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Multiple cysteine residues are necessary for sorting and transport activity of the arsenite permease Acr3p from *Saccharomyces cerevisiae*

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ABSTRACT

The yeast transporter Acr3p is a low affinity As(III)/H^+ and Sb(III)/H^+ antiporter located in the plasma membrane. It has been shown for bacterial Acr3 proteins that just a single cysteine residue, which is located in the middle of the fourth transmembrane region and conserved in all members of the Acr3 family, is essential for As(III) transport activity. Here, we report a systematic mutational analysis of all nine cysteine residues present in the *Saccharomyces cerevisiae* Acr3p. We found that mutagenesis of highly conserved Cys151 resulted in a complete loss of metalloid transport function. In addition, lack of Cys90 and Cys169, which are conserved in eukaryotic members of Acr3 family, impaired Acr3p trafficking to the plasma membrane and greatly reduced As(III) efflux, respectively. Mutagenesis of five other cysteines in Acr3p resulted in moderate reduction of As(III) transport capacities and sorting perturbations. Our data suggest that interaction of As(III) with multiple thiol groups in the yeast Acr3p may facilitate As(III) translocation across the plasma membrane.

1. Introduction

Arsenic is a highly toxic metalloid and human carcinogen which is commonly found in the environment from both geological and anthropogenic sources [1–3]. Thus, it is crucial to develop biological strategies to avoid accumulation of arsenic in crops and prevent arsenic poisoning in humans as well as to decontaminate polluted areas. On the other hand, arsenic displays an antitumor activity and is one of the most effective drugs in the treatment of acute promyelocytic leukemia with a clinical potential to cure other types of cancer and autoimmune diseases [4–6]. Compounds containing the related metalloid antimony remain the first line drugs against leishmaniasis [7]. Understanding of molecular mechanisms of arsenic and antimony transport in various organisms is necessary to cope with emerging resistance to metalloid-based drugs and to generate new plant species with low or increased ability to accumulate arsenicals and antimonials.

The Acr3 family of arsenite (As(III)) transporters, belonging to the bile/arsenite/riboflavin transporter (BART) superfamily [8], is the major detoxification system for arsenic in both prokaryotes and eukaryotes [9,10]. The Acr3 transporters are widespread in bacteria, archaea, fungi and lower plants [11–14]. Although the Acr3 orthologs have not been found in the genomes of flowering plants, heterologous expression of the yeast *ACR3* gene in rice and *Arabidopsis thaliana* significantly improved tolerance to As(III) by reducing its accumulation in transgenic plants [15,16]. Our genome database searches have identified new eukaryotic members of the *ACR3* gene family in chlorophyta (*Coccomyxa subellipsoidea*), slime mold (*Polysphondylium pallidum*), heterokonts (*Phytophthora infestans*) and choanoflagellates (*Monosiga brevicollis*) (Fig. 1A).

The Acr3 permeases have ten-transmembrane span topology [11,17] and usually localize to the plasma membrane to mediate As(III) efflux and confer high-level resistance to this toxic metalloid in *Bacillus subtilis* [18], *Alkaliphilus metalliredigens*, *Corynebacterium glutamicum* [11], and *Saccharomyces cerevisiae* [19–21]. In the arsenic hyperaccumulating fern *Pteris vittata* Acr3 is targeted to the vacuolar membrane to sequestrate As(III) into the intracellular compartment instead of extrusion out of the cell [14]. The *Shewanella oneidensis* Acr3 is not able to transport As(III) and confers resistance only to arsenate (As(V)) [22], while the Acr3 ortholog from *Synechocystis* sp. mediates tolerance to As(III), As(V) and antimonite (Sb(III)) [23]. Recently, we have shown that the *S. cerevisiae* Acr3p is also able to extrude Sb(III) but confers only a moderate level of Sb(III) resistance [21]. Consistently, we have provided in vitro evidence that Acr3p catalyzes low affinity As(III)/H^+ and Sb(III)/H^+ antiport coupled to the proton-motive force but the rate of Sb(III) transport is much slower compared to As(III) [24]. However, the involvement of particular amino acid residues in As(III) and Sb(III) translocation via the *S. cerevisiae* permease Acr3p has not been investigated yet.

