Exploration of Yeast Alkali Metal Cation/H\(^+\) Antiporters: Sequence and Structure Comparison

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ABSTRACT. The Saccharomyces cerevisiae genome contains three genes encoding alkali metal cation/H\(^+\) antiporters (Nha1p, Nhx1p, Kha1p) that differ in cell localization, substrate specificity and physiological function. Systematic genome sequencing of other yeast species revealed highly conserved homologous ORFs in all of them. We compared the yeast sequences both at DNA and protein levels. The subfamily of yeast endosomal/prevacuolar Nhx1 antiporters is closely related to mammalian plasma membrane NHE proteins and to both plasma membrane and vacuolar plant antiporters. The high sequence conservation within this subfamily of yeast antiporters suggests that Nhx1p is of great importance in cell physiology. Yeast Kha1 proteins probably belong to the same subfamily as bacterial antiporters, whereas Nha1 proteins form a distinct subfamily.

Abbreviations

<table>
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<tr>
<th>aa</th>
<th>amino acid</th>
<th>tmd(s)</th>
<th>transmembrane domain(s)</th>
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<tbody>
<tr>
<td>ORF</td>
<td>open reading frame</td>
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Microorganisms in antiporter names

<table>
<thead>
<tr>
<th>Cal</th>
<th>Candida albicans</th>
<th>Sca</th>
<th>Saccharomyces castellii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cgl</td>
<td>Candida glabrata</td>
<td>Sce</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>Ctr</td>
<td>Candida tropicalis</td>
<td>Sku</td>
<td>Saccharomyces kudriavzevi</td>
</tr>
<tr>
<td>Dha</td>
<td>Debaryomyces hansenii var. hansenii</td>
<td>Smi</td>
<td>Saccharomyces mikatae</td>
</tr>
<tr>
<td>Kla</td>
<td>Kluyveromyces lactis</td>
<td>Spa</td>
<td>Saccharomyces paradoxus</td>
</tr>
<tr>
<td>Pan</td>
<td>Pichia (Hansenula) anomala</td>
<td>Spn</td>
<td>Schizosaccharomyces pombe</td>
</tr>
<tr>
<td>Pso</td>
<td>Pichia sorbitophila</td>
<td>Yli</td>
<td>Yarrowia lipolytica</td>
</tr>
<tr>
<td>Sba</td>
<td>Saccharomyces bayanus</td>
<td>Zro</td>
<td>Zygosaccharomyces rouxii</td>
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The maintenance of intracellular alkali-metal-cation homeostasis is a crucial task for the survival of a yeast cell. In yeast cells, potassium is the major cytoplasmic cation involved, among others, in the regulation of cell volume and intracellular pH, whereas sodium is toxic. Thus, yeast cells spend energy to accumulate high intracellular concentrations of K\(^+\), on the one hand, and efficiently eliminate surplus Na\(^+\) on the other. To maintain an optimum cytoplasmic concentration of potassium and a stable high intracellular K\(^+\)/Na\(^+\) ratio, yeast cells employ transport systems mediating cation efflux and influx with different substrate specificities and using diverse mechanisms, e.g., ATPases, symporters, antiporters and channels (Rodriguez-Navarro 2000; Sychrova 2004).

For effective Na\(^+\) transport, the cells of most organisms use Na\(^+\)/H\(^+\) antiporters as well as ATPases (Brett et al. 2005a). Although alkali metal cation/H\(^+\) antiporters represent conserved transport systems existing in almost all types of organisms (archaea, bacteria, fungi, parasites, insects, plants, and mammals), their structure, substrate specificity and probable cell function have diverged during their evolution. While most microorganisms and plants use the inward gradient of protons created by the plasma membrane H\(^+\)-ATPase as a driving force to pump alkali metal cations out, animal cells usually consume the Na\(^+\) gradient resulting from Na\(^+\)/K\(^+\)-ATPase activity in order to force the excess protons out and regulate intracellular pH. Cells of higher eukaryotes usually express several Na\(^+\)/H\(^+\) antiporters in parallel, e.g., the 9 isoforms in human cells (Orlowski and Grinstein 2004) or 3–5 isoforms in many plants (Blumwald 2000; Mansour et al. 2003). Yeast cells usually express only a few types of alkali metal cation/H\(^+\) antiporters and thus become a suitable model to study their molecular properties and diverse functions.

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Systematic sequencing of the *Saccharomyces cerevisiae* genome revealed three ORFs encoding putative Na⁺/H⁺ antiporters — YDR456w, YJL094c and YLR138w (Andre 1995; Nelissen et al. 1997; Paulsen et al. 1998). None of these genes is essential, and a triple mutant harboring deletions of all three genes is viable though extremely sensitive to external salts of alkali metal cations (Marešová and Sychrová 2005).

