Genetic Determinants of Mitochondrial Response to Arsenic in Yeast Saccharomyces cerevisiae

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Abstract

We have used yeast Saccharomyces cerevisiae as a tool to identify the importance of mitochondrial processes involved in arsenic-induced carcinogenicity in humans. We screened 466 single-gene knockout strains of yeast S. cerevisiae known to be involved in biogenesis of mitochondria for sodium arsenite (AsIII) and sodium arsenate (AsV) sensitivity. We identified 72 arsenite-sensitive and 81 arsenate-sensitive mutants. We categorized the identified mutants based on the various mitochondrial processes, including nucleic acid metabolism, oxidative phosphorylation, protein synthesis, and vacuolar acidification. We have identified 65 human orthologues to proteins involved in arsenite sensitivity and 3 human orthologues to arsenite resistance. Furthermore, 23 human orthologues to arsenate sensitivity and 20 human orthologues to arsenate-resistant proteins, including MSHE, COX10, GCSH, PPXO, and MTHFD1, were also identified. Using PathwayAssist software, we did cellular network analysis between identified mitochondrial proteins. Three types of interactions, (a) protein-protein interactions, (b) common transcriptional regulators, and (c) common target genes, were identified. We found that RTG (retrograde) genes involved in mitochondria-to-nucleus signaling regulate both arsenite sensitivity and resistance. Furthermore, our study revealed that ABF1, a multifunctional transcriptional factor, regulates genes involved in both arsenite and arsenate sensitivity and resistance. However, REB1 and RAP1 transcriptional regulators were common to only arsenate- and arsenite-sensitive genes, respectively. These studies indicate that multiple pathways involved in mitochondrial biogenesis protect yeast S. cerevisiae from arsenic-induced toxicity. Together, our studies suggest that evolutionary conserved mitochondrial networks identified in yeast S. cerevisiae must play an important role in arsenic-induced carcinogenesis in humans.

Note: Supplementary data for this article are available at Cancer Research Online.

Materials and Methods

Medium, Solutions, and Strains

All yeast strains were grown in YPD complete medium (1% bacto-yeast extract, 2% bacto-peptone, 2% dextrose, and 2% bacto-agar for plates), with addition of 100 μL/mL. Ampicillin. Sodium arsenite(III) and sodium arsenate(V) were from Sigma and prepared as stock solutions in sterile water.

For growth inhibition assays, S. cerevisiae RKY3109 (MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2Δ8gln hom3-10 ade8 ade2Δ1) and isogenic mitochondrial DNA (mtDNA)-depleted rho+ cells (RKY3109 rho−) were...
used. For a test of arsenic sensitivity of mutants in retrograde response pathway, wild-type (WT) strain PSY142 (MATa leu2 lys2 ura3) and isogenic retrograde mutants \(\text{rtg1}^{D}\) (PSY142 \(\text{rtg1}^{D}::\text{URA3}\)), \(\text{rtg2}^{D}\) (PSY142 \(\text{rtg2}^{D}::\text{URA3}\)), and \(\text{rtg3}^{D}\) (PSY142 \(\text{rtg3}^{D}::\text{URA3}\)) were used as well as WT strain (JRY2334) and its isogenic ABF1 mutants, \(\text{abf-101}\) (JRY4996) and \(\text{abf-102}\) (JRY3765).

High-throughput screening was done with WT \(S.\text{cerevisiae}\) BY4741 (MATa his3D1 leu2D0 met15D0 ura3D0) and a collection of nonessential haploid \(\text{MATa}\) deletion strains (21) derived from parental strain BY4741. Collection was a gift from Dr. Lene Rasmussen (Roskilde University, Roskilde, Denmark). Four hundred and sixty-six strains from this collection, known to be involved in mitochondrial biogenesis and function, were identified and used for the screening purpose (20).

