Yeast SMF1 Mediates H\textsuperscript{+}-coupled Iron Uptake with Concomitant Uncoupled Cation Currents*

(Received for publication, August 4, 1999, and in revised form, September 14, 1999)

Xing-Zhen Chen‡§, Ji-Bin Peng‡§, Adiel Cohen‡, Hannah Nelson‡, Nathan Nelson‡, and Matthias A. Hediger¶

From the %Membrane Biology Program and Renal Division, Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115 and the *Department of Biochemistry, Tel Aviv University, Ramat Aviv, 69978 Tel Aviv, Israel

Yeast membrane proteins SMF1, SMF2, and SMF3 are homologues of the DCT1 metal ion transporter family. Their functional characteristics and the implications of these characteristics in vivo have not yet been reported. Here we show that SMF1 expressed in Xenopus oocytes mediates H\textsuperscript{+}-dependent Fe\textsuperscript{2+} transport and uncoupled Na\textsuperscript{+} flux. SMF1-mediated Fe\textsuperscript{2+} transport exhibited saturation kinetics (K_m = 2.2 \mu M), whereas the Na\textsuperscript{+} flux did not, although both processes were electrogenic. SMF1 is also permeable to Li\textsuperscript{+}, Rb\textsuperscript{+}, K\textsuperscript{+}, and Ca\textsuperscript{2+}, which likely share the same uncoupled pathway. SMF2 (but not SMF3) mediated significant increases in both Fe\textsuperscript{2+} and Na\textsuperscript{+} transport compared with control oocytes. These data are consistent with the concept that uptake of divalent metal ions by SMF1 and SMF2 is essential to yeast cell growth. Na\textsuperscript{+} inhibited metal ion uptake mediated by SMF1 and SMF2 expressed in oocytes. Consistent with this, we found that increased sensitivity of yeast to EGTA in the high Na\textsuperscript{+} medium is due to inhibition of SMF1- and SMF2-mediated metal ion transport by uncoupled Na\textsuperscript{+} pathway. Interestingly, DCT1 also mediates Fe\textsuperscript{2+}-activated uncoupled currents. We propose that uncoupled ion permeabilities in metal ion transporters protect cells from metal ion overload.

Metal ions are important for all living cells. In man, metal ion deficiency leads to anemia (1), whereas metal ion overload is toxic and leads to hemochromatosis (3), Menkes’ disease (2), Wilson’s disease (4), and neurodegenerative diseases (5–7). Metal ions such as iron, manganese, zinc, and cobalt are involved in many catalytic reactions, gene regulation, and signal transduction pathways (8–10). An adequate supply of metal ions to cells is important and is provided by specialized transporters.

The recently cloned mammalian metal ion transporter DCT1 (11, 12), originally named Nramp2 (natural resistance-associated macrophage protein 2) (13–15), is present in both plasma membranes and endosomal vesicles for translocation, via transferrin-dependent and -independent pathways, of metal ions into the cytoplasm of cells and for maintenance of systemic metal ion homeostasis. It has been found that a mutation in DCT1 at position 185 (G185R) causes microcytic anemia in mkn --/- mice and Belgrade rats (12). This mutation was subsequently shown to result in loss of Fe\textsuperscript{2+} transport ability (16). DCT1-mediated iron absorption in the intestine depends on the body iron status, which is regulated in part by the hemochromatosis gene HFE, a major histocompatibility complex gene (17, 18). A single point mutation in HFE (C282Y) results in iron overload in hemochromatosis patients (3).

SMF1, SMF2, and SMF3 are yeast homologues of the Nramp proteins with 51–54% identity in amino acid sequence to each other and 33–36% identity to DCT1. SMF1 was originally thought to be localized in the yeast mitochondrial membrane (19) and was named SMF, which stands for suppressor of mitochondria import function. However, more recent studies using an antibody demonstrated that SMF1 is located in the yeast plasma membrane, where it is thought to mediate uptake of Mn\textsuperscript{2+} and Zn\textsuperscript{2+} into the cytoplasm (20). There was indirect evidence that other divalent metal ions such as Cd\textsuperscript{2+}, Co\textsuperscript{2+}, and Cu\textsuperscript{2+} are also substrates of SMF1 (21). In analogy to HFE in mammalian cells, the product of the yeast BSD2 (bypass superoxide dismutase deficiency gene 2), localized in the endoplasmic reticulum, regulates metal ion absorption by exerting a negative control on SMF1 activity (21, 22). Despite these findings, a functional characterization of SMF1 has not yet been reported.

