The Saccharomyces cerevisiae ACR3 Gene Encodes a Putative Membrane Protein Involved in Arsenite Transport*

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The cluster of three genes, ACR1, ACR2, and ACR3, previously was shown to confer arsenical resistance in Saccharomyces cerevisiae. The overexpression of ACR3 induced high level arsenite resistance. The presence of ACR3 together with ACR2 on a multicopy plasmid was conducive to increased arsenate resistance. The function of ACR3 gene has now been investigated. Amino acid sequence analysis of Acr3p showed that this hypothetical protein has hydrophobic character with 10 putative transmembrane spans and is probably located in yeast plasma membrane. We constructed the acr3 null mutation. The resulting disruptants were 5-fold more sensitive to arsenate and arsenite than wild-type cells. The acr3 disruptants showed wild-type sensitivity to antimony, tellurite, cadmium, and phenylarsine oxide. The mechanism of arsenical resistance was assayed by transport experiments using radioactive arsenite. We did not observe any significant differences in the accumulation of $^{76}$AsO$_3^-$ in wild-type cells, acr1 and acr3 disruptants. However, the high dosage of ACR3 gene resulted in loss of arsenite uptake. These results suggest that arsenite resistance in yeast is mediated by an arsenite transporter (Acr3p).

Arsenicals are toxic compounds, which are commonly present in the environment at increasing concentrations as a result of industrial pollution (1). The pentavalent arsenate is a phosphate analog which interferes with phosphorylation reactions and competes with phosphate in transport (2–4). The more potent trivalent arsenite reacts with the sulphydryl groups of proteins and inhibits many biochemical pathways (3, 5). Both arsenic salts were observed to induce morphological transformation and some cytogenetic effects (6–8).

Arsenical resistance phenomenon was described in many organisms from bacteria to mammalian cells (9–13). Resistance to arsenate, arsenite, and antimonite in prokaryota is believed to be encoded by one gene, the so-called arsenic resistance operon (14, 17). The hypothetical product of the acr1 gene shows similarity to the hypothetical membrane protein encoded by Bacillus subtilis ORF1 named yqcl, a putative element of chromosomal copy of acr operon (26, 27).

Recently we have reported the isolation of three S. cerevisiae genes, ACR1, ACR2, ACR3, conferring the increased resistance to arsenic compounds in presence on a multicopy plasmid (26). The putative product of the ACR1 gene shows similarity to the yeast transcriptional regulatory proteins, encoded by YAP1 and YAP2 genes, and the acr1 null mutant exhibits arsenate and arsenites hypersensitivity phenotype (26). The ACR2 gene is required for high level arsenite resistance but not for arsenite resistance (26). The hypothetical product of the ACR3 gene shows high similarity to the hypothetical membrane protein encoded by Bacillus subtilis ORF1 named yqcl, a putative element of chromosomal copy of acr operon (26, 27).

Here we report the disruption of ACR3 gene and the phenotypic analysis of acr3 null mutants, including transport experiments with radioactive arsenite. Our results suggest that Acr3p is a putative membrane protein, involved in arsenite transport.

**EXPERIMENTAL PROCEDURES**

Materials—Restriction endonuclease enzymes, T4 DNA ligase, and calf intestinal alkaline phosphatase were from Promega or Life Technologies, Inc. All other chemicals were obtained from commercial sources. Radioactive sodium ortho-arsenite ($^{76}$AsO$_3^-$) was purchased from POLATOM, Otwock-Swierk, Poland.

Plasmids and Strains—Plasmids and S. cerevisiae strains used in this study are described in Table I. E. coli strain JM109 (recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi-1 [lac-proAB] F' [traD36 proAB+ lacZM15]) was used for molecular cloning.

Media—S. cerevisiae strains were grown at 28 °C in complete YPD, YPG, or YPE media supplemented with 2% glucose, 2% glycerol, or 2% ethanol, respectively. Alternatively, the minimal YNB medium with 2% glucose supplemented with auxotrophic requirements was used (30).

Sporulation medium contained 0.5% Difco yeast extract, 2% potassium thioglycollate, and 1% agar. The pH was adjusted to 6.5. For growth of S. cerevisiae, each plate contained 10 μl of mating solution (1:1) from mating pairs of strains. After growth, the mating plates were washed with 0.1 M K$_2$HPO$_4$, and the colonies were transferred to sporulation medium. After 10 days of incubation at 30 °C, the colonies were harvested and sporulated. Sporulation was confirmed by the presence of ascospores in the plates. The cells were then harvested by filtration and used for further analysis.

