The Human ZIP1 Transporter Mediates Zinc Uptake in Human K562 Erythroleukemia Cells*

Received for publication, February 26, 2001, and in revised form, April 9, 2001 Published, JBC Papers in Press, April 11, 2001, DOI 10.1074/jbc.M101772200

L. Alex Gaither and David J. Eide†
From the Department of Nutritional Sciences, University of Missouri, Columbia, Missouri 65211

The ZIP superfamily of transporters plays important roles in metal ion uptake in diverse organisms. There are 12 ZIP-encoding genes in humans, and we hypothesize that many of these proteins are zinc transporters. In this study, we addressed the role of one human ZIP gene, hZIP1, in zinc transport. First, we examined 65Zn uptake activity in K562 erythroleukemia cells overexpressing hZIP1. These cells accumulated more zinc than control cells because of increased zinc influx. Moreover, consistent with its role in zinc uptake, hZIP1 protein was localized to the plasma membrane. Our results also demonstrated that hZIP1 is responsible for the endogenous zinc uptake activity in K562 cells. hZIP1 is expressed in untransfected K562 cells, and the increase in mRNA levels found in hZIP1-overexpressing cells correlated with the increased zinc uptake activity. Furthermore, hZIP1-dependent 65Zn uptake was biochemically indistinguishable from the endogenous activity. Finally, inhibition of endogenous hZIP1 expression with antisense oligonucleotides caused a marked decrease in zinc uptake or intracellular zinc transport.

Members of the ZIP family are found at all phylogenetic levels, including archaeabacteria, eubacteria, and eukaryotes. There are currently ~85 members reported in the sequence data bases, and these fall into four subfamilies based on their amino acid similarities. Most members are predicted to have eight transmembrane domains and share a predicted topology where the amino and carboxyl termini are extracytoplasmic. The greatest degree of conservation is found in transmembrane domains IV–VII. Transmembrane domains IV and V are particularly amphipathic and contain conserved and functionally critical histidine residues flanked by equally important polar or charged amino acids. These residues are thought to line an aqueous cavity in the transporter through which the substrate moves. Notably, ZIP proteins do not contain ATP-binding sites or ATPase domains. Therefore, these proteins must function through either secondary active transport or facilitated diffusion.

There are 12 known ZIP members in the human genome, and five members have been found in the mouse (10). Three of the human proteins, hZIP1, hZIP2, and hZIP3, are very closely related to the fungal and plant proteins known to be zinc uptake transporters. The recent studies of fungal and plant ZIP transporters indicated that the ZIP superfamily plays remarkably conserved roles in metal ion transport and especially zinc uptake. These observations suggested that the mammalian ZIP proteins play similar roles. To test this hypothesis, we expressed the hZIP2 protein in human K562 erythroleukemia cells and showed that hZIP2 localizes to the plasma membrane (12). Moreover, hZIP2 expression resulted in a novel zinc uptake activity not found in these cells. Thus, hZIP2 is a metal ion transporter capable of zinc uptake. In this report, we continue our characterization of mammalian ZIP transporters by functional expression of the hZIP1 protein. Our results demonstrate that hZIP1, like hZIP2, is a zinc transporter. We also found that hZIP1 is the endogenous zinc uptake transporter normally found in K562 cells. Given the ubiquitous expression of hZIP1 in human tissues, we propose that hZIP1 is the major zinc transporter for many human cell types.

EXPERIMENTAL PROCEDURES

DNA Manipulations—An hZIP1 cDNA clone was isolated from a prostate library using high throughput PCR screening (Genome Systems Inc.) and sequenced in its entirety. The hZIP1 ORF was amplified

† To whom correspondence should be addressed: Dept. of Nutritional Sciences, 217 Gwynn Hall, University of Missouri, Columbia, MO 65211. Tel.: 573-882-9686; Fax: 573-882-0185; E-mail: eided@missouri.edu.

‡ To whom correspondence should be addressed: Dept. of Nutritional Sciences, University of Missouri, Columbia, MO 65211. Tel.: 573-882-9686; Fax: 573-882-0185; E-mail: eided@missouri.edu.