In the present study, we expressed SMF1, SMF2, and SMF3 in Xenopus oocytes and used both a radiotracer approach and the two-microelectrode voltage-clamp technique to investigate the function of these proteins. We show that SMF1 mediates H\textsuperscript{+}-dependent Fe\textsuperscript{2+} transport and uncoupled Na\textsuperscript{+} currents. SMF2 also mediates significant H\textsuperscript{+}-coupled Fe\textsuperscript{2+} transport and uncoupled Na\textsuperscript{+} currents, which are much smaller than those mediated by SMF1. SMF3 exhibited no detectable activities when expressed in oocytes. Because Na\textsuperscript{+} inhibited metal ion uptake in oocytes expressing SMF1, we investigated the effect of Na\textsuperscript{+} on yeast growth.

**EXPERIMENTAL PROCEDURES**

**Oocyte Preparation—**Yeast SMF1, SMF2, and SMF3 cDNAs were subcloned from pTLN2 into pNWP plasmid, and rat DCT1 cDNA was in pSPORT1. Capped SMF and DCT1 cRNAs were synthesized by in vitro transcription from their cDNAs. Oocytes were extracted from stage V–VI Xenopus laevis and were defolliculated using a Ca\textsuperscript{2+}-free solution (90 mM NaCl, 3 mM KCl, 0.82 mM MgSO\textsubscript{4}, 10 mM HEPES, pH 7.5) containing 2 mg/ml collagenase (Roche Molecular Biochemicals, Mannheim, Germany) for ~2 h at 18 °C. Oocytes were injected, on the same day (at least 4 h after defolliculation) or on the following day, with 50 nl of H\textsubscript{2}O containing 15 ng cRNA of SMF1, SMF2, or SMF3 or 25 ng of cRNA of DCT1. Equal amounts of H\textsubscript{2}O were injected into control oocytes. Injected oocytes were incubated at 18 °C using antibiotic Barth’s solution containing 90 mM NaCl, 2 mM KCl, 0.82 mM MgSO\textsubscript{4}, 0.41 mM CaCl\textsubscript{2}, 0.33 mM Ca(NO\textsubscript{3})\textsubscript{2} \textsubscript{2}, 10 mM HEPES, 50 µM gentamicin, 10
units/ml penicillin, and 10 μg/ml streptomycin, pH 7.5.

**Radiotracer Measurements**—Uptake experiments were performed at 2–4 days following injection. Uptake solutions for radiotracer experiments contained 100 mM NaCl + choline-Cl, 10 mM HEPES, 2 mM KCl, 0.5 mM MgCl2, 0.5 mM L-ascorbic acid (freshly prepared), pH 5.5–7.5, by Tris-Base or HEPES. L-ascorbic acid was added to solutions to maintain iron in the 2+ form. 8–10 oocytes were incubated in 0.5 ml of solutions containing radioactive 55FeCl3. Uptake lasted 30 min and was terminated by washing oocytes through six consecutive ice-cold uptake solution containing 100 mM NaCl, pH 7.5.

**Electrophysiology**—Experiments utilizing the two-microelectrode voltage clamping technique were performed as described (23). Resistance of microelectrodes filled with 3 M KCl was 0.5–2 MΩ. In experiments involving voltage jumping or holding, currents and voltages were digitized at 0.3 or 200 ms/sample, respectively. After ~3 min of membrane potential stabilization following microelectrode impalements, the oocyte was clamped to the holding potential (Vh) of ~50 mV. 100-ms voltage pulses between ~160 and +60 mV, in increments of +20 mV, were then applied, and steady-state currents were obtained as the average values in the interval from 80 to 95 ms after the initiation of the voltage pulses. Solutions used in electrophysiology were the same as in tracer assay except that they contained no L-ascorbic acid when metal ions other than iron were present. Experimental results were expressed in the form of means ± S.E. (n), where n indicates the number of oocytes used. Statistical analysis was performed as described (23).