The abbreviations used are: ACR, arsenic compounds resistance; ORF, open reading frame; PSORT, prediction of protein localization sites; bp, base pair(s); kb, kilobase(s); MFS, major facilitator superfamily.
supplemented when necessary with 100 μg/ml ampicillin (31). Media were solidified by addition of 1.5% Difco Bacto-agar.

DNA Manipulations—Cloning procedures (plasmid purification, endonuclease digestion, gel electrophoresis, ligation, dephosphorylation, E. coli transformation, and DNA transfer to nylon membrane) were carried out as described previously (31). Transformation of yeast cells was performed by the lithium acetate method (32). Yeast genomic DNA was isolated by the method of Kaiser et al. (33). DNA probe was labeled with biotin-7-dATP using Bionick 228 Labeling System, Life Technologies, Inc. Hybridization and chemiluminescent detection of biotinylated DNA on nylon membrane were performed with Southern-Light System, TROPIX.

Transformation of yeast cells. Verification of ACR3 gene was carried out according to Sherman and Hicks (34).

ACR3 Gene Disruption—Disruption of the ACR3 gene was carried out by the “one-step” method according to Rothstein (33). A 1141-bp BamHI-KpnI fragment was excised from the ACR3 gene, located on the pA1031 plasmid, and replaced by the corresponding 106-bp deletion mutation of ACR3 gene (26). The resulting construct was dubbed as pRW2. The URA3 gene was removed from the pAF101 plasmid (29) as a 1211-bp BglII-KpnI fragment. The pA1031 plasmid was constructed by deleting a 106-bp PvuII-SmaI fragment to abolish KpnI restriction site from the polylinker of pA103 plasmid. The 5206-bp BglII-BglII fragment of pRW2 plasmid was used for the transformation of yeast cells. Verification of ACR3 disruption was obtained by Southern analysis.

Tetrad Analysis—Micromanipulation and dissection of asci were done according to Sherman and Hicks (34).

Arsenic Resistance in Yeast

Determination of Cell Viability—Cell viability was determined by the methylene blue exclusion method (35).

Arsenic Sensitivity Assays—Strains were grown overnight in liquid YPD medium, previously described (30), and diluted to the optical density of 0.45 at 600 nm. In the growth inhibition experiments (Fig. 4), 10 μl of these cell suspensions were added to 20 ml of fresh liquid YPD containing increasing concentrations of arsenite and arsenate. The cultures were incubated at 28 °C with vigorous shaking, and the absorbance at 600 nm was measured after 24, 48, and 72 h. To determine the maximum non-inhibitory concentration of arsenic salts, 5 μl of the cell suspensions, prepared as described above, were placed on solid YPD medium with various concentrations of arsenicals (0–4 mM sodium arsenite and 0–25 mM sodium arsenate). Plates were incubated for 4 days at 28 °C.

Uptake of 129AsO32—Strains were grown in SD medium supplemented with auxotrophic requirements (lacking uracil in the case of strains bearing plasmids) at 28 °C with vigorous shaking for 16 h to the concentration of ~2 × 107 cells/ml. Cells were induced with 0.1 mM arsenite at 28 °C for 1.5 h without shaking, washed twice in cold distilled water, and suspended in 1 ml of 10 mM phthalic acid buffer (pH 5.5) with 100 mM glucose. The uptake of arsenite was carried out on 1-ml samples containing 0.1 ml of cells, 0.1 ml of 1 mM glucose, 0.2 ml of 50 mM phthalic acid buffer (pH 5.5), and 0.6 ml of distilled water. After 20 min of preincubation at 28 °C, radioactive arsenite in the form of 129AsO32− was added to a final concentration of 5 μM. Aliquots (0.1 ml

