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Transmembrane topology of the arsenite permease Acr3 from *Saccharomyces cerevisiae*

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ABSTRACT

Acr3 is a plasma membrane transporter, a member of the bile/arsenite/riboflavin transporter (BART) superfamily, which confers high-level resistance to arsenicals in the yeast *Saccharomyces cerevisiae*. We have previously shown that the yeast Acr3 acts as a low affinity As(III)/H^+ and Sb(III)/H^+ antiporter. We have also identified several amino acid residues that are localized in putative transmembrane helices (TM) and appeared to be critical for the Acr3 activity. In the present study, the topology of Acr3 was investigated by insertion of glycosylation and factor Xa protease cleavage sites at predicted hydrophilic regions. The analysis of the glycosylation pattern and factor Xa cleavage products of resulting Acr3 fusion constructs provide evidence supporting a topological model of Acr3 with 10 TM segments and cytoplasmically oriented N- and C-terminal domains. Next, we investigated the role of the hydrophilic loop connecting TM8 and TM9, the large size of which is unique to members of the yeast Acr3 family of metalloid transporters. We found that a 28 amino acid deletion in this region does not affect Acr3 folding, trafficking substrate binding, or transport activity. Finally, we constructed a homology-based structural model of Acr3 using the crystal structure of the *Yersinia frederiksenii* homologue of the human bile acid sodium symporter ASBT.

1. Introduction

Resistance to highly toxic metalloid arsenite (As(III)) mediated by the Acr3 permeases is the most common tolerance mechanism found in both prokaryotes and eukaryotes [1–3]. Homologues of Acr3 are particularly widespread in archaea, bacteria, unicellular eukaryotes, fungi, and lower plants, but are absent in flowering plants and animals [4–6]. The *Saccharomyces cerevisiae* Acr3 is a founder member of the Acr3 (arsenical resistance 3) family, which belongs to the bile/arsenite/riboflavin transporter (BART) superfamily [7]. The *ACR3* gene was isolated on a multicopy plasmid conferring resistance to high concentrations of As(III) in budding yeast [8]. Cells lacking Acr3 were hypersensitive to arsenicals and accumulated more As(III) compared to wild type cells, suggesting that Acr3 confers resistance to arsenicals by lowering intracellular concentration of toxic metalloids [9,10]. Subsequently, we showed that Acr3 is a plasma membrane protein that functions as a metalloid antiporter coupled to the electrochemical potential gradient of protons [11,12].

Although the physiological function of Acr3 proteins has been thoroughly studied, little is known about the structure of these transporters and the molecular mechanism of metalloid/proton exchange. Recently, we reported identification of several amino acid residues that are important for transport activity of the yeast Acr3 [2,6]. First, we investigated the role of cysteine residues in the antiport activity of Acr3 because As(III) easily binds to the thiol group of this residue. We found that a highly conserved Cys151, located in the predicted fourth transmembrane span (TM4), is indispensable for As(III) transport mediated by the yeast Acr3. In addition, we showed that Cys90 and Cys169, which possibly reside within the cytosolic loops of Acr3, are required for Acr3 sorting from the endoplasmic reticulum (ER) to the plasma membrane and its full transport activity, respectively [6]. Next, we analyzed the role of 26 conserved reactive hydrophobic and polar amino acid residues located in the putative transmembrane segments of Acr3 by site-directed mutagenesis [2]. Here, we found that replacement of highly conserved residues, such as Phe266 (TM7), Phe352 (TM9), Ser349 (TM9), Glu353 (TM9), Glu380 (TM10), with alanine significantly reduced or abolished the As(III)/H^+ exchange. What is more, detailed

Abbreviations: aa, amino acids; As(III), arsenite; As(V), arsenate; Endo H, endoglycosidase H; ER, endoplasmic reticulum; fXa, factor Xa protease; L, hydrophilic loop; NP-40, Nonidet P-40; Sb(III), antimonite; TM, transmembrane segment.

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mutational analysis of Glu353 showed that this residue is crucial for As(III)/H^+ translocation. On the other hand, similar analysis of Glu380 variants of Acr3 suggested that a negatively charged residue located in the middle of a putative TM10 is also necessary for As(III)/H^+ exchange [2].

Similar mutational studies of Acr3 from *Corynebacterium glutamicum* identified only two residues, Cys129 and Glu305 (corresponding to Cys151 and Glu353 in the *S. cerevisiae* Acr3), that are necessary for transport activity [4,13]. Surprisingly, mutagenesis of Glu332 (corresponding to Glu380 in the *S. cerevisiae* Acr3) only slightly affected transport activity of the *C. glutamicum* Acr3 [13]. This may suggest some functional differences between Acr3 transporters from various organisms. Indeed, members of the Acr3 family display slightly different substrate specificities. Acr3 proteins from *C. glutamicum* or *Alkaliphilus metalliredigens* mediate only transport of As(III) [4,13]. In contrast, the *S. cerevisiae* Acr3 exhibits similar low affinity for both As(III) and antimonite (Sb(III)) but transports As(III) three times faster than Sb(III) [11,12]. Interestingly, the *Schewanella oneidensis* Acr3 confers resistance solely to pentavalent arsenate (As(V)) and does not require the conserved cysteine in TM4 for its activity [14].

