Identification and localization of the *sod2* gene product in fission yeast

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Abstract Sod2, the Na^+/H^+ antiporter of the fission yeast *Schizosaccharomyces pombe*, was identified by addition of a hemagglutinin tag to the carboxyl terminus of the protein. The tagged protein was expressed in the sod2-deficient strain of *S. pombe*. Transformants retained tolerance to lithium (1–10 mM) at external pH values from 3.5 to 6.5. Both Na^+-dependent proton uptake and active sodium extrusion were also restored in transformed cells, suggesting that a functional antiporter was present. The protein was present in a membrane fraction. In SDS PAGE it migrated as a single 47 kDa band. The protein could be efficiently solubilized with the non-ionic detergent, dodecyl maltoside. Immunofluorescent microscopy revealed an asymmetric distribution with preferable accumulation in polar tip areas. The results are the first identification and localization of the Na^+/H^+ exchanger in yeast cells.

Key words: Lithium tolerance; Localization; Na^+/H^+ antiporter; Sod2; Sodium transport; *Schizosaccharomyces pombe*

1. Introduction

Sod2 is the gene product of the fission yeast *Schizosaccharomyces pombe* which is responsible for sodium and lithium tolerance. The *sod2* gene was previously cloned and characterized using selection for increased LiCl resistance [1]. Analysis of the DNA sequence predicted that *sod2* encodes an integral membrane protein of 12 transmembrane segments. The sequence of the hypothetical protein shows only a weak overall similarity with the mammalian and bacterial Na^+/H^+ exchangers [1]. Disruption of the *sod2* gene results in an inability to extrude cytoplasmic Na^+ and to take up external protons in exchange for internal sodium ions [1]. These observations suggest that the hypothetical *sod2* gene product is a Na^+/H^+ antiporter that should reside in the plasma membrane of *S. pombe*.

Recently, genes more closely related to *sod2* have been isolated from the yeasts *Zygosaccharomyces rouxii* [2] and *Saccharomyces cerevisiae* [3]. In both cases, the alleles were crucial for lithium and sodium tolerance [2,3]. Thus, sod2 may represent a new family of eukaryotic Na^+/H^+ exchangers that use ΔpH (acidic outside) to export Na^+ ions out of the cytosol. In bacteria, Na^+/H^+ antiporters also use a pre-established proton motive force to expel toxic Na^+ or Li^+ from the cytosol [4–7]. In contrast, the mammalian Na^+/H^+ antiporter removes intracellular protons at the expense of ΔpNa (higher $[Na^+]$ outside the cell) [8,9]. Prokaryotic Na^+/H^+ antiport also effectively increases the pH buffer capacity of cytosol [10] and contributes to the buffering of the proton motive force [11].

Although genetic and physiological analysis has clearly indicated that *sod2* and *sod2*-like genes encode functional Na^+/H^+ antiporters, no corresponding proteins have been identified to this date [1–3]. For this reason studies on the structure and regulation of expression of these proteins are still in their infancy. In this report we present the identification and immunochemical localization of sod2 of *S. pombe*. The antiporter was identified by addition of a hemagglutinin (HA) epitope to the carboxyl-terminus. We present evidence for a plasma membrane presence and for a predominantly polar localization of sod2 in the cell.

2. Materials and methods

2.1. Strains and media

S. pombe was maintained on yeast extract adenine (YEA) or low sodium minimal KMA medium using standard methods [12,13]. KMA medium contained (per liter): potassium hydrophthalate, 3 g; K_2HPO_4, 3 g; yeast nitrogen base without amino acids, 7 g; glucose, 20 g; adenine, 100 mg, and histidine, 100 mg. Leucine 200 mg/l was added to maintain the *sod2::ura4* strain which is deficient in sod2 [1]. Standard LB medium was used for *E. coli* cultures.