Table I

| Strain/plasmid | Genotype/description | Source |
|---------------|----------------------|--------|
| FY23          | MATA ura3–52 trp1Δ63 leu2Δ1 GAL2 | F. Winston |
| FY73          | MATA ura3–52 his3Δ200 GAL2 | F. Winston |
| DN3           | MATA ura3–52 his3Δ200 acr3–Δ1::URA3 GAL2 | F. Winston |
| FD236         | Cross DN6 × FY23 | This study |
| FD236–6A      | MATA ura3–52 trp1Δ63 leu2Δ1 his3Δ200 acr3–Δ1::URA3 GAL2 | This study |
| FD236–6B      | MATA ura3–52 leu2Δ1 acr3–Δ1::URA3 GAL2 | This study |
| FD236–6C      | MATA ura3–52 trp1Δ63 his3Δ200 GAL2 | This study |
| FD236–6D      | MATA ura3–52 GAL2 | This study |
| FD236–7A      | MATA ura3–52 trp1Δ63 his3Δ200 acr3–Δ1::URA3 GAL2 | This study |
| FD236–7B      | MATA ura3–52 trp1Δ63 acr3–Δ1::URA3 GAL2 | This study |
| FD236–7C      | MATA ura3–52 leu2Δ1 GAL2 | This study |
| FD236–7D      | MATA ura3–52 leu2Δ1 his3Δ200 GAL2 | This study |
| PB110         | MATA ura3–52 trp1Δ63 leu2Δ1 his3Δ200 acr1::HIS3 GAL2 | (26) |
| pFL44L        | Multicopy, shuttle vector, AmpR, URA3 | (28) |
| pFL44S        | Multicopy, shuttle vector, AmpR, URA3 | (28) |
| pFL39         | Centromeric, shuttle vector, AmpR, TRP1 | (28) |
| pA10          | The 5.5-kb insert containing ACR1, ACR2, and ACR3 genes cloned in BamHI site of pFL44L | (28) |
| pA1           | The 4.2-kb insert containing ACR1, ACR2, and ACR3 genes cloned in BamHI site of pFL44L | (28) |
| pA103         | The 2.1-kb HindIII fragment of pA10 insert comprising ACR3 gene cloned into pFL44S | (28) |
| pA1031        | pA103 plasmid with deleted 106-bp PvuII-SmaI fragment from the polylinker | This study |
| pA123         | pA1 plasmid with deleted ACR1 gene | (26) |
| pRW2          | pA1031 plasmid with deletion mutation of ACR3 gene | This study |
| pRW3          | The 2057-bp EcoRI fragment, containing ACR3 gene, inserted in the EcoRI site of pFL39 | This study |
| pAF101        | AmpR, URA3 | (29) |

Fig. 1. The predicted topology of the Acr3 protein. The predicted Acr3 protein comprises 8 integral transmembrane spans (shaded boxes) and 2 possibly integral spans (white boxes) (37). Putative N-glycosylation sites (G) and phosphorylation sites by protein kinase C (C) and casein kinase II (K) are indicated.
Sequence Analysis—The protein sequence analysis was performed using the software PC/GENE (IntelliGenetics, Inc.).

**RESULTS**

**Isolation and Disruption of the ACR3 Gene**—The ACR3 gene was cloned as a multicopy plasmid-borne DNA fragment capable of conferring arsenical resistance in *S. cerevisiae* cells (26). This fragment occurred to be a part of XVI chromosome containing three unknown ORFs named ACR1, ACR2, and ACR3 (26). We had also shown that the high dosage of ACR3 gene led to arsenical hyper-resistance phenotype (26). The hypothetical product of ACR3 gene is a 45.8-kDa protein with the expected isoelectric point of 8.9. According to the method of Kyte and Doolittle (36), the Acr3p is predicted to have 10 potential membrane-spanning segments with 64.1% membrane-buried residues of a total of 404 amino acids. This prediction was confirmed by the method of Klein et al. (37) which detected 8 integral transmembrane spans and 2 possibly integral spans (Fig. 1). A computer program PSORT (Prediction of Protein Localization Sites, version 6.3b) (38) classified the Acr3p as a plasma membrane protein with the certainty of 0.6. The Acr3p also comprises 3 potential N-glycosylation sites, 4 putative protein kinase C phosphorylation sites, and 4 potential phosphorylation sites for casein kinase II (Fig. 1). The codon bias index is 0.116 for the ACR3 gene, indicating a moderate level of expression (39).

To explore the effect of the loss of ACR3, the gene was disrupted in *S. cerevisiae* strain FY73. For this purpose, the *BglII* fragment of plasmid pRW2, containing the ACR3-disrupted allele, was used to transform yeast cells. One of the integrative *URA3* transformants DN6 showing arsenical hypersensitivity phenotype was crossed with the isogenic strain *FY23*. The resulting diploid strain FD236 was sporulated, and 14 tetrads were isolated. Two tetrads, numbers 6 and 7, were randomly chosen to present phenotypes, although all 14 tetrads shared the following features. The progeny of every spore was viable (Fig. 3). All tetrads indicated 2:2 segregation ratio for *URA3* phenotype (two spores showed *URA3* and two *ura3* phenotypes). The *Ura3* cells were also hypersensitive to arsenite and arsenate.