1 The abbreviations used are: hZIP, human ZIP; RT-PCR, reverse transcription-polymerase chain reaction; ORF, open reading frame; CMV, cytomegalovirus; HA, hemagglutinin; PBS, phosphate-buffered saline; MOPS, 4-morpholinepropanesulfonic acid; GFP, green fluorescent protein; FACS, fluorescence-activated cell sorting.
by PCR using the primers 5′-GGCCCAGGTTGGAGGGCCCT- GGAGAGCC-3′ and 5′-AAGGAAGGGCGGCTTATTG- GATGGAAGCC-3′ and cloned into the HindIII and NotI sites of the pRe-CMV mammalian expression vector (Invitrogen). hZIP1 was epitope-tagged at its amino terminus with the cDNA copy of the hemagglutinin (HA) by PCR to generate CMV-HA-hZIP1. This fragment was digested with HindIII and XhoI, cloned into the pRe-CMV vector, and confirmed by sequencing.

Cell Culture Methods—K562 erythroleukemia cells (ATCC CCL-243) were grown in complete RPMI 1640 medium (Life Technologies, Inc.) supplemented with 1% glutamine and 10% fetal bovine serum (Sigma). Cells were cultured in 10-cm2 dishes, incubated in humidified 5% CO2 isolectric incubators, and transfected by electroporation (Invitrogen) using 3 × 107 cells and 20 µg of purified plasmid DNA unless noted otherwise. Stable transfected cell lines were generated by the limiting dilution method. The stable cell lines were subsequently maintained in 350 µg/ml G418. Cell numbers were determined with a hemocytometer, and cultures were examined weekly for mycoplasma contamination using Hoechst dye and fluorescence microscopy.

65Zn Uptake Assays and Atomic Absorption Spectroscopy—Cells were grown to 50% confluence, harvested by centrifugation, and washed once in cold uptake buffer (15 mM HEPES, 100 mM glucose, and 150 mM KCl, pH 7.0). The cells were resuspended in uptake buffer and incubated for 10 min in a shaking 37°C water bath. The cells were then mixed with an equal volume of prewarmed uptake buffer containing the specified concentration of 65ZnCl2 (Amersham Pharmacia Biotech) and incubated for 15 min unless indicated otherwise. Assays were stopped by adding an equal volume of ice-cold uptake buffer supplemented with 1 mM EDTA (stop buffer). Cells were collected by filtration on glass-fiber filters (Schleicher & Schuell) and washed three times in stop buffer (10 ml of total wash volume). Cell-associated radioactivity was measured with a Packard Auto-Gamma 5650 14-c counter. Chloride salt stock solutions (100 mM) of cadmium, copper, cobalt, magnesium, manganese, and nickel were prepared in distilled water. A ZnCl2 stock was prepared at 100 mM in 0.02 N HCl, and an FeCl3 stock was prepared at 50 mM in 0.1 N HCl. Sodium ascorbate (1 mM) was used to reduce Fe3+ to Fe2+ where indicated. Ascorbate treatment alone did not alter zinc uptake activity (data not shown). Sodium bicarbonate stocks (1M) were prepared fresh for use. For experiments with complete medium as the uptake buffer, the 65Zn was added to the medium and incubated at 20°C for 24 h before use to ensure equilibrium of the 65Zn with medium components. The statistical significance of differences in values was determined using STATVIEW software (Abacus Concepts, Inc., Berkeley, CA) and subjected to one-way analysis of variance followed by Scheffe’s test.

Characterization of the hZIP1 Zinc Transporter—To test the ability of hZIP1 to transport zinc, the hZIP1 ORF was cloned into the mammalian expression vector pRe-CMV, allowing expression from the CMV promoter. This plasmid was transfected into human K562 erythroleukemia cells, and stable G418-resistant clonal cell lines were isolated. The hZIP1-expressing transfectants (hereafter designated as CMV-hZIP1) and stable vector-only transfectants (CMV) were assayed for accumulation of 65Zn. These assays were performed in uptake buffer. As found previously (12), K562 cells had an endogenous zinc uptake activity when assayed under these conditions (Fig. 1A). Consistent with the ability of hZIP1 to transport zinc, CMV-hZIP1 cells accumulated almost 2-fold more 65Zn over a 60-min period than did the endogenous activity in CMV control cells. No zinc accumulation was seen in either cell type at 4°C, indicating that zinc accumulation by both systems was temperature-dependent and therefore likely to be transporter-mediated rather than due to cell-surface binding.