**Growth Inhibition Assay**

The growth inhibition assay was carried out as described previously (22). Strains were grown overnight in 5 mL liquid YPD medium, and for each strain, \(A_{600}\) was measured. Appropriate amount of this culture was inoculated in 6 mL of fresh YPD medium (to have density of approximately \(A_{600} = 0.2\)) and the cultures were grown at 30°C with vigorous shaking for 2 h or until the \(A_{600} = 0.45\) to 0.50. Log-phase culture (25 mL) was inoculated in 25 mL of fresh YPD medium. Five milliliter from this were dispensed in each tube containing 0, 0.25, 0.50, and 0.75 mmol/L sodium arsenite. The cultures were incubated at 30°C with vigorous shaking for 24 h. Cell viability was done by plating appropriate dilutions on YPD agar plates, and after 2 days at 30°C, colony-forming units in each plate were counted. A percentage survival on different sodium arsenite concentrations was calculated relative to untreated cultures and a graph of percentage survival was plotted against the concentration of arsenite.

**High-Throughput Screening**

Before the actual screening could be done, minimum inhibitory concentration (MIC) of arsenic [sodium arsenite (AsIII) and sodium arsenate (AsV)] for the WT strain (BY4741) was determined as follows: BY4741 was inoculated in 96-well plates containing 200 \(\mu\)L YPD in each well. Plates were incubated for 2 days at 30°C. Using 96-pin replicator, cultures were spotted onto YPD plates containing varying concentrations of sodium arsenite (1.25, 2.5, 5.0, and 10 mmol/L) or sodium arsenate (5, 10, 15, and 25 mmol/L) and plates were incubated for 3 to 6 days at 30°C. Growth of the WT BY4741 strain on the plates containing either compound was compared with its growth on the control plate (without any arsenite or arsenate). The standardization experiments were repeated thrice and MIC of sodium arsenite(III) and sodium arsenate(V) was determined as

Figure 1. Arsenite sensitivity of WT (RKY3109) and rho- yeast cells. Logarithmically growing cultures of both strains were inoculated into three different concentrations of sodium arsenite (medium without any sodium arsenite was used as negative control ("untreated")) and exposed for 24 h. Appropriate dilutions of cells were then plated on YPD plates and grown at 30°C, and after 2 d, colonies were counted. Percentage of survival was calculated relative to untreated control. Data represent the average from three independent experiments.

Figure 2. Classification of identified arsenite-and arsenate-sensitive yeast mitochondrial mutants. Both arsenite-sensitive (A) and arsenate-sensitive (B) mutants were classified based on their biological function using data available in SGD and CYGD databases. Venn diagram (C) shows number of mutants exhibiting uniquely arsenite or arsenate sensitivity as well as number of mutants that are sensitive to both.
Figure 3. Cellular interaction analysis of the genes identified in sodium arsenite screen. PathwayAssist software was used to analyze three different types of interactions for the genes identified in sodium arsenite screen: common transcriptional regulators (A), common gene targets (B), and direct protein-protein interactions (C) of genes identified in arsenite screen.
follows: sodium arsenite(III) MIC = 5 mmol/L and sodium arsenate(V) MIC >25 mmol/L.

Based on the MICs of arsenic obtained from the above experiments, the concentrations to identify sensitive phenotype were determined to be 2.5 mmol/L for sodium arsenite(III) and 15 mmol/L for sodium arsenate(V). To identify resistant phenotype of the mutant strains, concentration was determined to be 5 mmol/L for sodium arsenite(III) and 30 mmol/L for sodium arsenate(V).

For high-throughput analysis of sensitivity to arsenic, the collection of deletion mutants was prepared as follows: 466 strains (showing growth defects on nonfermentable carbon substrate) were isolated from the entire collection of haploid deletion strains by picking up small portion of growth from the solid agar in 96-well microtiter plates and inoculating in another 96-well microtiter plate containing 200 µL fresh sterile YPD liquid medium. Each plate in its first column contained the WT BY4741, so each plate had a total of 88 mutants. The plates (in triplicates) were then incubated for 48 h at 30°C. After incubation, one set was used for the screening and the other two sets were stored as frozen glycerol stock (with addition of glycerol in 15% final concentration). Screening was done by spotting the cultures (using 96-pin replicator) onto YPD plates containing either sodium arsenite (2.5 or 5 mmol/L) or sodium arsenate (15 or 30 mmol/L) as well as on YPD plates without any arsenic (control plates). Plates were incubated for 4 to 15 days at 30°C and phenotype of each mutant was scored as sensitive or resistant compared with control plate and internal control (BY4741 on each plate). Each experiment was done thrice independently.