![Figure 2](image2.png) **Fig. 2.** Presentation of the events leading to the disruption of the ACR3 gene (*A, B*) and Southern blot analysis of diploid FD236 and tetrad number 6 (*C*). *A*, a 1.1-kb *BamHI*-KpnI fragment was removed from the ACR3 gene and replaced by the *URA3* marker gene. *B*, a 4.6-kb fragment of XVI chromosome after the integration of *acr3* null allele. Abbreviations for restriction enzymes: *B*, *BamHI; K, KpnI; E*, *EcoRI; G*, *BglII*. *C*, the total DNA was digested with *EcoRI*. Southern analysis was performed according to “Experimental Procedures.” A 2.1-kb *EcoRI* fragment of pA103 containing the ACR3 gene was used as a probe. Lanes: *a*, FD236; *b*, FD236-6D; *c*, FD236-6C; *d*, FD236-6B; *e*, FD236-6A. Wild-type strains (*lanes b and c*) exhibit the 4.6-kb *EcoRI* fragment, whereas the *acr3-*Δ1::*URA3* strains (*lanes d and e*) show two bands of 3.2- and 1.4-kb *EcoRI* fragments, because of the additional *EcoRI* restriction site in the *URA3* gene that replaced the ACR3 gene. A band pattern of diploid FD236 (*lane a*) demonstrates the presence of a wild-type copy of ACR3 and the null allele of this gene.

![Figure 3](image3.png) **Fig. 3.** Arsenite, arsenate, and uracil phenotype of the meiotic segregants from the tetrads obtained by sporulation of the FD236 diploid. Shown are colonies of tetrads number 6 and 7, each containing four spores: *A, B, C, D*, and progeny of spores 6A and 7A bearing the plasmid pRW3. Plates were prepared as described under “Experimental Procedures.”

**Table II**

| Compound tested          | MNC<sup>a</sup>  | Δacr<sup>b</sup>  | Wild type<sup>c</sup> |
|--------------------------|------------------|-------------------|-----------------------|
| Sodium arsenite          | 0.5 mM           | 2.5 mM            | 2.5 mM                |
| Sodium arsenate          | 2 mM             | 10 mM             | 10 mM                 |
| Potassium antimonyl tartrate | 60 mM       | 60 mM             | 60 mM                 |
| Phenylarsine oxide       | 0.07 mM          | 0.07 mM           | 0.07 mM               |
| Potassium tellurite      | 5 mM             | 5 mM              | 5 mM                  |
| Cadmium chloride         | 0.3 mM           | 0.3 mM            | 0.3 mM                |

<sup>a</sup> Maximum non-inhibitory concentration (MNC) is the highest drug concentration tested at which normal growth occurred.

<sup>b</sup> FD236–6A strain.

<sup>c</sup> FD236–6D strain.
demonstrated that Ura\textsuperscript{−} segregants which grew at a wild-type rate in the presence of arsenic salts had a wild-type restriction profile, whereas Ura\textsuperscript{−} cells showing hypersensitivity to arsenicals possessed a profile of the disrupted gene (Fig. 2C). These results demonstrate the monogenic character of the disruption and that the arsenical sensitivity segregates with the null mutation of ACR3 gene. To ensure that the increased arsenical sensitivity is caused only by the lack of ACR3 gene, we transformed a few of the above disruptants with the centromeric plasmid pRW3 bearing the ACR3 gene. After transformation we observed that the wild-type copy of ACR3 present on the plasmid in cytoplasm restored arsenical resistance to wild-type level (Fig. 3).

**Phenotype Analysis of acr3 Null Mutants and Arsenite Transport in Intact Cells**—To study more thoroughly the phenotypic differences between the disruptants and the wild-type strains, we determined the highest non-inhibitory concentrations of arsenicals (Table II) and the rate of growth in the presence of various amounts of arsenic salts (Fig. 4, A–D). On the medium containing glucose the acr3 null mutants were 5-fold more sensitive to both arsenate and arsenite than wild-type cells (Table II). When the only source of energy was glycerol or ethanol, both the disruptants and wild-type cells were about 10 times more sensitive to arsenicals (data not shown). We have also tested other metals; however, the acr3 mutants showed wild-type sensitivity to potassium antimonyl tartrate, phenylarsine oxide, potassium tellurite, and cadmium chloride (Table II). Besides we noticed that yeast cells tolerated very high concentrations of antimony salt (up to 60 mM) on glucose, (Table II). Besides we noticed that yeast cells tolerated very high concentrations of antimony salt (up to 60 mM) on glucose, whereas yeast sensitivity to antimony increased about 60 and 6 times on the medium with glycerol or ethanol, respectively (data not shown).