2.2. Addition of the HA tag to the 3′-coding region of sod2

The pKS-sod2 plasmid contains the *sod2* gene (together with the 187 bp upstream and the 692 bp downstream flanking regions) as a 2.3 kb *Hin*dIII-*Hin*dIII fragment [1]. Prior to tag addition, the *Sal*I and *Eco*RI sites of the vector's polycloning site were eliminated by digestion with the respective enzymes and filling in with Klenow, followed by self-ligation. This yielded the plasmid pSK-sod2-SE. Mutagenesis used a series of PCR reactions (with pSK-sod2 as a template) to insert *Sal*I and *Eco*RI sites at the stop codon of the *sod2* gene. The oligonucleotide primers used to create these sites (underlined) were HAf, 5′-C GTc **gac** GAA **tt**C TCT TTT AAT GTC AAT TCG GAT TTC C-3′, HAr, 5′-TT AAA AGA **Gaa** TTC **gtc g**AC GTA ATC TTC CTG TGA CTT ATC-3′, the universal M13-20 Bluescript primer and H590, 5′-GTT GAA GGC TTG CCT GTT TGG CG-3′ (a primer for an upstream coding region of sod2). One PCR reaction was with HAr and H590 and a second PCR reaction was with HAf and M13-20. The resulting two PCR products were purified, combined and amplified via PCR to give a larger product containing both new restriction enzyme sites. After digestion with *Bsg*I and *Bsp*EI, the 430 bp fragment from the PCR product containing *Sal*I and *Eco*RI sites was inserted into pSK-sod2-SE instead of the original *Bsg*I-*Bsp*EI segment, resulting in pNic which had the inserted *Sal*I and *Eco*RI sites.

The pD37 plasmid [14] was used as a source of the triple HA DNA sequence. PCR was with the oligonucleotides 5′S (5′-A CGC **gtc gac** GGC CGC ATC TTT TAC CCA TAC G-3′) and 3′E (5′-CC **Gga att c**TA GCA CTG AGC AGC GTA ATC TGG-3′) and added *Sal*I and

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Abbreviations: ADH, alcohol dehydrogenase; $C_{12}E_8$, octaethylene glycol monododecyl ether; DM, dodecyl maltoside; ΔpH and ΔpNa, transmembrane H^+ and Na^+ concentration gradients; EDTA, ethylenediaminetetraacetic acid; HA, hemagglutinin; OG, octyl glucoside; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid

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*Eco*RI sites (underlined) to the 5′ and 3′ ends of the tag sequence, respectively. The PCR product was digested with *Sal*I and *Eco*RI, and the 119 bp *Sal*I-*Eco*RI fragment was inserted directionally into pNic. The resulting plasmid, pNicol, was digested with *Swa*I, and a 813 bp *Swa*I-*Swa*I fragment containing the HA tag, was ligated to the pKS-sod2 vector. This resulted in an intermediate construct containing *sod2* with the HA tag added to the 3′-terminus. Finally, the tagged *sod2* gene was cloned into the pWH5 shuttle vector [15] as a *Hin*dIII-*Hin*dIII insert yielding the plasmid pWH5-sod2T for expression in the *S. pombe sod2*::*ura4* strain. Exchange of *Swa*I-*Swa*I fragments was also used to add the HA tag to *sod2* cloned in pSOD2.11 [1] where it is under the control of the ADH promoter. The resulting plasmid, pARTA, is shown in Fig. 1. All constructs were sequenced to verify proper orientation and fidelity of PCR reactions.

2.3. Growth experiments

Lithium tolerance at different external pH was determined in liquid KMA media supplemented with the indicated amounts of LiCl. To stabilize the desired pH during growth, the following buffers were included at a final concentration of 0.1 M: citrate/MES (pH 3.0, 3.5, 4.5, and 5.0); succinate/MES (pH 4.0, 5.5, and 6.0); and MES/Trizma base (pH 6.5 and 7.0). Leucine (200 μg/ml) was added for growth of sod2::ura4. Cells (5×10^6) were inoculated into 2 ml of medium and incubated at 30°C with vigorous aeration for 24 h. Then, growth was assessed by measuring the increase in absorbance of the cell suspension at 600 nm. The final pH of the medium was determined and in all cases the resulting acidification of the medium did not exceed 0.15 pH units.