To determine if the higher zinc accumulation observed in CMV-hZIP1 cells was due to increased zinc influx rather than decreased zinc efflux, we assayed initial rates of 65Zn uptake over a shorter time period (0–60 s) (Fig. 1B). hZIP1-expressing cells accumulated zinc at an initial rate of ~6 pmol/min/106 cells, whereas in CMV transfectants, that rate was only 2
malian cells encounter agents. This buffer condition is very different from what mammalian cells can withstand previously (12), the endogenous zinc uptake activity in K562 cells is concentration-dependent and saturable. As we reported previously (12), the endogenous zinc uptake activity in K562 cells is concentration-dependent and saturable. This system shows Michaelis-Menten kinetics, with an apparent $K_m$ of 3.5 μM and a $V_{max}$ of 11 pmol/min/10⁶ cells (Fig. 1C). We determined the concentration dependence of hZIP1 activity by measuring the $^{65}$Zn uptake rate over a range of zinc concentrations in CMV-hZIP1 cells and subtracting the endogenous activity from those values. hZIP1-dependent uptake activity was also concentration-dependent and saturable. The apparent $K_m$ for $^{65}$Zn uptake in CMV-hZIP1 cells was 3 μM, and the $V_{max}$ was 23 pmol/min/10⁶ cells. Therefore, expression of hZIP1 in K562 cells caused an increase in $^{65}$Zn uptake activity that was time-, temperature-, and concentration-dependent and saturable. These results are consistent with our hypothesis that hZIP1 is a zinc transporter in human cells.

The experiments in Fig. 1 (A–C) indicate that hZIP1 is capable of transporting zinc in a buffer lacking any zinc-binding agents. This buffer condition is very different from what mammalian cells encounter in vivo. Although the concentration of total zinc in blood plasma is normally 10–20 μM, most of that zinc is bound to proteins such as albumin and α₂-macroglobulin. A reasonable estimate of the free Zn²⁺ concentration in plasma is ~0.2 nM (18), and the relatively high $K_m$ value of hZIP1 activity called into question the relevance of this transporter in zinc acquisition in vivo. Therefore, it was important to determine whether hZIP1 could function under more physiological conditions. To address this question, we first examined the effects of hZIP1 expression on zinc accumulation by cells grown in complete culture medium containing 10% fetal bovine serum. The total zinc content of this medium was determined by atomic absorption spectrophotometry to be 11 μM, and the albumin concentration was ~60 μM. Cell-associated zinc levels were measured in CMV and CMV-hZIP1 cells grown to 50% confluence in complete medium. When extracellular zinc was removed prior to analysis by washing the cells with PBS, CMV-hZIP1 cells were found to have accumulated ~60% more zinc than control cells (Fig. 1D). When PBS plus 1 mM EDTA was used as a more stringent wash buffer for the removal of surface-bound zinc, cell-associated zinc was reduced in both cell types. However, hZIP1-expressing cells consistently accumulated more zinc than control cells. This conclusion was also supported by the analysis of uptake rates using complete medium as the assay buffer. The zinc uptake rate in CMV-hZIP1 cells was ~2-fold higher than in controls (Fig. 1E). As expected, these rates were ~20–30-fold lower than in synthetic buffer, probably due to decreased substrate availability in medium containing zinc-binding agents such as albumin. This conclusion was supported by the inability to saturate the transport process in complete medium by adding zinc up to 60 μM (Fig. 1F). Taken together, these results indicate that hZIP1 can function as a zinc transporter under physiological conditions as simulated by complete medium.