**Simultaneous Voltage-clamped Tracer and Current Measurements**—Before starting tracer uptake, oocyte was clamped at ~50 mV and perfused with substrate-free solution. Then the membrane potential was held at other test values (+10, −20, −50, −70, or −80 mV). After the perfusion was stopped, the uptake solution (200 μl) was added manually using a pipettor, which washed out the substrate-free solution. The uptake lasted 2–5 min in the chamber whose volume is about 200 μl and was terminated by perfusing (washing) the oocyte with the substrate-free solution. Currents were continuously measured during uptake. Oocyte was then dissolved in 250 μl of 10% SDS and mixed with 2.5 ml of scintillation mixture.

**Yeast Strains, Media, and Reagents**—The wild-type strain used in this study is *Sacharomyces cerevisiae* W303 (MATa ade2 his3 leu2 ura3). The other strains used are ΔSMF1 (MATa ade2 his3 leu2 trp1 ura3 SMF1::URA3), ΔSMF2 (MATa ade2 his3 leu2 trp1 ura3 SMF2::URA3), ΔSMF3 (MATa ade2 his3 leu2 trp1 ura3 SMF3::URA3), and ΔSMF1+ΔSMF2+ΔSMF3 (MATa ade2 his3 leu2 trp1 ura3 SMF1::URA3 SMF2::URA3 SMF3::URA3). During the preparation of the triple disruptant ΔSMF1+ΔSMF2+ΔSMF3, the URA3 gene was inactivated twice by 5-fluoroorotic acid treatment.

Yeast (*S. cerevisiae*) cells were grown in a YPD medium containing 1% yeast extract, 2% Bactopeptone, and 2% dextrose. The medium was buffered by 50 mM Mes, and the pH was adjusted by NaOH (24, 25).

### Results

**Transports of Fe**

**Transport of Fe** by SMF1—*Xenopus* oocytes expressing SMF1 exhibited significant increases in 55Fe uptake compared with H2O-injected control oocytes (Fig. 1a). The increase was higher in the absence of external Na+ (Na+o) than in the presence of 100 mM Na+. At pH 5.5 and in the absence of Na+, SMF1-mediated iron uptake was 30–40 times higher than control levels.

Iron transport was saturable and followed the Michaelis-Menten relationship (Fig. 1b). The apparent affinity constant (Km) was high, with an apparent affinity constant (Km) of 2.2 ± 0.2 μM (n = 10). The H+ dependence of Fe2+ uptake (at 5 μM Fe2+) also followed the Michaelis-Menten relationship with a Km of 6.0 ± 0.2 μM (or pH 6.2 ± 0.2). This suggests that one H+ is coupled to the uptake of one Fe2+. Uptake of Fe2+ was completely inhibited by 1 mM Mn2+, Zn2+, and Cd2+, weekly inhibited by 50 μM Al3+, and was not inhibited by 1 mM La3+ or 50 μM Cd2+ (Fig. 1d). In addition, no increase in Fe3+ uptake was observed in SMF1-expressing oocytes compared with control oocytes. These results indicate that various divalent but not trivalent metal ions are transported by SMF1. Despite the relatively low transport level of SMF1 in *Xenopus* oocytes, these characteristics of SMF1 are similar to those of the mammalian homolog DCT1.

Metal ion transport mediated by SMF1 was electrogenic and voltage-dependent (Fig. 2, a and b). Consistent with radiotracer experiments, Fe2+ and Mn2+ generated currents. Interestingly, although Na+ inhibited iron uptake, Na+ applied in the absence of metal ions induced large SMF1-specific inward currents at ~50 mV (Fig. 2c). At pH 5.5 (but not at pH 7.5) and in the presence of 100 mM Na+, addition of Fe2+ apparently evoked a net outward current that is likely composed of Fe2+-inhibited inward Na+ current and Fe2+-stimulated H+-Fe2+ exchange.
cotransport inward current, with the former being larger than the latter. In the absence of metal ions and in the presence of 100 mM Na\(^+\), a reduction in medium pH also apparently stimulated a net outward current, indicating that protons inhibit the Na\(^+\) current. Our results show mutual inhibition between the H\(^+\)-Fe\(^{2+}\) cotransport and Na\(^+\) permeation, suggesting that the observed Na\(^+\) fluxes are mediated by SMF1 and not by SMF1-stimulated endogenous Na\(^+\) pathways.