2.4. ^{22}Na transport assay

Cells were grown in KMA medium and harvested at a density of 1.2×10^7 cells/ml. They were washed twice with double distilled water, and resuspended in the experimental buffer at a concentration of 2.5×10^9 cells/ml. Experimental buffer contained 0.1 M KNO_3, 0.5 mM NaCl, 50 mM succinate/MES (pH 4.0). After addition of 0.5 μCi/ml carrier-free ^{22}Na, cells were incubated in a laboratory rotator at room temperature for 2 h. Sodium extrusion was initiated by addition of 40 mM glucose. Adenine (final concentration of 400 μg/ml) was added to cell 10 min prior glucose addition. At the indicated times, 0.1 ml aliquots of cell suspension were withdrawn and added to 5.9 ml of double distilled water. Samples were filtered immediately through the 0.8 μm pore size Millipore AA filters, and the radioactivity was measured with a β-counter. Cells treated with toluene were used to subtract nonspecific absorption of the isotope. All experimental points were duplicated in each experiment.

2.5. Measurement of sodium-dependent proton uptake

Na^+-dependent H^+ uptake in *S. pombe* cells was measured as described [16] with minor modifications. Logarithmic cells (app. 10^7 cells/ml) were washed twice with double distilled water and resuspended to a density of 5×10^8 cells/ml in 20 mM MES/Tris (pH 6.1) in the presence or absence of 100 mM NaCl. After 2 h of incubation at room temperature, cells were briefly pelleted in a microfuge, concentrated 5-fold in 0.5 ml of 1 mM MES/Tris (pH 6.1) and quickly added to 2 ml of the same buffer in a stirred cuvette. The pH of the suspension was measured using a Fisher Scientific Accumet 925 pH meter. Data were collected with an Apple Macintosh Plus computer.

2.6. Membrane isolation

Plasma membrane-enriched fractions from *S. pombe* were prepared essentially as described in [17,18] with minor modifications. Yeast cells were grown in KMA medium to an OD_{600} of 2 at 30°C. The following procedures were carried out at 4°C. Cells were pelleted ($3500\times g$, 5 min) and washed with double distilled water and resuspended at 200 mg/ml in disruption buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, protease inhibitor cocktail [19], and 1 mM dithiothreitol). They were then passed through a French press at 20 000 psi. Unbroken cells were pelleted by centrifugation at $3500\times g$ for 5 min, and the supernatant was centrifuged at $14\,000\times g$ for 20 min. (This second centrifugation was omitted in some experiments to obtain crude membrane preparations). Enriched membranes then were pelleted at $200\,000\times g$ for 1 h. and resuspended in a small volume of the same buffer. They were quickly frozen in liquid nitrogen and stored at −70°C.

2.7. Electrophoretic methods

Protein samples for SDS-PAGE were prepared as described earlier [18]. Proteins were resolved using a 12% polyacrylamide gel and visualized by Coomassie blue staining. The two-dimensional electrophoresis was performed using Mini-PROTEAN II 2-D Cell (Bio-Rad) as described by the manufacturer. Western blotting and immunoblotting were performed as described in [20]. Anti-HA monoclonal antibody, 12CA5 (Boehringer Mannheim, Laval, Que., Canada) was used at a dilution of 1:500, and peroxidase-conjugated goat anti-mouse antibody (Bio/Can, Mississauga, Ont., Canada) was used at a dilution 1:1000. Immunoreactive proteins were visualized using the Amersham Enhanced Chemiluminescence kit as recommended by the manufacturer.

2.8. Indirect immunofluorescence

Formaldehyde-fixed cultures of mid-logarithmic yeast cells were prepared for indirect immunofluorescence (IIF) as described in [21] with the following exceptions. Cell walls were digested for 30 min at 30°C with zymolyase 100T (ICN Biomedicals, Aurora, OH) in phosphate buffered saline containing 28.6 mM β-mercaptoethanol and 1.2 M sorbitol. Spheroplasts were labeled with anti-HA monoclonal antibody (BAbCO, Richmond, CA) at a dilution of 1:100 and visualized with rhodamine conjugated goat anti-mouse antibody (1:200). For some experiments spheroplasts were labeled with anti-H^+-ATPase antibody (1:100) and visualized with rhodamine conjugated anti-rabbit antibody (1:200). A laser confocal microscope (Bio-Rad) connected to a Silicon Graphics computer was used to obtain images of yeast cells.