If hZIP1 is a transporter protein involved in zinc uptake across the plasma membrane, it should be localized on the plasma membrane. However, a previous report (19) described preliminary studies localizing a GFP-hZIP1 fusion protein to an intracellular compartment. These studies lacked evidence that the fusion protein retained function, leaving open the possibility that the GFP moiety may disrupt the protein’s normal localization. To reexamine this question of hZIP1 localization...
It was intriguing that the \( K_m \) Cells—permeabilized cells (data not shown), indicating that the amino functional hZIP1 protein is located on the plasma membrane. Similar results were obtained with permeabilized and non-functional hZIP1 protein (Fig. 2, A and C). In contrast, cells expressing the tagged allele showed a bright rim of fluorescence at the cell periphery only (Fig. 2, B and D). These data demonstrated that functional hZIP1 protein is located on the plasma membrane. Similar results were obtained with permeabilized and non-permeabilized cells (data not shown), indicating that the amino terminus of the protein is extracellular, as predicted (12).

**hZIP1 is the Endogenous Zinc Uptake System in K562 Cells**—It was intriguing that the \( K_m \) of the endogenous zinc uptake system in K562 cells was similar to that observed for both hZIP1- and hZIP2-dependent activities (this report and Ref. 12). We showed previously that hZIP2 is not expressed in K562 cells and that its uptake activity is clearly distinguishable from the endogenous system (12). These observations suggested that perhaps hZIP1 is responsible for endogenous zinc uptake in K562 cells. To test this hypothesis, we assayed for hZIP1 mRNA expression in K562 cells by Northern blot analysis and found that it was expressed in these cells (Fig. 3A). Furthermore, cells expressing hZIP1 from the CMV promoter had ~2-fold more hZIP1 mRNA compared with controls. This 2-fold higher level of expression was also confirmed by RT-PCR (Fig. 4D) and was consistent with the 2-fold increase in zinc accumulation in CMV-hZIP1 cells (Fig. 1). As a further test, we determined if hZIP1 activity was distinguishable from the endogenous system. First, we compared the sensitivity of hZIP1, hZIP2, and the endogenous system to inhibition by other metals. Zinc uptake activity was measured in the absence or presence of a 2-fold molar excess of various divalent cations (Fig. 3B). Mg\(^{2+}\) had no effect on any system. Although hZIP2 was strongly inhibited by Co\(^{2+}\) and Mn\(^{2+}\), both hZIP1 and the endogenous system were unaffected. Similarly, Cu\(^{2+}\) and Fe\(^{3+}\) greatly inhibited hZIP2 activity, but had lesser effects on hZIP1 and the endogenous system. Although hZIP1 and the endogenous system were inhibited by Ni\(^{2+}\), hZIP2 activity was not altered. Thus, both hZIP1 and the endogenous uptake activity share remarkably similar profiles of inhibition by other metal ions that are distinct from those of hZIP2.

We demonstrated previously that HCO\(_3\) treatment stimulates hZIP2 uptake activity, but inhibits uptake by the endogenous system (12). Here, we found that hZIP1 and the endogenous system were not stimulated by HCO\(_3\) treatment and were inhibited to a similar degree (Fig. 3C). As a further comparison of hZIP1 and endogenous activities, we determined if zinc uptake by either system was energy-dependent. Treatment with several different electron transport/oxidative phosphorylation inhibitors (i.e. oligomycin, antimycin A, CN\(^{-}\), N\(_{3}\), rotenone, and cyanide 3-chlorophenylhydrazone) did not decrease zinc uptake by either system despite causing marked decreases in ATP levels (i.e. <10% of normal levels) (data not shown). Thus, hZIP1 and the endogenous uptake system are both energy-independent. The similar properties of hZIP1-dependent and endogenous zinc uptake activities strongly suggest that endogenous hZIP1 activity in K562 cells is responsible for zinc uptake in these cells.