In the absence of metal ions, a large conductance in oocytes expressing SMF1 was evoked by Na\(^+\) (Fig. 3, a–d). The outward currents observed in the presence of external choline-Cl (Fig. 3b) are likely due to effluxes of K\(^+\) and Na\(^+\), because SMF1 is also permeable to K\(^+\) (see below). The Na\(^+\) currents were voltage-dependent and exhibited no saturation by hyperpolarization (Fig. 3e). They were not significantly affected by depletion of extracellular Ca\(^{2+}\), Mg\(^{2+}\), K\(^+\), or CL\(^-\) or by depletion of intracellular Ca\(^{2+}\) (through injection of 25 nl of 50 mM EGTA into the oocyte cytoplasm), indicating that they are uncoupled currents. Na\(^+\) currents were Na\(^+\)-dependent but did not saturate at concentration up to 100 mM Na\(^+\) at any V\(_{m}\) tested (−160 to +60 mV) (Fig. 3f).

We also tested the functions of SMF2 and SMF3 by oocyte expression studies. Compared with SMF1, SMF2 exhibited lower (but significant) Fe\(^{2+}\) uptake and uncoupled Na\(^+\) currents (Fig. 4). In contrast, SMF3 exhibited no detectable iron uptake or uncoupled sodium currents in oocytes. It is possible that SMF3 is primarily localized in intracellular membranes, in analogy to Nramp1 (28).

**Inhibition by La\(^{3+}\) and Cation Selectivity**—In oocytes expressing SMF1, Na\(^+\) currents were partially inhibited by application of 1 mM La\(^{3+}\) (Fig. 5 and Table I). In contrast, Fe\(^{2+}\) uptakes were not inhibited by La\(^{3+}\) (Fig. 2d). La\(^{3+}\) also inhibited an outward current in oocytes expressing SMF1 (Fig. 5b) but not in H\(_2\)O-injected oocytes (data not shown). In average (n = 5), 47% of the Na\(^+\) current in oocytes expressing SMF1 was inhibited by 1 mM La\(^{3+}\) (Fig. 5c). Na\(^+\) currents in H\(_2\)O-injected oocytes were not significantly inhibited by La\(^{3+}\), further supporting the concept that Na\(^+\) currents are mediated by SMF1 but not through endogenous Na\(^+\) pathways. Taken together, our data indicate the presence of separate modes for La\(^{3+}\)-sensitive uncoupled Na\(^+\) entry and La\(^{3+}\)-insensitive H\(^+\)-coupled Fe\(^{2+}\) entry by SMF1.

To test the ion selectivity for the Na\(^+\) permeation pathway, Li\(^+\), Rb\(^+\), or K\(^+\) was used to replace Na\(^+\). SMF1 was permeable to all of these alkaline cations, which generated similar amplitudes at −50 mV (Fig. 5d), indicating that the cation pathway is nonselective to monovalent cations. La\(^{3+}\) inhibited these currents as well and had lower inhibitory effects on the corresponding currents mediated by H\(_2\)O-injected oocytes (Table I).
Interestingly, SMF1 and SMF2 are also permeable to Ca$^{2+}$ (Fig. 6a). The permeation of Ca$^{2+}$ via SMF1 was partially inhibited by La$^{3+}$ and Na$^{+}$ and was not driven by protons (Fig. 6). These data indicate that Ca$^{2+}$ and monovalent cations share the same nonselective cation pathway.

**TABLE I**

*Alkaline cation-stimulated currents and inhibition by lanthanum in SMF1-expressing or H$_2$O-injected Xenopus oocytes*

|        | Na$^+$ | Li$^+$ | Rb$^+$ | K$^+$ |
|--------|--------|--------|--------|--------|
| SMF1   | 165 ± 33$^a$ | 192 ± 59 | 259 ± 34 | 264 ± 51 |
| −La    | 87 ± 12 | 94 ± 14 | 101 ± 14 | 100 ± 10 |
| +La    | 8 ± 1  | 17 ± 7  | 80 ± 15  | 50 ± 6   |
| H$_2$O | 11 ± 2 | 12 ± 2 | 48 ± 1 | 31 ± 2 |

$^a$ Mean ± S.E.