3. Results and discussion

3.1. Expression of the recombinant sod2 protein

Earlier studies attempted to generate antibodies against the sod2 protein to use in characterization of this protein [1]. These attempts were not successful either because of the low amount of this protein present or because of low antigenicity of this protein [1]. Na^+/H^+ antiporters are apparently present in only small amounts in most cell types. The major Na^+/H^+ antiporter in *Escherichia coli*, NhaA constitutes less than 0.2% of total membrane protein when expressed from its own promoter [22]. In addition the mammalian Na^+/H^+ antiporter is present in small amounts in mammalian plasma membrane [8]. In this study, we added the highly immunoreactive triple HA epitope to the carboxyl terminus of sod2. Initially, the *sod2* gene with the attached HA tag was expressed from its

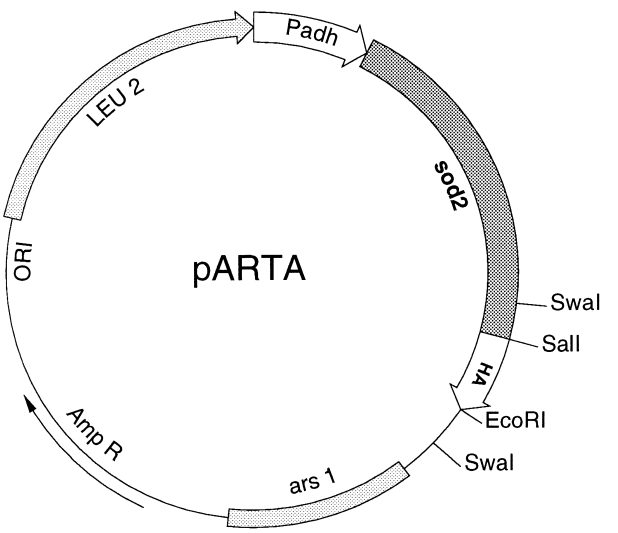

Fig. 1. Schematic diagram of pARTA vector used for expression of sod2 in *S. pombe*. Padh, ADH promoter; HA, triple hemagglutinin epitope.

natural promoter. The sod2-deficient *S. pombe* strain, sod2-::ura4, was transformed with plasmid pWH5-sod2T. Membranes as well as total cell lysates were prepared from the resulting transformant strain (SpWHAT), and the expression of protein was examined by SDS PAGE and Western blot analysis. In membrane preparations, a weak immunoreactive band of approximately 47 kDa was identified using anti-HA antibody. To improve and confirm this observation the strong constitutive ADH promoter was exploited to govern expression of the tagged sod2 protein in the next series of experiments. The plasmid used pARTA, is depicted in Fig. 1.

3.2. *Physiological effects of the HA-tagged sod2 on growth of S. pombe*

Similar to other Na^+/H^+ antiporters, sod2 can transport Li^+ instead of Na^+ [1]. This makes it possible to monitor the physiological function of sod2 in vivo by measuring lithium tolerance of *S. pombe* transformed with *sod2*. Since Li^+ is much more toxic than Na^+, one can use relatively low LiCl concentrations eliminating possible osmotic effects of salt addition. We measured the pH-dependent profiles of yeast growth in the presence of different concentrations of LiCl (Fig. 2A, B). Sod2::ura4, which is deficient in sod2, is highly sensitive to external lithium (Fig. 2A). At 2 mM LiCl added, only marginal growth can be seen after 24 h in medium of acidic (3.5–4.5) pH. LiCl concentrations of 5 mM completely arrested growth at pH 3.5–6.5. Transformation of sod2::ura4 with pARTA plasmid restored the cell's resistance to as much as 10 mM of LiCl at pH 3.5–6.0 (Fig. 2B). These results suggest that the tagged sod2 protein is active in cells transformed with the pARTA plasmid.

3.3. *Direct demonstration of the activity of HA-tagged sod2*

Na^+/H^+ antiport can be directly demonstrated in whole yeast cells by several techniques [1,23]. To confirm that the HA-tagged sod2 (sod2-HA) is active we used two independent strategies. First, we measured the ΔpNa-driven H^+ flux across the *S. pombe* membrane at an external pH of 6.1. An artificial ΔpNa (high $[Na^+]$ inside the cell) was created by loading cells with 0.1 M NaCl at pH 6.1 and by subsequent rapid transfer into the same buffer devoid of NaCl. At this external pH, the ΔpH on the plasma membrane is less than 0.5 pH units under in vivo conditions [24], so the cells can be loaded with considerable amounts of Na^+. After dilution into Na^+ free buffer, we found that cells possessing sod2-HA, exhibited Na^+-dependent proton uptake that developed within approximately one minute after applying the artificial ΔpNa (Fig. 2C, filled symbols). Cells without sod2 did not alkalinize the external medium.