In the growth inhibition experiment (Fig. 4, A–D) we noticed that after 24 h of incubation the acr3 disruptants were unable to grow even in the presence of the lowest concentration of arsenate and arsenite (0.25 mM) tested. The growth of cells expressing the wild-type ACR3 gene was not inhibited by this concentration of arsenicals (Fig. 4, A and B). After 48 h wild-type cells could tolerate 1 mM arsenite and arsenate, while the acr3 disruptants did not grow in the presence of 0.5 mM arsenite and 1 mM arsenate (Fig. 4, C and D). However, after 3 days of incubation the null mutants were able to recover from the inhibition at 0.5 mM arsenite and 1 mM arsenate (data not shown).

We also investigated the viability of cells incubated at arsenical concentrations indicated in Fig. 4. After 24 h the survival rate was similar for all strains and conditions tested. Within next 24 h we did not observe any differences in cell viability either in the case of wild-type cells treated with arsenite and arsenate or in disruptants treated with arsenate. Nevertheless, 48 h of incubation with 0.75 mM and 1 mM arsenite led to the dramatic death of 50% acr3 mutant cells (data not shown).

The ACR3 gene product is a putative membrane protein with significant identity and similarity to the hypothetical protein encoded by the element of putative chromosomal operon from *B. subtilis* (26). The Acr3p protein also shares 36.6% identity with *Mycobacterium tuberculosis* potential membrane protein of unknown function (accession number Z80225) and 26.2% identity with hypothetical protein from cyanobacterium *Synechocystis* (accession number S76146). Cells with the disrupted copy of ACR3 gene are hypersensitive to arsenate and arsenite. So, we assume that Acr3p may be an oxyanion transporter conferring resistance to arsenic salts. To verify this hypothesis, transport experiments using radioactive arsenite first developed for *E. coli* (5) and then for *Leishmania* (24) were adapted to *S. cerevisiae*.

The uptake of \(^{76}\text{AsO}_3^{2−}\) was observed in *acr1* disruptants, acr3 null mutants, and wild-type cells bearing no plasmid, control vector (pFL44L), and multicopy plasmids (pA10, pA123 and pA1031) comprising ACR genes (Table I, Fig. 5). Under our experimental conditions there was no significant difference in the accumulation of \(^{76}\text{AsO}_3^{2−}\) in wild-type and acr mutant cells. On the other hand the presence of multicyclics of ACR genes, which were previously shown to confer high level arsenic salts resistance (26), resulted in lack of arsenite accumulation, whereas cells containing the empty vector exhibited arsenite uptake at the wild-type level. Besides we did not observe any differences in arsenite accumulation of cells harboring pA10 (ACR1 –2, -3), pA123 (ACR2, -3), and pA103 (ACR3) clones. These results indicate that the product of the ACR3 gene itself is sufficient to decrease uptake of arsenite. On the basis of these findings, the ACR3 gene of *S. cerevisiae* seems to encode an arsenite transporter.
DISCUSSION

We have previously shown the isolation and initial characterization of the cluster of three genes, ACR1, ACR2, and ACR3, involved in yeast arsenical resistance (26). The ACR3 gene seems to encode a putative membrane protein which confers high level arsenite resistance when present in multicopies. Resistance phenotype is expanded into arsenate when ACR3 is accompanied by ACR2 on the same high-copy plasmid. We have suggested that the role of Acr3p in arsenical resistance is crucial and related to cell membrane (26).