Interestingly, SMF1 and SMF2 are also permeable to Ca$^{2+}$ (Fig. 6a). The permeation of Ca$^{2+}$ via SMF1 was partially inhibited by La$^{3+}$ and Na$^{+}$ and was not driven by protons (Fig. 6). These data indicate that Ca$^{2+}$ and monovalent cations share the same nonselective cation pathway.

**DCT1-mediated Uncoupled Currents**—Although yeast SMF1 and its mammalian homologue DCT1 (Nramp2) are functionally similar and both mediate H$^+$-driven Fe$^{2+}$ uptake, DCT1 did not mediate uncoupled Na$^+$ currents (data not shown). However, DCT1 is not merely a cotransport system. Based on a simultaneous measurements of Fe$^{2+}$-elicited currents and $^{55}$Fe uptake under voltage-clamp conditions (Fig. 7, a and b), the ratio of the Fe$^{2+}$-evoked current to $^{55}$Fe uptake at $V_m$ of +10 mV was close to 3:1, suggesting that the H$^+$:Fe$^{2+}$ stoichiometry...
The various yeast strains were grown on agar plates containing 0.25% yeast extract, 0.5% Bactopeptone, 2% dextrose, 2% agar, and 50 mm Mes, pH 6. NaCl and/or EGTA were added where indicated.

For the growth assay, the strains were grown in liquid YPD medium to mid-logarithmic phase. The cells were washed and resuspended in water, and 1 µl was seeded on agar plates with the indicated supplements. The concentration of applied MnCl₂, CuCl₂, or ZnCl₂ was 5 μM.

is 1:1 at this membrane potential. However, this ratio increased substantially with hyperpolarization and was unexpectedly high at hyperpolarized potentials (Fig. 7, b and c). This indicates that Fe⁷⁺ evokes a stoichiometrically uncoupled and voltage-dependent current. The Fe⁷⁺-induced inward currents and the charge:Fe⁺⁺ ratios were not significantly affected by removing external cation Na⁺, Ca⁺⁺, or Mg⁺⁺, or depleting intracellular Cl⁻ through overnight incubation in Cl⁻-free media (data not shown). Thus, the uncoupled currents of DCT1 are distinct from the SMF1-mediated Na⁺ currents. The nature of these uncoupled currents is currently under investigation.

**Effects of Na⁺ on Yeast Growth**—Uncoupled currents induced by expression of SMF1 in *Xenopus* oocytes could arise from factors not directly related to the functional properties of SMF1, including up-regulation of an endogenous *Xenopus* protein and conformational changes of SMF1 induced by abnormal lipid composition in the target membrane. Our electrophysiologic data indicate that Na⁺ permeation is mediated directly by SMF1. To further validate this concept, we examined whether the observed Na⁺ currents in oocytes expressing SMF1 can be verified in *S. cerevisiae in vivo*. For this purpose we constructed yeast disruptant mutants with each of SMF1, SMF2, and SMF3 genes individually inactivated and a mutant with all three genes inactivated. EGTA, which chelates vital metal ions such as Mn⁺⁺ and Cu⁺⁺ is known to inhibit yeast cell growth (29). The yeast disruptant mutants exhibited higher sensitivities to EGTA than the wild-type yeast. The higher sensitivities and associated growth arrest could be suppressed by exposure to low concentrations of manganese or copper in the medium (20, 30). We utilized these mutants to examine whether inhibition of SMF1-mediated Mn⁺⁺ or Cu⁺⁺ transport by Na⁺ increases sensitivity to EGTA. Of note, FET3 and FET4 (not SMFs) constitute the main yeast iron transport systems, with high and low affinities, respectively (31), whereas SMF1 and SMF2 mainly mediate uptake of Mn⁺⁺ and other metal ions. In the presence of 0.1 mM EGTA and 20 mM Na⁺, only the triple mutant ΔSMF1+2+3 failed to grow (Fig. 8). Among the individually disruptant mutants, only ΔSMF1 exhibited growth arrest in the presence of 0.5 mM EGTA. At 1 mm EGTA both ΔSMF1 and ΔSMF2 failed to grow. ΔSMF3 can grow at higher EGTA concentrations. These results are in agreement with our observation that oocytes expressing SMF2 exhibit lower activities (in both Fe⁺⁺ uptake and Na⁺ currents, Fig. 4) than SMF1-expressing oocytes and that SMF3-expressing oocytes exhibited no significant transport activities in oocytes. For all mutants the growth arrest was suppressed by addition of 5 μM Mn⁺⁺ or Cu⁺⁺ but not Zn⁺⁺.