As a further test of the role of hZIP1 as the endogenous zinc uptake system in K562 cells, we used antisense oligonucleotides to inhibit hZIP1 mRNA accumulation. When transfected into cells, antisense oligonucleotides can bind to target mRNA and stimulate its degradation by providing a substrate for RNase H (16). Six antisense oligonucleotides were designed to hybridize to regions in the 5’- and 3’-untranslated regions of hZIP1 mRNA (Table I). Five µg of each oligonucleotide was mixed with 10 µg of a GFP expression plasmid and transfected into K562 cells. After 24 h, successfully transfected GFP-ex-
pressing cells were isolated from the population by FACS. The efficiency of this sorting was aided by tagging the 5’-end of each oligonucleotide with fluorescein. Untransfected cells showed a peak below 10 fluorescence units (Fig. 4A). The profile of an unsorted population following transfection is shown in Fig. 4B. The sorting procedure enriched for transfected cells (gate M2) to almost 100% (data not shown). The transfected cells isolated in this manner were ≥80% viable as judged by their front and side light-scattering properties (Fig. 4C, gate R1). After sorting, the cells were cultured in complete medium for an additional 24 h prior to zinc uptake assays and mRNA analysis by RT-PCR.

Transfection with GFP alone had no effect on zinc uptake activity, nor did cotransfection with 5 μg of a control oligonucleotide of randomly chosen sequence (see C in Fig. 4D). Anti- hZIP1 oligonucleotides O2, O4, and O5 had no effect on zinc uptake activity or hZIP1 expression (data not shown). However, 5 μg of anti-hZIP1 oligonucleotide O1, O3, or O6 decreased zinc uptake by 30–40%. A mixture of 5 μg each of oligonucleotides O1, O3, and O6 caused a 90% decrease in zinc uptake. This effect was not due to nonspecific toxicity of the oligonucleotide treatment because 15 μg of the control oligonucleotide had no effect on zinc uptake activity. To determine if endogenous hZIP1 expression was inhibited by oligonucleotide treatment, total RNA was isolated from these cells and analyzed by quantitative RT-PCR. Neither 5 nor 15 μg of control oligonucleotide affected the hZIP1 mRNA level. Oligonucleotides O1, O3, and O6 and the mixture were all found to decrease hZIP1 mRNA levels, with no effect on a control mRNA, glyceraldehyde-3-phosphate dehydrogenase. Thus, oligonucleotides that inhibited accumulation of endogenous hZIP1 mRNA also inhibited zinc uptake activity. These results strongly suggest that hZIP1 is the endogenous zinc transporter in K562 cells.

Inhibition of zinc uptake by the mixture of oligonucleotides reduced zinc uptake activity to only 10% of normal levels. This result suggests that hZIP1 is the major zinc transporter in at least one mammalian cell type, K562 erythroleukemia cells. To determine what tissues may use hZIP1 as a zinc transporter in vivo, we assayed hZIP1 mRNA expression in various human tissues by semiquantitative RT-PCR. cDNAs prepared from a number of different tissues contained detectable levels of hZIP1 mRNA (Fig. 5). These included small intestine, kidney, liver, pancreas, and prostate, i.e., tissues known to be important in zinc metabolism. Thus, we conclude that hZIP1 is widely expressed in mammalian tissues. 10-Fold dilution of these cDNA samples caused hZIP1 mRNA to be undetectable in all tissue types under these conditions (data not shown). This result indicates that hZIP1 is expressed at similar levels in all of these tissues. The ubiquitous expression of hZIP1 in human tissues suggests that the hZIP1 protein plays an important housekeeping function in many cell types.

**DISCUSSION**

In a recent report, we used functional expression in K562 cells to characterize the biochemical properties of the hZIP2 zinc transporter (12). In the course of that study, we concluded that hZIP2 is not responsible for the endogenous zinc uptake activity in K562 cells. This conclusion was based on (a) the lack of detectable hZIP2 mRNA in K562 cells and (b) the clear differences in the biochemical properties of the hZIP2 and endogenous zinc transport systems. For example, HCO3- stimulated hZIP2 zinc uptake, but had no such effect on the endogenous system. In this study, we used a similar functional expression approach to demonstrate that, like hZIP2, hZIP1 encodes a zinc transporter. Consistent with our hypothesis, overexpression of hZIP1 in K562 cells led to an increase in zinc uptake activity, and the hZIP1 protein localized exclusively to the plasma membrane.

