, C. J. and Land, S. C. 1996. Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. Proc. Natl. Acad. Sci. USA 93, 9493-9498.

16. Izirary, R. A., Hobbs, B., Collin, F., Beazer-Barclay, Y. D., Antonellis, K. J., Scher, U. and Speed, T. P. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4, 249-264.

17. Jiang, Y., Vasconcelles, M. J., Wretzel, S., Light, A., Giloly, L., McDaid, K., Oh, C. S., Martin, C. E. and Goldberg, M. 2002. Mga2p processing by hypoxia and unsaturated fatty acids in Saccharomyces cerevisiae: impact on LORE-dependent gene expression. Eukaryotic Cell 1, 481-490.

18. Kastaniotis, A. J. and Zitomer, R. S. 2000. ROX1 mediated repression. Adv. Expil. Med. Biol. 475, 185-195.

19. Kastaniotis, A. J., Mennella, T. A., Konrad, C., Torres, A. M. and Zitomer, R. S. 2000. Roles of transcription factor Mot3 and chromatin in repression of the hypoxic gene ANB1 in yeast. Mol. Cell. Biol. 20, 7088-7098.

20. Kundaje, A., Xin, X., Lan, C., Lianoglou, S., Zhou, M., Zhang, L. and Leslie, C. 2008. A predictive model of the oxygen and heme regulatory network in yeast. PloS Comput. Biol. 4, e1000224.

21. Kwast, K. E., Burke, P. V., Staahl, B. and Poyton, R. O. 1999. Oxygen sensing in yeast: Evidence for the involvement of the respiratory chain in regulating the transcription of a subset of hypoxic genes. Proc. Natl. Acad. Sci. USA 96, 5446-5451.

22. Kwast, K. E., Lai, L. C., Menda, N., Jemmes, D. T., Aref, S. and Burke, P. V. 2002. Genomic analysis of anaerobically induced genes in Saccharomyces cerevisiae: functional roles of Rox1 and other factors in mediating anoxic response. J. Bacteriol. 184, 250-265.

23. Lai, L. C., Kosorukoff, A. L., Burke, P. V. and Kwast, K. E. 2005. Dynamical remodeling of the transcriptosome during short-term anaerobiosis in Saccharomyces cerevisiae: differential responle and role of Msn2 and/or Msn4 and other
초록: 저산소 환경에 대한 전체 유전자 발현 반응에서 미토콘드리아 호흡계의 연루
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세포는 다양한 인체 질환에 관련되어 있는 저산소 환경을 인지하고 반응하며 적응한다. 저산소 상태에 적응하기 위해서는 hypoxic 유전자의 발현을 증가시키고 aerobic 유전자의 발현을 감소시키는 유전자 발현 조절이 필요하다. 최근 연구에서 미토콘드리아 호흡계가 이러한 유전자 발현 조절에 관여됨이 밝혀지고 있다. 본 연구에서는 호흡이 가능한 곰팡이(Saccharomyces cerevisiae)와 호흡이 불가능한 돌연변이 곰팡이를 실험대상으로 하여 미토콘드리아 호흡계가 저산소 환경에서 유전자 발현 조절에 관여하는지를 DNA microarray 기법을 이용하여 전체 유전자 발현을 대상으로 조사하였다. 산소 농도가 감소함에 따라 많은 유전자의 발현이 변화가 있으며, 이러한 차별적인 발현 양상을 보이는 유전자는 여러 그룹으로 분류할 수 있었다. 대부분의 hypoxic 그리고 aerobic 유전자는 저산소 상태에 적응하는 발현 양상을 위해서는 미토콘드리아 호흡계가 필요하였다. 그러나 일부 hypoxic 그리고 aerobic 유전자는 미토콘드리아 호흡계와 무관하게 저산소 상태에 적응하는 발현 양상을 보였다. 이러한 결과는 미토콘드리아 호흡계가 저산소 환경에 적응하는 유전자 발현 조절에 필수적이며, 또한 여러 기전을 통하여 이러한 유전자 발현 조절에 관여함을 제시한다. 또한 microarray 실험 결과에서 도출된 산소 농도에 대한 차별적인 발현을 보이는 유전자에 대하여 gene ontology 및 promoter 분석을 수행하였고 이러한 추가 분석 결과는 산소에 의해 조절되는 유전자와 함께 세포가 저산소 환경에 적응하는 기작을 이해하는 데 유용한 자료가 될 것으로 기대된다.

Oliveira, A. L. and Sa-Correia, I. 2006. The YEASTRACT database: a tool for the analysis of transcription regulatory associations in Saccharomyces cerevisiae. Nucleic Acids Res. 34, D446-D451.

Waterland, R. A., Basu, A., Chance, B. and Poyton, R. O. 1991. The isoforms of yeast cytochrome c oxidase subunit V alter the in vivo kinetic properties of the holoenzyme. J. Biol. Chem. 266, 4180-4186.

Zhang, H., Bosch-Marce, M., Shimoda, L. A., Tan, Y. S., Baek, J. H., Wesley, J. B., Gonzalez, F. J. and Semenza, G. L. 2008. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J. Biol. Chem. 283, 10892-10903.

Zitomer, R. S., Limbach, M. P., Rodriguez-Torres, A. M., Balasubramanian, B., Deckert, J. and Snow, P. M. 1997. Approaches to study Rox1 repression of the hypoxic genes in yeast Saccharomyces cerevisiae. Methods 11, 279-288.