Thus, ΔSMF2 was the appropriate mutant to study the effect of Na⁺ on SMF1. Indeed, addition of 100 mM NaCl to the plates containing 0.5 mM EGTA caused growth arrest of ΔSMF2, consistent with our finding in *Xenopus* oocytes that Na⁺ can effectively compete with other metal ions for their uptake by SMF1.

**DISCUSSION**

The present studies demonstrate that SMF1, like its mammalian homologue DCT1/Nramp2, mediates proton-coupled metal ion transport when expressed in *Xenopus* oocytes. Transport is saturable with increasing iron concentration and is not inhibited by La³⁺. SMF1 also exhibits uncoupled cation (Na⁺, K⁺, Rb⁺, Li⁺, and Ca⁺⁺) currents. Uncoupled Na⁺ currents were not saturable with increasing Na⁺ concentration and inhibitable by La³⁺. These results indicate that the cation permeation is a channel-like behavior of SMF1 and that H⁻-Fe⁺⁺ cotransport and Na⁺ permeation are mediated by SMF1 through distinct mechanisms.

In analogy to DCT1, SMF1 mediates absorption of a variety of divalent metal ions, including Fe⁺⁺, Mn⁺⁺, Zn⁺⁺, Cd⁺⁺, and Cu⁺⁺. However, although DCT1 is thought to play an essential role in intestinal absorption of both Fe⁺⁺ and other divalent metal ions, iron uptake in yeast is probably mediated by the specialized FET3 and FET4 iron uptake systems. Thus, the role of SMF in yeast is most likely to absorb other essential metal ions present in the soil such as Mn⁺⁺, Zn⁺⁺, and Cu⁺⁺.

Our yeast studies show that SMF1 plays a major role in supporting cell growth in the presence of relatively low EGTA concentrations. Oocyte studies suggest that Na⁺ inhibition of yeast growth at low EGTA concentrations can be attributed to Na⁺ inhibition of metal ion uptake by SMF1. The physiological significance of this phenomenon can be explained by considering the growth conditions in nature. In the natural environment, yeast can be exposed to drastic changes in the salinity because of water evaporation. Under these conditions the concentration of metal ions in the extracellular environment can reach dangerous levels. Because yeast cells can tolerate NaCl concentrations of up to 0.9 M, competition of Na⁺ with metal ion uptake may prevent accumulation of metal ions to toxic levels.

Several other transporters also exhibit uncoupled ion currents such as chloride currents in the glutamate transporters (32), Na⁺ leak currents in the Na⁺-glucose transporters (33), and alkaline-cation currents in the serotonin transporter (34, 35) and the GABA transporter (36). Uncoupled Na⁺ and Li⁺ currents mediated by serotonin transporters were not saturable and exhibited channel-like behaviors (35, 37). The biological and physiological meaning of uncoupled cation-leak pathways in these transporters are still unclear. It was proposed that concomitant Cl⁻ flow with glutamate cotransport through glutamate transporters act as a feedback mechanism to reduce neuronal cell depolarization that would result in toxic vesicular glutamate release (38). The identification of these new components of transporter-associated currents provides new tools to elucidate the interaction between the transporter substrates and ions and will help to elucidate the structure-function relationship of these transporters.