YDR456w encodes Nhx1p (TC2.A.36) which is an endosomal/prevacuolar Na⁺/H⁺ exchanger participating in salt tolerance by the compartmentation of toxic cations inside the cell (Nass et al. 1997; Nass and Rao 1998). The physiological role of Nhx1p is more complex, since it was found to participate in intracellular protein trafficking (Bowers et al. 2000; Ali et al. 2004), to contribute to cell resistance to hyperosmotic shock (Nass and Rao 1999), to participate in the regulation of intracellular pH (Brett et al. 2005b) and also to transport potassium cations (Fukuda et al. 2004). Antiporters with a high similarity to yeast Nhx1p were found in nematodes (Nehrke and Melvin 2002), mammals and plants. In mammalian cells, the NHE family has at least 9 members localized either in the plasma or intracellular membranes (Orlowski and Grinstein 2004). Nhxl homologues were found in the plasma membrane of *Arabidopsis thaliana* (Shi et al. 2000), in the tonoplast membrane in many different plant species (Blumwald et al. 2000) and some of them (e.g., *OsNhxl* from *Oryza sativa*) were shown to complement *nhx1Δ* defects in *S. cerevisiae* (Kinclová-Zimmermannová et al. 2004).

The product of YJL094w, named Kha1p (TC2.A.37) is the least-characterized antiporter in *S. cerevisiae* cells. Though it was thought to mediate K⁺/H⁺ exchange at the plasma membrane level (Ramirez et al. 1998), it has been shown to be localized intracellularly, probably in the Golgi apparatus, and play an important role in the growth of the cell at alkaline pH levels (Marešová and Sychrová 2005). Similar sequences can be found in the genomes of many organisms (e.g., bacteria, *Neurospora crassa*, *Aspergillus nidulans*, *Xenopus laevis*, *Drosophila melanogaster*) but their corresponding products have not been characterized.

The NHA1 gene (YLR138w) encodes an antiporter (TC 2.A.37) that is localized in the plasma membrane and has a broad substrate specificity for at least 4 alkali metal cations (K⁺, Li⁺, Na⁺, Rb⁺) (Bañuelos et al. 1998; Kinclová et al. 2001b). Nha1p is also involved in the regulation of intracellular pH (Sychrová et al. 1999, Brett et al. 2005b), in the response of cells to osmotic shock (Kinclová et al. 2001b, Prof t and Struhl 2004), and its importance for the regulation of the cell cycle has been demonstrated (Simon et al. 2001).

So far, only genes encoding Nha1-type Na⁺/H⁺ antiporters have been identified and characterized in other yeast species, namely *Schizosaccharomyces pombe* (Jia et al. 1992), *Zygosaccharomyces rouxii* (Watanabe et al. 1995), *Candida albicans* (Soon et al. 2000), *Pichia sorbitophila* (Bañuelos et al. 2002) and *Debaryomyces hansenii* (Velkó v and Sychrová 2006). Here we present an inventory of all alkali metal cation/H⁺ antiporters resulting from a detailed search in yeast genome databases, together with a comparison of their deduced protein sequences and structures.

**MATERIALS AND METHODS**

The following databases and programs were used to search for the sequences encoding antiporters, to compare the DNA and protein sequences and to predict protein hydropathy profiles:

- **Candida database**: [http://genolist.pasteur.fr/CandidaDB/](http://genolist.pasteur.fr/CandidaDB/)
- **ClustalX**: [http://www.hgmp.mrc.ac.uk/Registered/Option/clustalx.html](http://www.hgmp.mrc.ac.uk/Registered/Option/clustalx.html) (Thompson et al. 1997)
- **EBI**: [http://www.ebi.ac.uk/](http://www.ebi.ac.uk/)
- **HMMPRED**: [http://www.enzim.hu/hmmpred/](http://www.enzim.hu/hmmpred/) (Tusnady and Simon 2001)
- **MEGA2.2**: [http://www.megasoftware.net/](http://www.megasoftware.net/) (Kumar et al. 2001)
- **MIPS**: [http://mips.gsf.de/genre/proj/yeast/index.jsp](http://mips.gsf.de/genre/proj/yeast/index.jsp) (Mewes et al. 1998)
- **TMHMM Server 2.0**: [http://www.cbs.dtu.dk/services/TMHMM/](http://www.cbs.dtu.dk/services/TMHMM/) (Sonhammer et al. 1998)
- **TMPred**: [http://www.ch.embnet.org/software/TMPRED_form.html](http://www.ch.embnet.org/software/TMPRED_form.html) (Hofmann and Stoffel 1993)