In the second approach we monitored ΔpH-dependent so-

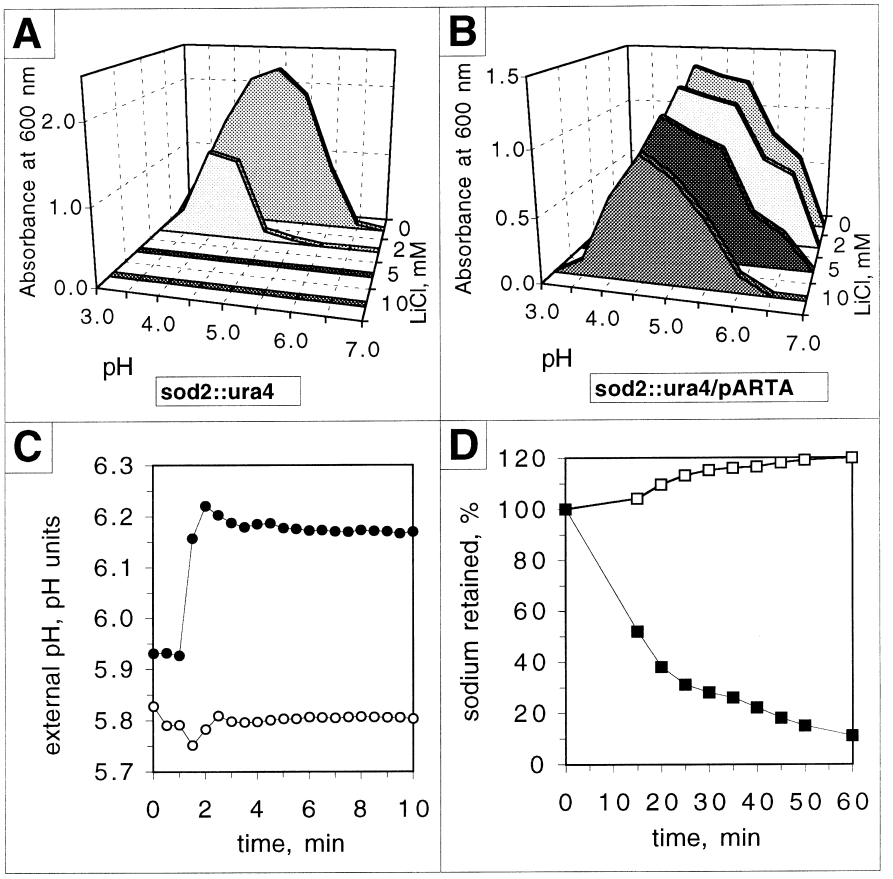

Fig. 2. Demonstration of the activity of HA-tagged sod2. A and B: Growth profiles of *S. pombe* in the presence or absence of external lithium in medium of varying external pH. A: Growth of sod2::ura4. B: Growth of the strain sod2::ura4 which was transformed with the plasmid pARTA (SpARTA). C: Na^+-dependent proton influx in SpARTA cells. NaCl-preloaded (filled circles) or control (empty circles) cells of *S. pombe* were placed in Na^+-free medium 1 min after measurements began and H^+ uptake was measured by monitoring the rise in medium pH. D: Active Na^+ efflux in SpARTA cells that were loaded with $^{22}Na^+$. Glucose (40 mM, closed symbols) or incubation buffer (open symbols) was added to cells at time zero. Each point represents an average of at least two independent determinations.