---

A. Cohen, H. Nelson, and N. Nelson, unpublished data.
Ca\(^{2+}\) flux via SMF1 was not driven by protons but rather through the uncoupled nonselective pathway. On the other hand, although the mammalian homologue DCT1 was not permeable to Ca\(^{2+}\), its H\(^{+}\)-coupled cotransport was inhibited by Ca\(^{2+}\), indicating that Ca\(^{2+}\) can interact with DCT1 as well. Hence, SMF1 and DCT1, when expressed in oocytes, likely possess structural similarity at their Fe\(^{2+}\) and Ca\(^{2+}\) binding sites. Unlike Na\(^{+}\), Ca\(^{2+}\) (up to 70 ms) had no inhibitory effects on yeast growth (30), suggesting that the Ca\(^{2+}\) permeability of SMF transporters in yeast are not significant enough to interfere with cell growth.

What is the physiological significance of the Na\(^{+}\) permeability of SMF1? We have shown that the mammalian homologue DCT1 also mediates uncoupled currents that substantially increase with hyperpolarization. However, in contrast to yeast SMF1, these DCT1-mediated currents are metal ion-gated. Although SMF1 and DCT1 mediate different uncoupled currents, they may utilize these characteristics for similar purposes, that is to prevent from excessive metal ion loading. The Na\(^{+}\) permeation via SMF1 may provide a protection by the preferential uptake of Na\(^{+}\) over the excessive uptake of toxic metal ions. Influx of more than 10 positive charges per Fe\(^{2+}\) entry under physiological (negative) potentials would significantly depolarize the cells, which results in an inhibition of Fe\(^{3+}\) uptake. Further studies are needed to define the physiological significance of the anomalous behaviors of the SMF and DCT metal ion transporters.

Acknowledgments—We thank P. Fong for kindly providing the pTLN2 oocyte expression vector and J.-Y. Lapointe and E. M. Wright for valuable discussions and suggestions.