**Data Analysis**

Categorization of genes based on the biological functions. The deletion mutants that showed arsenic-sensitive phenotype were categorized based on the biological functions of the genes. The information on the biological functions for each of these genes was obtained from Saccharomyces Genome Database (SGD)1 and Comprehensive Yeast Genome Database (CYGD).2

Interaction analysis of the genes using PathwayAssist software. The deletion mutants were analyzed using PathwayAssist software (version 3.0; Stratagene Corp.) at the Gene Expression Facility of Roswell Park Cancer Institute. Three different types of interactions that were included in the analysis are as follows: direct interactions between the proteins, common regulators for the genes of interest, and common targets of the genes of interest.

**Results**

mitDNA-depleted yeast cells (rho−) show higher sensitivity to sodium arsenite. It has been shown that mitochondria are direct target of arsenic-induced toxicity in mammalian cells (12, 13, 23, 24). As the first step in identifying mitochondrial processes involved in arsenic-induced toxicity, we used *S. cerevisiae* strains defective in mitochondrial oxidative phosphorylation. To show that mitochondrial oxidative phosphorylation is involved in arsenic-induced toxicity in yeast, we tested arsenite sensitivity of WT and its isogenic derivative rho− cells lacking the entire mtDNA as described in Materials and Methods.

Figure 1 shows the percentage survival for WT and rho− strain at different concentrations of sodium arsenite after 24-h exposure. At 0.25 mmol/L sodium arsenite, rho− cells showed significantly higher sensitivity than WT strain (25% survival versus 90% survival of the WT). At 0.50 mmol/L sodium arsenite, that difference was less exaggerated (10% survival of rho− cells versus 25% for WT), whereas at the highest concentration of 0.75 mmol/L sodium arsenite, growth of both strains was completely inhibited. These data clearly suggest an important role for mitochondrial function in response to arsenic.

Screening of single-gene deletion mutants of *S. cerevisiae* for arsenic sensitivity. To further identify mitochondria-mediated processes involved in response to arsenic, we screened for arsenic

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1 http://www.yeastgenome.org
2 http://mips.gsf.de/genre/proj/yeast/index.jsp
sensitivity of 466 deletion mutants of *S. cerevisiae* that have previously been identified to be defective in variety of mitochondrial functions (20). Before the actual screening, MIC of arsenic [sodium arsenite (AsIII) and sodium arsenate (AsV)] for the WT strain (BY4741) was determined as described in Materials and Methods. MIC for sodium arsenite and sodium arsenate was determined to be 5 and 25 mmol/L, respectively.

Screening of the strains was done as described in Materials and Methods. The growth of each strain was recorded in terms of +++ (luxuriant growth), ++ (moderate growth), + (scanty growth),...

**Figure 4.** Cellular interaction analysis of the genes identified in sodium arsenate screen. PathwayAssist software was used to analyze three different types of interactions for the genes identified in sodium arsenate screen: common transcriptional regulators (A), common gene targets (B), and direct protein-protein interactions (C) of genes identified in arsenate screen.
and – (no growth). WT strain was inoculated in the first column of each plate, which served as an internal control in the case of both arsenite and arsenate, and screening was repeated thrice. Scores from all three screens were consolidated and only strains that showed consistent sensitive phenotype in all three screens are included in the final list of sensitive mutants (Supplementary Tables S1 and S2). Under these conditions, screen identified 72 arsenite-sensitive and 81 arsenate-sensitive mutants (Supplementary Tables S1 and S2). All identified sensitive mutants were rescreened twice to confirm their sensitivity to arsenite and arsenate.

Identified sensitive mutants belong to different biological functions. The arsenite- and arsenate-sensitive mutants identified were classified based on their biological functions. This categorization was done using information from yeast databases SGD and CYGD. Identified genes were found to be involved in diverse cellular functions, such as signal transduction/protein modification, transcription, metabolism, transport, vacuolar processes, oxidative stress, cell cycle, and various other processes important in maintaining the proper mitochondrial function (Supplementary Tables S1 and S2).