It has been determined that the mutagenesis of single cysteine residue in the *C. glutamicum* (Cys129) and *A. metalliredigens* Acr3 (Cys138),

Abbreviations: As(III), arsenite; As(V), arsenate; Sb(III), antimonite; ER, endoplasmic reticulum; TM, transmembrane segment

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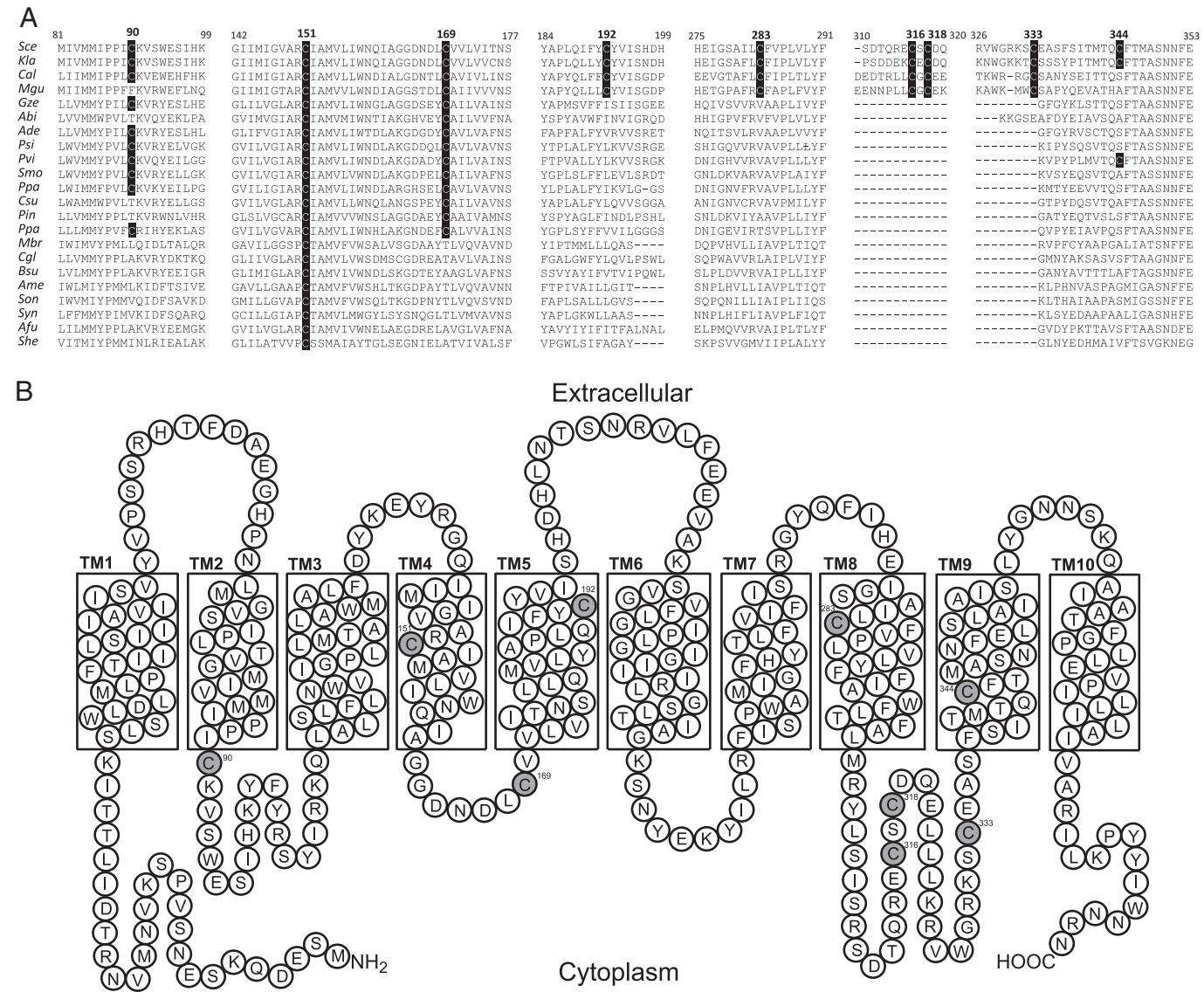