Perhaps of even greater significance is our demonstration that hZIP1 is the endogenous zinc transporter in K562 cells. This conclusion is based on several independent observations. First, we found that hZIP1 is normally expressed in these cells, and a 2-fold increase in mRNA level generated by expression from the CMV promoter correlated closely with a 2-fold increase in zinc uptake activity. Second, the endogenous uptake system and hZIP1 were indistinguishable in a number of different tests. For example, these systems have similar apparent $K_m$ values and are sensitive to inhibition by an array of metal ions to almost precisely the same degree. Finally, we found that inhibition of endogenous hZIP1 expression in K562 cells with antisense oligonucleotides also inhibited zinc uptake activity. Although antisense oligonucleotides have been reported to give artifactual results (16), our control experiments indicate that
the observation that iron can inhibit zinc uptake by these transporters. This hypothesis is supported by expression of hZIP1 or hZIP2 and found no effect. These results indicate that the mammalian members of the family are not functional in yeast, whereas hZIP2 was expressed in yeast (12), suggesting that the protein was produced, and there was no detectable increase in zinc uptake activity (data not shown). A similar lack of effect was observed when hZIP2 was expressed in yeast (12), suggesting that this protein plays a very specialized tissue-specific function. In marked contrast, hZIP1 is expressed in all 24 human tissues we examined. This observation, coupled with our results from K562 cells, suggests that hZIP1 may be the major endogenous zinc uptake transporter in many cells in the body. This conclusion is supported by Costello et al. (17), who previously provided evidence that hZIP1 is responsible for zinc uptake in prostate cells; treatment of those cells with prolactin and testosterone causes an increase in both hZIP1 mRNA levels and zinc uptake activity. Finally, Lioumi et al. (19) observed expression of hZIP1 mRNA in intestinal enterocytes. This location of expression suggests that hZIP1 may be involved in the uptake of dietary zinc from the intestine. It should be noted that hZIP1 was designated ZRT1 (for ZrT/IRT transporter-like) by these authors. In an earlier report, Costello et al. (17) named this gene hZIP1, and we have retained that nomenclature here.

One paradox that arose from our studies of hZIP1 and hZIP2 is that these transporters have a surprisingly low affinity for their substrate. Both transporters have a \( K_m \) value of \(-3 \mu M\) for free \( Zn^{2+} \) ions. Similar \( K_m \) values have been reported for zinc transporters in a large number of mammalian cell types (21). The paradox arises when we consider the free \( Zn^{2+} \) concentration in mammalian serum. Although the total zinc concentration of serum is \(-20 \mu M\), very little metal is present in an unbound form (18). In serum, \(-75\%\) \( Zn^{2+} \) is bound to albumin, and 20% is bound to \( \alpha_2 \)-macroglobulin. Much of the remaining zinc is complexed with amino acids such as histidine and cysteine. Because of the high chelation capacity of serum, the free \( Zn^{2+} \) concentration in serum is calculated to be in the low nanomolar range. Given this extremely low concentration of substrate, it was initially unclear how these transporters could contribute to zinc accumulation by mammalian cells under physiological conditions. The solution to this paradox comes

obtained when the properties of different transporters have been analyzed. In yeast, for example, both Zrt1 and Zrt2 are dependent on energy for zinc transport (1, 2). In contrast, neither hZIP1 nor hZIP2 (12) requires ATP for activity. This conclusion is based on the observation that metabolic inhibitors that reduce ATP levels to below 10% of normal levels (data not shown) had no effect on uptake activity of either of these proteins. We determined that zinc uptake by hZIP2 is stimulated by increased HCO\(_3^-\) levels, suggesting that zinc uptake occurs via a \( Zn^{2+}/HCO_3^- \) symport mechanism (12). In contrast, hZIP1 activity was not affected by HCO\(_3^-\) levels in our experiments here, suggesting that this protein may use a different transport mechanism. Alternatively, sufficient levels of HCO\(_3^-\) may already be present in our standard assay conditions, through equilibration with atmospheric CO\(_2\), to saturate the hZIP1 transporter. Thus, it remains unresolved if hZIP1 and hZIP2 use the same or different transport mechanisms.