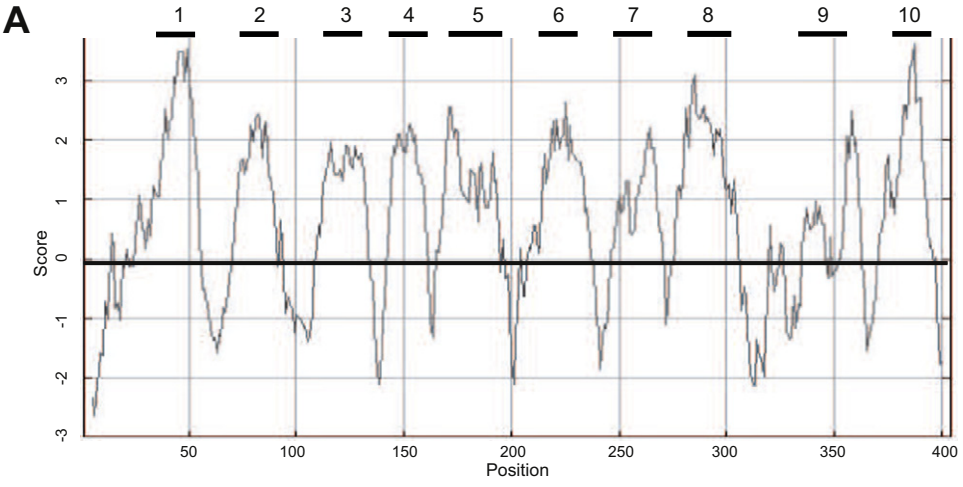
It has been experimentally shown that the Acr3 orthologues from *Bacillus subtilis* and *A. metalliredigens* exhibit ten-transmembrane topology [4,15]. In contrast, *in silico* analysis of potential membrane-spanning regions in the *S. cerevisiae* Acr3 revealed nine to ten hydrophobic regions, depending on the topological analysis program used (Fig. 1). In the nine-span topology, the potential transmembrane region of residues 332–366 (span no. 9 in the ten-span model), which contains several key residues important for transport activity of the yeast Acr3, is predicted to reside in the cytoplasmic loop. Consequently, the C-terminus is located outside the cell. In addition, the yeast members of the Acr3 family possess a unique structural feature in the form of large extra segment (from 7 to 63 amino acid residues) located in the predicted cytoplasmic loop (L8) connecting TM8 and TM9.

In the present study, we experimentally determined the membrane topology of the yeast Acr3 using two strategies, glycosylation mapping and proteolytic cleavage analysis. Our results clearly showed that the *S. cerevisiae* Acr3 transporter has ten transmembrane regions with both termini localized in the cytoplasm suggesting that members of the Acr3 family share a common topology. In addition, we constructed the *acr3* deletion mutant lacking 28 residues in the cytoplasmic loop L8 and found that this region is not important for folding, trafficking, or antiport activity of Acr3. Finally, having established the membrane topology of the yeast Acr3, we built a homology structural model of Acr3 using the structure of the bile acid sodium symporter ASBT from *Yersinia frederiksenii* as a template.

2. Materials and methods

2.1. Growth conditions

The yeast strain used in this study was RW104 (*MAT***a** *acr3Δ::kanMX ura3 leu2 trp1 his3 ade2 can1*) [16]. Standard yeast media, growth and transformation conditions were used [17]. Plasmids bearing wild type or mutated versions of *ACR3-GFP* were tested for their ability to



B

TM	TopPred	HMMTop	TMPred	SPLIT	TOPCOIN	SOSUI	TMHMM	OCTOPUS	DAS-TMfilter
1	35-55	29-53	35-55	24-56	33-53	30-52	35-57	34-55	32-55
2	70-90	70-89	70-90	71-94	70-90	71-93	72-94	70-90	74-87
3	114-134	110-134	110-133	108-134	113-133	111-133	114-133	111-131	112-131
4	141-161	143-162	142-159	141-163	142-162	143-165	143-162	137-158	146-156
5	167-187	171-195	170-193	170-195	168-188	172-194	169-191	171-190	170-192
6	212-232	214-238	211-229	213-236	215-235	215-237	211-233	206-226	215-233
7	249-269	249-268	245-268	247-270	248-268	246-268	245-267	247-268	254-268
8	282-302	277-301	277-295	275-307	281-301	280-302	277-299	281-301	278-303
9	332-352	337-361	333-363	352-366	341-361	-	-	-	-
10	371-391	370-389	370-391	373-394	371-391	373-395	371-393	365-386	376-394

Fig. 1. Hydropathy analysis of the yeast Acr3. (A) A hydropathy profile of Acr3 was generated according to the algorithm of Kyte and Doolittle [34] using a window of 9 residues. The most hydrophobic regions are boxed and numbered. (B) Comparison of predicted transmembrane segments (TM) in Acr3 using indicated algorithms [35–43]. The numbers represent the position of predicted hydrophobic segments in the protein sequence of Acr3.