Of all the mutants identified as sensitive in both arsenite and arsenate screens, close to 20% were defective in transcription, 11% mutants were defective in signal transduction/protein modification, and <5% were involved in both oxidative stress and cell cycle. About 13% of the mutants were defective in metabolic pathways, including ergosterol biosynthesis, ubiquinone metabolism, and protein synthesis. Majority of the mutants identified in the screens belonged to the functional group of vacuolar process, which made up ~30% of the sensitive mutants. The genes under this category encoded for subunits of vacuolar ATPases or were involved in vacuolar protein sorting. Almost 16% of mutants identified were defective in other processes involved in mitochondrial function (Fig. 2A and B).

Of all mutants that were screened, 61 mutants were found to be sensitive to both arsenite and arsenate (Supplementary Table S3). It is likely that large numbers of common mutants between arsenate and arsenite are due to the fact that arsenate is converted to arsenite during metabolism in yeast (25). Genes that were unique to arsenite or arsenate sensitivity are listed in Supplementary Tables S4 and S5, respectively.

Cellular interaction analysis of the identified genes. All the genes whose inactivation led to arsenic sensitivity were analyzed for various cellular interactions. Using PathwayAssist software, three different kinds of cellular interactions were analyzed. These include common regulators, common targets, and direct interactions between identified genes. This analysis provides an approach to compare different kinds of cellular interactions, including protein-protein interactions as well as genetic interactions. Genes...
that did not show any interactions were excluded from the analysis. It is noteworthy that absence of interaction does not mean that these genes do not play a role in arsenic toxicity.

Analysis of common transcriptional regulators showed that 25 arsenite-responsive and 32 arsenate-responsive genes were regulated by common transcriptional regulators (Figs. 3A and 4A). These transcriptional regulators include RTG3, ABF1, SMP1, REBI1, LEU3, HAP5, MCM1, and NDD1. The "common targets" analysis yielded targets of two or more genes found in our screen. These targets were shown to regulate cellular processes and transcription of other transcription factors (Figs. 3B and 4B). The "direct interactions" analysis yielded a network of direct and multiple interactions between the genes identified in our screen. Direct interactions between 40 of 72 arsenite-responsive genes and 56 of 81 arsenate-responsive genes were observed (Figs. 3C and 4C). The type of interactions that were observed included direct binding interactions, genetic interactions, expression, protein modification, and regulation.

Effect of sodium arsenite on mutants defective in mitochondria-to-nucleus retrograde response. Above interaction analysis showed RTG3 as one of the common transcriptional regulators involved in arsenic response. Interestingly, Rtg3p is involved in mitochondria-to-nucleus retrograde response. In addition, our arsenite sensitivity assay of cells depleted for mtDNA (rho− cells) showed their increased sensitivity (Fig. 1). It is noteworthy that the mitochondria-to-nucleus retrograde signaling pathway is activated in cells lacking mtDNA and this signaling is controlled by Rtg3p along with Rtg1p and Rtg2p (26). We therefore asked whether genes involved in mitochondria-to-nucleus response pathway also play a role in arsenic sensitivity. For this purpose, we measured percentage survival of rtg1, rtg2, and rtg3 null mutant strains as well as their isogenic WT strain (Fig. 5A).

Indeed, rtg3Δ mutant shows dose-dependent sensitivity. Interestingly, rtg2Δ mutant shows complete growth inhibition. In contrast to rtg2 and rtg3, rtg1Δ mutant shows resistance. Together, these data suggest that different components of the retrograde signaling pathway play different roles in the response of the cell to arsenic: whereas Rtg2 and Rtg3 seem to be involved in protection from arsenic exposure, Rtg1 seems to sensitize cells to arsenic.

Arsenic sensitivity of ABF1 null mutants. In addition to RTG3, our interaction analyses identified ABF1 gene as a key regulator of arsenic response. ABF1 is an essential regulatory factor that contributes to transcriptional activation of a large number of genes involved in multiple functions, including silencing and replication. We tested the sensitivity of two mutant alleles of ABF1, abf1-101 and abf1-102, to sodium arsenite. As described previously, cultures of both mutants as well as their corresponding isogenic WT strains were exposed to various concentrations of sodium arsenite for 24 h followed by plating on YPD medium for colony count. Percentage of survival was calculated relative to untreated control. Interestingly, whereas abf1-101 mutant shows greater sensitivity to sodium arsenite than its corresponding WT, abf1-102 mutant shows resistance to same concentrations of sodium arsenite (Fig. 5B).