Fig. 1. Conservation of cysteine residues in the Acr3 family and hypothetical topology model of the Acr3 plasma membrane protein from *S. cerevisiae*. (A) Alignment of protein sequences of Acr3 orthologs was performed with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo) [30]. The alignment includes representative eukaryotic members of the Acr3 family from fungi (Ascomycota and Basidiomycota), vascular plants (spikemoss, fern and spruce), Bryophyta (moss), Chlorophyta (green algae), Stramenopiles (heterokonts), Amebozoa (slime mold) and Choanoflagellida (choanoflagellates) as well as a few examples of prokaryotic Acr3 proteins from gram positive and gram negative bacteria and archaea (Euryarchaeota and Crenarchaeota). The following protein sequences of Acr3 family members obtained from NCBI (accession numbers in parentheses) were used in the alignment: *Saccharomyces cerevisiae* S288c (Sce, DAA11615), *Kluyveromyces lactis* NRRL Y-1140 (Kla, XP_455090), *Candida albicans* SC5314 (Cal, XP_715501), *Meyerozyma guilliermondii* ATCC 6260 (Mgu, XP_001481996), *Gibberella zeae* PH-1 (Gze, XP_386755), *Agaricus bisporus* var. *burnettii* JB137-S8 (Abi, EKM76040), *Auricularia delicata* TFB-10046 SS5 (Ade, EJD54751), *Picea sitchensis* (Psi, ADE76924), *Pteris vittata* (Pvi, ADP20955), *Selaginella moellendorffii* (Smo, XP_002970839), *Physcomitrella patens* subsp. *patens* (Ppa, YP_001138464), *Coccomyxa subellipsoidea* C-169 (Csu, EIE24472), *Phytophthora infestans* T30-4 (Pin, EEY55020), *Polysphondylium pallidum* PN500 (Ppa, EFA79047), *Monosiga brevicollis* MX1 (Mbr, EDQ91878), *Corynebacterium glutamicum* ATCC 13032 (Cgl, YP_225795), *Bacillus subtilis* (Bsu, BAA12433), *Alkaliphilus metalliredigens* QYMF (Ame, YP_001319657), *Shewanella oneidensis* MR-1 (Son, AAN53615), *Synechocystis* sp. PCC 6803 (Syn, BAA18405), *Archaeoglobus fulgidus* DSM 4304 (Afu, AAB90761), and *Staphylothermus hellenicus* DSM 12710 (She, YP_003668173). For simplicity, only fragments of protein sequences containing conserved cysteine residues are shown. The conserved cysteine residues are highlighted. Sequence coordinates are from the *S. cerevisiae* Acr3p. (B) The transmembrane topology of Acr3p was predicted by the HMMTOP method (http://www.enzim.hu/hmmtop) [29], which best fits the Acr3 topology determined for bacterial species [11,17,25]. Cysteine residues subjected to mutagenesis are in gray circles.

located in the middle of the fourth transmembrane domain and conserved in all members of the Acr3 family, resulted in a complete loss of transport activity [11,25]. This suggests that interaction of As(III) with a single thiol group is required to activate As(III) transport capacity of bacterial Acr3 proteins. In addition, a conserved glutamate (Glu305) in the *C. glutamicum* Acr3 has been proposed to be involved in H^+ translocation [25]. Here, we analyzed the effect of mutagenesis of nine cysteine residues present in the yeast permease Acr3p and found that lack of Cys151, which corresponds to Cys129 in the *C. glutamicum* Acr3 and Cys138 in the *A. metalliredigens* Acr3, also completely abolished the ability of the *S. cerevisiae* Acr3p to mediate As(III) efflux. In addition, we showed the importance of seven other cysteine residues for proper Acr3p trafficking to the plasma membrane as well as for its transport activity.

2. Materials and methods

2.1. Mutagenesis of ACR3 gene

The pUG35 plasmids (CEN, *URA3*, Amp^R) containing the *ACR3*-GFP fusion gene under the control of the native promoter (pACR3-GFP) [21] or the *MET25* promoter (pMET-ACR3-GFP) were used as templates