There are many yeast proteins (Pdr5p, Sng2p, Sge1p etc.) which were proposed to be integral membrane proteins and conducive to resistance phenotypes when present in multicopies (40–43). These proteins belong to either the ABC (ATP-binding cassette) family or the MFS (major facilitator superfamily) family (43–45). The putative Acr3p, lacking ATP-binding cassette, may belong to the MFS superfamily. However, the Acr3p is not present in the group of 100 S. cerevisiae transport proteins classified as the MFS proteins (43–45). MFS proteins comprise 500–600 amino acids with 12 transmembrane helices and share sequence similarities suggesting the common ancestral origin (43–45). The Acr3p possesses only 10 transmembrane spans and shows amino acid similarities only to the hypothetical membrane protein encoded by P. aeruginosa (46). Disruption of many drug resistance yeast genes (PDR5, SGE1, for example) increased the sensitivity of S. cerevisiae cells when tested for drug resistance phenotype (42, 47). Inactivation of the chromosomal ars operon by Mud1 insertion in the araB gene was shown to decrease resistance of E. coli cells to arsenate and arsenite (48). Phenotype of the acr3 disruption is identical with the acr1 null mutation (26). Both mutant strains are 4–5 times more sensitive to arsenite and arsenate than the wild-type isogenic strain. The Acr1p is similar to the yeast transcriptional regulatory proteins: yAP1, yAP2, pap1 involved in the multidrug and oxidative stress resistance phenotypes (26). The yAP1 protein was shown to regulate the transcription of YCF1 gene, encoding an ATP-binding cassette transporter (49). The disruption of either the YCF1 or YAP1 gene is conducive to the same cadmium hypersensitivity phenotype (49). In the case of ACR genes the disruption of positive regulatory gene (ACR1) or the regulated target gene (ACR3) by analogy would lead to the same arsenical sensitivity phenotype, as a result of the lack of expression of a gene encoding an oxyanion transporter.

Reduced accumulation of arsenite from bacterial cells bearing multicopies of the arsA and/or the arsB elements was shown to reflect active extrusion of arsenite (3–5). Results obtained in uptake experiments with multicopies of the ACR3 gene are consistent with the facts mentioned above. Cells containing multicopies of ACR3 gene never accumulated radioactively labeled arsenite. However, we did not find any significant difference in arsenite accumulation between wild-type cells and acr3 disruptants. We assume that under our experimental conditions it is not possible to notice any disparities in arsenite uptake when we investigate strains either with one copy of the ACR1 and ACR3 genes or with disrupted alleles. On the basis of these findings we conclude that the ACR3 gene encodes the protein involved in arsenate transport in yeast cells.

The arsenical resistance in bacteria is mediated by an ATP-driven pump which consists of two subunits: ArsA and ArsB (14). When the ArsA protein catalytic subunit is absent, the transport of oxyanions is carried out through the ArsB protein coupled to the proton motive force (23). Prokaryotic cells extrude arsenicals only in the form of unconjugated arsenite (23)

### Table III

| Description | ArsB | Yqcl | Acr3p |
|-------------|------|------|-------|
| Description | Arsenite transporter-S. aureus | Putative ArsB-S. subtilis chromosome | Arsenite transporter-S. cerevisiae |
| Non-polar aa (%) | 59.8 | 62.0 | 52.6 |
| Polar aa (%) | 28.2 | 24.3 | 31.4 |
| Basic aa (%) | 6.1 | 8.0 | 9.3 |
| Acidic aa (%) | 4.6 | 4.8 | 5.8 |
| Total residues | 429 | 346 | 404 |
| Expected isoelectric point | 9.6 | 9.9 | 8.9 |
| Calculated molecular mass (kDa) | 46.5 | 38.3 | 46.5 |
| Transmembrane spans (37) | 12 | 10 | 10 |

aa, amino acids.
Arsenic resistance is reduced to arsenite by the ArsC protein prior to excretion (50). The active extrusion system of oxyanions was reported in L. tarentolae (24). Dey et al. (25) have shown the existence of an ATP-dependent As(III)-glutathione pump in membrane vesicles of L. tarentolae. They proposed a model of arsenical resistance in the Trypanosomatidae consisting of three steps: reduction of As(V) to As(III) by a hypothetical reductase, conjugation of As(III) to the thiol of reduced glutathione, and active extrusion of the complex by an ATP-driven pump (25). An energy-dependent efflux pump mediates arsenicals resistance also in mammalian cells, but unconjugated arsenite was the only product of transport (13). On the other hand, the human multidrug resistance protein, which is an ATP-driven pump mediating an extrusion of glutathione conjugates, was shown to confer arsenite resistance as well (51).

On the basis of our results and the observations from other organisms we propose the following model of arsenical resistance in the yeast S. cerevisiae which requires at least three genes: ACR1, ACR2, and ACR3. The ACR3 gene, positively regulated by the Acr1p, encodes an oxyanion transporter similar to the bacterial ArsB protein. The Acr3p probably extrudes unconjugated oxyanions of arsenite out of cells, thus reducing the intracellular concentration of toxic anions and providing resistance phenotype. This transport is probably coupled to the protonmotive force as it was suggested for the ArsB-dependent resistance phenotype. This transport experiments.

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