REFERENCES
1. Hartman, K. R., and Barker, J. A. (1996) *Am. J. Hematol.* 51, 269–275
2. Chelly, J., Tumer, Z., Tonnesen, T., Petterson, A., Ishikawa-Bruch, Y., Tommerup, N., Horn, N., and Monaco, A. P. (1993) *Nat. Genet.* 3, 14–19
3. Feder, J. N., Gnrke, A., Thomas, W., Tsuchihashi, Z., Ruddy, D. A., Basava, A., Dormishian, F., Domingo, R., Jr., Ellis, M. C., Fullan, A., Hinton, I. M., Jones, N. L., Kimmel, B. E., Kronmal, G. S., Lauer, P., Lee, V. K., Loeb, D. B., Mapa, F. A., McClelland, E., Meyer, N. C., Mintier, G. A., Moeller, N., Moore, T., Morkang, E., Wolf, R. K., et al. (1996) *Nat. Genet.* 13, 399–408
4. Cox, D. W. (1996) *Prog. Liver Dis.* 14, 245–264
5. Good, P. F., Werner, P., Hsu, A., Olanow, C. W., and Perl, D. P. (1996) *Am. J. Pathol.* 149, 21–28
6. Hirach, E. C. (1994) *Mol. Neurobiol.* 9, 335–412
7. Babcock, M., de Silva, D., Oaks, R., Davis-Kaplan, S., Jiralserspong, S., Montermin, L., Pandolfo, M., and Kaplan, J. (1997) *Science* 276, 1709–1712
8. Orgad, S., Nelson, H., Segal, D., and Nelson, N. (1998) *J. Exp. Biol.* 201, 115–120
9. Palmeter, R. D., Cole, T. B., Quaife, C. J., and Findley, S. D. (1996) *Proc. Natl. Acad. Sci. U.S.A.* 93, 14934–14939
10. Rodrigues, V., Cheah, P. Y., Ray, K., and Chia, W. (1995) *EMBO J.* 14, 3097–3102
11. Gunshin, H., Mackenzie, B., Berger, U. V., Gunshin, Y., Nussberger, S., Romero, M., Boron, W. F., Gallan J. L., and Hediger, M. A. (1997) *Nature* 388, 482–488
12. Fleming, M. D., Trenor, C. C., III, Su, M. A., Fenzler, D., Beier, D. R., Dietrich, W. F., and Andrew, N. C. (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95, 1148–1153
13. Vidal, S. M., Malo, D., Vogan, K., Skamene, E., and Gros, P. (1993) *Cell* 73, 469–485
14. Vidal, S., Belouchi, A. M., Cellier, M., Beatty, B., and Gros, P. (1995) *Mamm. Genome.* 6, 224–230
15. Grumert, S., Cellier, M., Vidal, S., and Gros, P. (1995) *Genomics* 25, 514–525
16. Fleming, M. D., Romano, M. A., Su, M. A., Garrick, L. M., Garrick, M. D., and Andrews, N. C. (1996) *Proc. Natl. Acad. Sci. U.S.A.* 93, 1148–1153
17. Wood, R. J., and Han, O. (1998) *J. Nutr.* 128, 1841–1844
18. Rolle, A., and Hediger, M. A. (1999) *J. Physiol.* 518, 1–12
19. West, A. H., Clark, D. J., Martin, J., Neupert, W., Harl, F. U., and Horwich, A. L. (1992) *J. Biol. Chem.* 267, 24625–24633
20. Supek, F., Supekova, L., Nelson, H., and Nelson, N. (1996) *Proc. Natl. Acad. Sci. U.S.A.* 93, 5105–5110
21. Liu, X. F., Supek, F., Nelson, N., and Culotta, V. C. (1997) *J. Biol. Chem.* 272, 11763–11769
22. Liu, X. F., and Culotta, V. C. (1999) *J. Biol. Chem.* 274, 4683–4686
23. Chen, X.-Z., Shayakul, C., Berger, U. V., Tian, W., and Hediger, M. A. (1998) *J. Biol. Chem.* 273, 20972–20981
24. Nelson, H., and Nelson, N. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87, 3503–3507
25. Nouni, T., Beltran, C., Nelson, H., and Nelson, N. (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88, 1938–1942
26. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) *J. Bacteriol.* 153, 163–168
27. Elble, R. (1992) *BioTechniques* 13, 18–20
28. Searle, S., Bright, N. A., Roach, T. I., Atkinson, P. G., Barton, C. H., Meloen, R. H., and Blackwell, J. M. (1998) *J. Cell Sci.* 111, 2855–2866
29. Kinner, E., Grumet, S., Raymond, M., and Gros, P. (1997) *J. Biol. Chem.* 272, 28933–28938
30. Supek, F., Supekova, L., Nelson, H., and Nelson, N. (1997) *Exp. Biol. 200*, 321–330
31. Dix, D. R., Bridgman, J. T., Broderius, M. A., Byersoder, C. A., and Eide, D. J. (1994) *J. Biol. Chem.* 269, 26092–26099
32. Fairman, W. A., Vandenberg, R. J., Arriaga, J. L., Kavanaugh, M. P., and Amara, S. G. (1995) *Nature* 375, 599–603
33. Chen, X.-Z., Coady, M. J., Jackson, F., Berteloot, A., and Lapointe, J.-Y. (1995) *Biophys. J.* 69, 2405–2414
34. Mager, S., Min, C., Henry, D. J., Chavkin, C., Hoffman, B. J., Davidson, N., Andrews, N. C. (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95, 1148–1153
35. Galli, A., Petersen, C. I., deBlaquiere, M., Blakely, R. D., and DeFelice, L. J. (1995) *Nature* 375, 3401–3411
36. Cammack, J. N., and Mager, S. (1996) *Proc. Natl. Acad. Sci. U.S.A.* 93, 723–727
37. Lin, F., Lester, H. A., and Mager, S. (1997) *Proc. Natl. Acad. Sci. U.S.A.* 94, 7284–7288
38. Picaud, S. A., Larsson, H. P., Grant, G. B., Lecar, H., and Werblin, F. S. (1995) *J. Neurophysiol.* 74, 1760–1771
Yeast SMF1 Mediates $H^+$-coupled Iron Uptake with Concomitant Uncoupled Cation Currents
Xing-Zhen Chen, Ji-Bin Peng, Adiel Cohen, Hannah Nelson, Nathan Nelson and Matthias A. Hediger

J. Biol. Chem. 1999, 274:35089-35094.
doi: 10.1074/jbc.274.49.35089

Access the most updated version of this article at http://www.jbc.org/content/274/49/35089

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 38 references, 19 of which can be accessed free at http://www.jbc.org/content/274/49/35089.full.html#ref-list-1