Human orthologues of identified yeast genes. One of the major goals of using yeast as a model system in this study was to identify human orthologues to the yeast genes that may play a role in arsenic response. Our search for human orthologues identified 32 proteins in arsenite-sensitive category and 40 human orthologues in the arsenate-sensitive category (Supplementary Tables S6 and S7; Fig. 6). Five human orthologues were to genes that are uniquely sensitive to arsenite, 13 were to genes uniquely sensitive to arsenate, and 27 of them overlapped in both cases. Human orthologues were found to belong to category of transcription factors, signal transduction, oxidative stress, metabolism, vacuolar processes, and transport processes. However, to confirm biological significance of this analysis, selected genes need to be analyzed using small interfering RNA system in cell culture to silence their expression and subsequently analyze their sensitivity to arsenite or arsenate as well as any other phenotypic changes. This experimental approach will potentially give valuable insight in genes and pathways responsible for protecting human cells against arsenic toxicity.

Discussion
Arsenic is considered a "paradoxical" carcinogen as it can induce tumors in humans but not in any other animal model (2). The mechanism of human carcinogenicity remains unclear. Recent study described mitochondria as an important target for arsenic-induced carcinogenicity (27). Using yeast as a model system, we show that cells defective in oxidative phosphorylation (OXPHOS) and other mitochondrial functions show significantly greater sensitivity to arsenic. This suggests that mitochondrial function plays an important role in protecting cells against arsenic and that impaired OXPHOS leads to arsenic sensitivity.

We analyzed the effect of arsenite and arsenate on null mutants deleted in nuclear genes involved in a variety of mitochondrial functions. After determining the MIC for sodium arsenite and sodium arsenate (5 and >25 mmol/L, respectively), high-throughput screen of 466 such null mutants defective in mitochondrial function yielded a set of mutants sensitive to both arsenite and arsenate. Identified arsenite- and arsenate-responsive genes belong to various functional classes, including transcription, signal transduction/protein modification, oxidative stress, cell cycle, metabolism, and cellular transport. Among the arsenite-sensitive genes, 61 exhibited cross-sensitivity to arsenite, which indicates that the remaining unique genes in each case may play various roles in protecting yeast against arsenic-induced toxicity. Commonality of large number of genes can be accounted for from the fact that arsenite is reduced to arsenite in the metabolic pathway (28). It also suggests existence of common pathways involved in protecting cells against arsenite- and arsenate-induced toxicity.

Genes whose deletion leads to both arsenite and arsenate sensitivity. Various genes identified encoded for proteins belonging to a network of transcription factors, such as SWI/SNF complexes. SWI3 and SNF6 identified in our screen are global transcription activators. They form a subunit of the SWI/SNF chromatin remodeling complex that regulates transcription of many important genes (29–31). SWI6 is a transcription cofactor, which regulates transcription at G1-S transition by formation of complexes with DNA-binding proteins Swi4p and Mbp1p (32). SRB8 is a subunit of RNA polymerase II mediator complex and is essential for transcriptional regulation (33). Mutants defective in these transcription factors showed sensitivity to arsenic, which suggests that these genes regulate the transcription of various genes that protect cells from arsenic.

Signal transduction/protein modification also seems to be affected by arsenic. SNF1 and SNF4 are a part of protein kinase and are required for glucose repression in yeast (34). Deletion of these genes has been known to have pleiotropic effects on cell cycle...
and cellular energetic (35). These deletion strains showed sensitivity to arsenic, implying their role in arsenic toxicity.

Arsenic increases oxidative stress in the cell (36). Deletion of genes involved in protection of the cell against oxidative stress will increase vulnerability of cells to oxidative damage. We identified two such genes (i.e., SOD2 and GRX5). SOD2 is a mitochondrial superoxide dismutase that protects the cell from oxidative stress (37). GRX5 is a mitochondrial glutaredoxin and is essential for the activity of iron/sulfur centers (38). Cells deleted in GRX5 are constantly subjected to oxidative damage and this effect is severed in the presence of oxidant (38). Identification of SOD2 and GRX5 null mutants sensitive to arsenite and arsenate implies that oxidative stress plays an important role in arsenic-induced cell death.