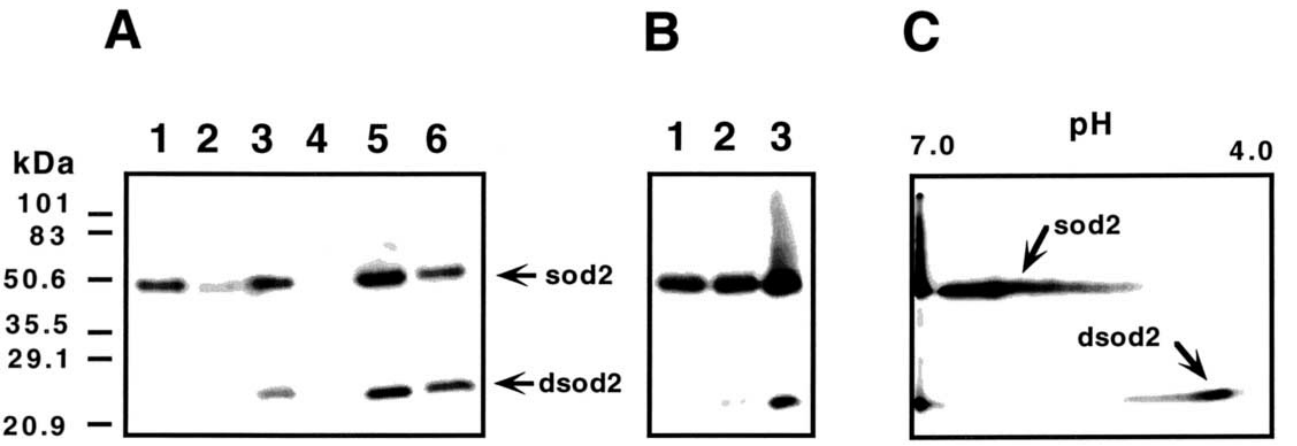

Fig. 3. Identification and characterization of the sod2 protein. SDS PAGE and immunoblotting against sod2-HA protein were performed using crude SpARTA membranes prepared as described in Section 2. The location of intact sod2 and a proteolytic fragment of sod2 (dsod2) is indicated by the arrows. A: Solubilization with varying detergents and conditions: lane 1, 1.3% SDS (positive control); lane 2, water (negative control); lane 3, 1.3% octyl glucoside; lane 4, 1.3% octyl glucoside in the presence of 0.1 M NaCl; lane 5, 1.3% dodecyl maltoside; and lane 6, 1.3% $C_{12}E_8$. B: Enrichment of sod2-HA protein in *S. pombe* membranes. Lane 1, 100 μg of protein of crude membranes; lane 2, membranes (80 μg of protein) 'pre-extracted' with water; lane 3, membranes (50 μg of protein) extracted with DM after pre-extraction with water. C: Two-dimensional electrophoresis of DM solubilized membranes prepared as described in Section 2. The estimated value of the pH gradient is indicated above the gel.

dium extrusion from *S. pombe*. This assay was performed at an acidic external pH, which provides a large ΔpH on the plasma membrane to drive Na^+/H^+ antiporter [24]. The H^+-ATPase of the yeast plasmalemma is known to be activated upon glucose addition. This causes pumping of protons out of the cytosol and creates a significant ΔpH (alkaline inside the cell) [25,26]. We used this method to generate a ΔpH and to initiate active ^{22}Na export from starved, ^{22}Na preloaded SpARTA cells at an external pH of 4.0. Cells with sod2-HA rapidly extruded the isotope in response to glucose addition (Fig. 2D, filled symbols). In parallel measurements, an aliquot of incubation buffer was added to cells instead of glucose and no ^{22}Na extrusion occurred (Fig. 2D, open symbols). This confirmed that, without added glucose, the level of internal ^{22}Na was unchanged. No glucose-activated sodium extrusion was observed in the *sod2::ura4* mutant which was not transformed with sod2-HA (data not shown). Thus, the results of the ^{22}Na transport measurements verify that the sod2-HA protein is active.

3.4. Characterization of the sod2-HA protein

The addition of an HA epitope to the COOH-terminus of sod2 enabled clear visualization of the protein in membrane fractions of the overexpressing strain, SpARTA. The full length antiporter migrated in SDS PAGE gels as a band with an apparent molecular weight of 47 kDa (Fig. 3). This is 82% of the calculated molecular weight and is consistent with the electrophoretic behavior of numerous integral membrane proteins such as NhaA, the major Na^+/H^+ antiporter of *Escherichia coli* [22]. No immunoreactive sod2-HA protein was detected in soluble fractions obtained in membrane isolation (data not shown), suggesting that the expressed sod2-HA protein is entirely associated with yeast membranes.