Genes from various other pathways, such as cell cycle (PAei3 and SPS2) ergosterol biosynthesis (ERG2 and ERG3), heme synthesis (HEM14), amino acid synthesis (AAT2), ubiquinone biosynthesis (COQ2 and COQ6), and protein degradation (DOA4), also seem to contribute to arsenic sensitivity when deleted. The largest groups of mutant were found to belong to proteins involved in vacuolar processes.

The genes included in the functional group of vacuolar processes encoded for various subunits of the vacuolar H\(^+\)-ATPase and proteins involved in vacuolar protein sorting. Vacuolar H\(^+\)-ATPase hydrolyses cytosolic ATP and transports protons across the membrane. This movement leads to generation of electrochemical gradient that acidifies the interior of a vacuole (39). In yeast, vacuolar acidification is involved in secondary transport of variety of ions and metabolites (40). It is known that arsenic is removed from the cells via sequestration of As(GS)\(_3\) into vacuoles (28).

Mutants that are defective in vacuolar H\(^+\)-ATPases may not be able to carry out acidification of the vacuoles and might therefore not allow for sequestration of Ast(GS)\(_3\) into the vacuoles. This would essentially lead to accumulation of arsenic in the cell leading to cell death. Another process that was affected in response to arsenic was vacuum protein sorting. Proteins synthesized on the endoplasmic reticulum in the cytosol are transported to Golgi complex and then directed to various organelles. The distribution of proteins to their destination is carried out by set of proteins called sorting proteins (39). In our screen, mutants lacking these genes are defective in vacuolar sorting function, which may result in loss of transport of proteins to the mitochondria, leading to improper functioning of mitochondria. The fact that these mutants are sensitive to arsenic suggests that proper functioning of mitochondria is necessary for the cells to nullify the toxic effects of arsenic.

Genes whose deletion leads to sensitivity exclusively to arsenite or arsenate. Many of the mutants were found to be uniquely sensitive to either arsenite or arsenate. This exhibition of specific sensitivity to valence state of the arsenic implies that cells have distinct pathways of protection against effects of arsenite and arsenate.

**Arsenate-specific sensitivity.** MTM1 is a gene involved in activation of mitochondrial Sod2p (41). Deletion of this gene results in sensitivity specifically to arsenite. PHO80 encodes for a cyclin that forms a cyclin-cyclin-dependent kinase complex with Pho85p and phosphorylates Pho4p-Swi5p, which in turn activates transcription of genes expressed in G\(_1\) phase and G\(_1\)-M boundary (42). SSD1 gene functions in maintaining the cell wall integrity and deletion of this gene alters the composition and cell wall architecture of the yeast cell surface (43). Detection of these genes suggests that oxidative stress, cell wall integrity, and cell cycle phase are important components in determining the sensitivity of the cells to arsenic.

**Arsenite-specific sensitivity.** Unlike arsenite, arsenate-specific sensitivity was shown by mutants deleted in genes involved in transcription, signal transduction, lipid metabolism, protein synthesis, and vacuolar processes. Some of them are described as follows. RNR4 encodes for a subunit of RNR complex that catalyzes the reduction of ribonucleotides to deoxyribonucleotides (44). SNF2 encodes for catalytic subunit of SWI/SNF chromatin remodeling.
complex (45). *CRD1* is a cardiolipin synthase involved in production of cardiolipin, an important constituent of mitochondrial membrane, which is required for maintaining the mitochondrial membrane potential and function (46). *PHO85* is a cyclin-dependent protein kinase, which plays a role in the metabolism of phosphate and glycogen and influences progress of the cell cycle (47).

**Cellular interactions involved in arsenic response.** Using PathwayAssist software cellular interaction analysis software, we identified multiple genes whose deletion resulted in arsenic-sensitive phenotype. Among transcriptional regulators, we identified Rtg3p as a common regulator of arsenic response. Rtg3p, together with Rtg1p and Rtg2p, monitors the functional state of mitochondria and are described as the regulators of mitochondria-to-nucleus response. To date, no known human homologues of Rtg proteins have been identified; however, mitochondria-to-nucleus retrograde response pathway is conserved across the species. Because mitochondria are an important target of arsenic-induced toxicity (27), we determined whether all three proteins involved in mitochondria-to-nucleus retrograde response were involved in arsenic-induced cellular response. Our results suggest that *RTG2* and *RTG3* genes play a key role in protecting cells from toxic effects of arsenic. Surprisingly, our study revealed that *RTG1* has distinctly different function as null mutation in *RTG1* gene results in resistance to arsenite.