In initial experiments to characterize the sod2 protein we tested a series of detergents and conditions designed to solubilize the protein from the membrane (Fig. 3A). Membranes were diluted to a final concentration of 15 mg/ml by addition of two volumes of the buffer supplemented with protease inhibitor cocktail [19] and 2% detergent. After 20 min of incubation at 0°C, samples were centrifuged for 1 h at 100 000 × *g*. Supernatants were precipitated with cold 5% TCA to remove the solubilizing detergent and 100 μg aliquots of the protein were subjected to SDS PAGE and immunoblotting with anti-HA antibody. Some degradation of the sod2 protein occurred during solubilization, despite the presence of a protease inhibitor cocktail. The released proteolytic fragment (degraded sod2, dsod2) has an apparent molecular weight of 23.5 kDa, irrespective of the detergent examined (see Fig. 3A, lanes 3, 5, and 6). Our solubilization trials revealed that sod2 can be most effectively solubilized by dodecyl maltoside (Fig. 3A, lane 5). The same detergent was also used successfully to isolate functionally active bacterial antiporter [22]. Octyl glucoside and $C_{12}E_8$ also solubilized the antiporter, though not as efficiently as dodecyl maltoside. It is noteworthy that high ionic strength completely prevented solubilization of sod2 by octyl glucoside (see Fig. 3A, lanes 3 and 4).

Dodecyl maltoside (DM) was therefore chosen for experiments on solubilization and enrichment of sod2-HA from the SpARTA plasma membrane (Fig. 3B). Crude membranes (Fig. 3B, line 1) were defrosted and pre-extracted using 40-fold dilution with cold disruption buffer containing protease inhibitor cocktail without added detergent. This first step removed 50–60% of the total protein from the membranes. The proteins removed likely represent soluble polypeptides which were trapped in membrane vesicles formed during the French press treatment of yeast. Freeze thawing may allow release of such proteins from the vesicle lumen. No sod2 was found in supernatant fractions after the same treatment (data not shown). After this treatment pelleted membranes (Fig. 3B, line 2) were solubilized with DM as described above. Typically the resulting DM extracts contained 5–6 times less total protein than the starting crude membrane preparations. When similar amounts of protein were used for Western blotting, the immunoblot signal of sod2 became stronger rather than weaker due to the relative enrichment of the protein (Fig. 3B, line

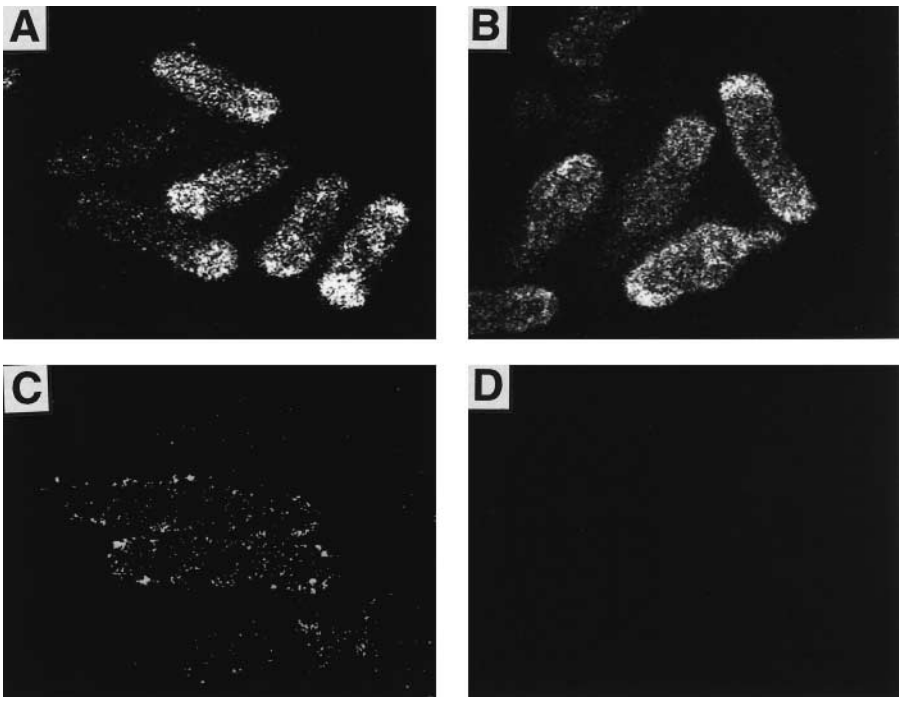

Fig. 4. Confocal microscopy of indirect immunofluorescence of *S. pombe*. A-C: SpARTA; D: sod2::ura4 yeast strains. Fixed sphaeroplasts were labeled with primary antibodies against the HA epitope (A, B, D) or against H^+-ATPase (C) and visualized by rhodamine-conjugated secondary antibody as described in Section 2. Exposure was 15 s (A) or 30 s (B–D).

3). Thus, the solubilization procedure allowed an estimated 3–6-fold enrichment of the sod2-HA protein.

We noted the presence of a 23.5 kDa proteolytic product of the sod2-HA protein. The size of this protein suggests that the cleavage site is localized between the 6th and 7th putative membrane-spanning domains of the protein. We examined the characteristics of this fragment and the intact protein using two-dimensional gel electrophoresis. Based on the predicted amino acid sequence of sod2-HA, the isoelectric point for the intact protein is predicted to be 5.58 while that for the sod2-HA fragment is approximately 4.67 depending on the exact cleavage site. The results (Fig. 3C) confirmed that sod2-HA and the proteolytic fragment were of the predicted sizes and isoelectric points. There were two anti-HA immunoreactive spots, and the one corresponding to the sod2-HA fragment was far more 'acidic' than the whole protein.

3.5. Immunolocalization of sod2 in S. pombe

We used SpARTA (Fig. 4A–C) and the *sod2::ura4* knockout (Fig. 4D) to examine immunolocalization of the sod2 protein. It was necessary to examine sod2 under the stronger ADH promoter because the signal from ·sod2 under the control of its own promoter was not sufficient for analysis (not shown). Control experiments used an antibody against the yeast plasmalemmal H^+-ATPase to label a typical yeast plasma membrane protein (Fig. 4C). We found that the sod2-::ura4 cells that were without the pARTA plasmid, did not react with anti-HA antibody (Fig. 4D). Fig. 4AFig. 4B show two different exposures of the SpARTA cells labeled with anti-HA antibody. The most prominent feature of the sod2 distribution is its clear asymmetry. The antiporter is accumulated in the tip regions of the cells (Fig. 4A,B). In some cases this occurs at only one end of the cell. In other cases it is at both ends of cells that are sometimes slightly larger than cells with labeling at only one end. This difference could represent different localization at different stages of the cell cycle. One can also see that besides the tip distribution, sod2 was also present in the plasma membrane and in smaller amounts in what may be the endoplasmic reticulum surrounding the nucleus. The internal signal may represent biosynthesis of sod2. In addition because sod2 was overexpressed, this membrane protein could cause 'accommodative' yeast membrane biogenesis [27]. Similar membrane biogenesis has been directly demonstrated in the cells of *E. coli* overexpressing fumarate reductase [28]. Fig. 4C shows immunolocalization of the H^+-ATPase. In contrast to sod2, it showed a plasma membrane localization with no concentration at the cell poles or tips. The localization of sod2 at the cell tips is very interesting. Electric currents are always associated with tip growth in fungi [29]. It has been suggested that H^+ influx occurs at the growing cell tip [30] and an apical localization of antiporters vs. a distal localization of proton pumps was suggested to explain this local proton inflow into the apical region [30]. However, direct evidence for this differential distribution of membrane ion transporters has been lacking so far. Our observations thus provide the first such evidence inferring the involvement of H^+ and Na^+ fluxes. We found that the H^+-ATPase that pumps protons out of the cell is distributed randomly over the cell surface (Fig. 4C). Therefore, the concentration of sod2 at the cell tip may lead to a local inwardly directed H^+ flux into the tip region.

In the present communication, we identified the yeast Na^+/H^+ antiporter, sod2. The protein was 'tagged' by the addition of an HA epitope. It retained its antiporter activity which was sufficient to protect yeast against relatively high concentrations of LiCl. The non-denaturing detergent, dodecyl maltoside effectively solubilized sod2-HA and we were able to detected the protein in Western blots using anti-HA antibody.

Sod2 had an asymmetrical localization and was concentrated in the tips of *S. pombe*. We consider this study to be the first advances toward the isolation and characterization of the sod2 protein. An effort to purify functional sod2 protein is now in progress.

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