Beside RTG3, we identified other common regulators. This includes *ABF1*, a DNA-binding protein that is involved in transcriptional activation, gene silencing, and DNA replication and repair (48). ABF1 regulates the expression of at least 10 genes that were involved in different processes, including ergosterol metabolism and vacuolar protein sorting. Indeed, our experiment revealed (Fig. 5B) that *ABF1* gene is involved in arsenic response. Another gene, *REB1*, a DNA-binding protein that binds to genes transcribed by both RNA polymerase I and RNA polymerase II (49), regulates the expression of proteins involved in vacuolar acidification and protein sorting. The fact that the handful of genes whose deletion causes sensitivity to arsenic are commonly regulated suggests that these regulators may play an important role in the response of the cells to arsenic. It also suggests that mutation in the genes encoding the common regulator may lead to altered expression of the subsequent genes affecting the ability of the cells to deal with arsenic in its environment.

Many common targets of the arsenic-responsive genes suggest that deletion of these genes leads to altered expression of the target genes, which in turn renders the cells sensitive to arsenic. The common targets are regulated by more than one gene and could suggest that the target genes may have essential functions and may signify redundancy of the regulating genes. Direct interactions analysis between genes of our interest suggested that they form a part of a complex regulatory network and alteration of any one may affect expression of various other genes that directly or indirectly interact with it. This altered expression may account for the sensitivity of the cells to arsenic in our screen, implying that all these genes work in tandem with each other to protect the cells against arsenic.

**Human orthologues to arsenic-responsive yeast genes.** One of the goals of our study was to identify the possible pathways of arsenic toxicity in humans. Our search in the HomoloGene database revealed several human orthologues to the identified yeast genes whose deletion causes sensitivity to arsenite and arsenate. These include genes involved in transcription, signal transduction, oxidative stress, metabolism, and vacuolar processes.

Human orthologue of yeast gene *RRM2* is *RRM2*, which encodes for M2 subunit of enzyme ribonucleotide reductase (RR). The activity of this enzyme depends on the synthesis of M2 subunit, which is controlled in cell cycle–dependent fashion (50). RR plays a key role in DNA synthesis and is responsible for maintaining the deoxynucleotide triphosphate pool for DNA synthesis and repair. It may be hypothesized that decrease in activity of RR due to deletion or mutation of *RRM2* may affect the DNA synthesis and repair ability of the cells. In addition, it is known that p53 regulates RR by interaction with p53R2 and *RRM2* in humans (51). Sensitivity of yeast *rrn4* null mutant to arsenate suggests that inactivation of *RRM2* gene in human cells should result in arsenic-sensitive phenotype. People with *RRM2* polymorphic variant may have suboptimal RR activity and may be susceptible to arsenic-induced carcinogenesis and other diseases.

Among many other yeast gene that has a human orthologue is *SOD2*, a mitochondrial superoxide dismutase 2. Superoxide dismutase converts superoxide byproducts from the OXPHOS chain to hydrogen peroxide and diatomic oxygen. *SOD2* in humans has been implicated to play a role in cancer (52, 53). As previously described, arsenic has been known to increase oxidative stress in the cells and alteration in the antioxidant *SOD2* gene can lead to sensitivity to arsenic, which points toward the involvement of *SOD2* in the mechanism of arsenic-induced carcinogenesis in humans.

Identification of multiple homologues belonging to diverse functional groups indicates that several biological processes are involved in protecting the human cells against arsenic-induced toxicity. Our mitochondria-wide screen of genes in yeast indicates that human cells use multiple pathways to protect cells against arsenic-induced cell death.

In conclusion, we identified various processes involved in response to arsenic in yeast, some of which have been described in arsenic carcinogenesis in humans. Human orthologues to these identified genes in yeast may serve as an important tool to investigate the mechanisms of arsenic carcinogenesis in humans.

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Mitochondrial Response to Arsenic


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