An important unanswered question is what role these proteins play in zinc transport in vivo. hZIP2 expression has been detected only in prostate (12) and uterine (20) epithelial cells, suggesting that this protein plays a very specialized tissue-specific function. In marked contrast, hZIP1 is expressed in all 24 human tissues we examined. This observation, coupled with our results from K562 cells, suggests that hZIP1 may be the major endogenous zinc uptake transporter in many cells in the body. This conclusion is supported by Costello et al. (17), who previously provided evidence that hZIP1 is responsible for zinc uptake in prostate cells; treatment of those cells with prolactin and testosterone causes an increase in both hZIP1 mRNA levels and zinc uptake activity. Finally, Lioumi et al. (19) observed expression of hZIP1 mRNA in intestinal enterocytes. This location of expression suggests that hZIP1 may be involved in the uptake of dietary zinc from the intestine. It should be noted that hZIP1 was designated ZRT1 (for ZrT/IRT transporter-like) by these authors. In an earlier report, Costello et al. (17) named this gene hZIP1, and we have retained that nomenclature here.

One paradox that arose from our studies of hZIP1 and hZIP2 is that these transporters have a surprisingly low affinity for their substrate. Both transporters have a \( K_m \) value of \(-3 \mu M\) for free \( Zn^{2+} \) ions. Similar \( K_m \) values have been reported for zinc transporters in a large number of mammalian cell types (21). The paradox arises when we consider the free \( Zn^{2+} \) concentration in mammalian serum. Although the total zinc concentration of serum is \(-20 \mu M\), very little metal is present in an unbound form (18). In serum, \(-75\%\) \( Zn^{2+} \) is bound to albumin, and 20% is bound to \( \alpha_2 \)-macroglobulin. Much of the remaining zinc is complexed with amino acids such as histidine and cysteine. Because of the high chelation capacity of serum, the free \( Zn^{2+} \) concentration in serum is calculated to be in the low nanomolar range. Given this extremely low concentration of substrate, it was initially unclear how these transporters could contribute to zinc accumulation by mammalian cells under physiological conditions. The solution to this paradox comes.

---

**Table I**

| Oligonucleotide | Sequence (5’ → 3’) | Position relative to ORF |
|-----------------|-------------------|-------------------------|
| O1              | C*T*C*GAGCTCG*C*G*A | −39 → −26 (5’-UTR)     |
| O2              | T*A*G*CCCTGAC*C*T*T | −23 → −12 (5’-UTR)     |
| O3              | C*C*C*ATGATC*C*T*T | −7 → +5 (ATG)          |
| O4              | C*C*C*TAGATTC*C*G*A | +963 → +978 (UAG)      |
| O5              | C*T*C*TACTCT*C*A*G | +1192 → +1112 (3’-UTR)|
| O6              | A*T*T*TATCTG*C*A*G | +1122 → +1135 (3’-UTR)|
| C               | T*C*G*ATCGA*C*A*G | NA                      |

---

2 L. A. Gaither, unpublished observation.
from considering the capacity of these transporters relative to the zinc requirements of the cell. Steady-state cell accumulation of zinc is ~100 pmol/10^6 cells (Fig. 1D), which is equivalent to 1 x 10^8 atoms of zinc/cell. This value is similar to those obtained by others (22). With a doubling time of 24 h, the uptake rate required to maintain this level of zinc in growing cells is ~0.1 pmol/min/10^6 cells, i.e., a value almost identical to the uptake rate observed using complete medium as the assay buffer (Fig. 1E) and far lower than the rate (11 pmol/min/10^6 cells) measured in buffer (Fig. 1C). Thus, our studies demonstrated that the capacity (i.e., V_max) for zinc uptake is so high relative to the cellular demand for zinc that sufficient levels can be obtained despite the chelation capacity of serum and the apparent low affinity of the transporters.

With the characterization of hZIP1 and hZIP2, our understanding of zinc homeostasis in mammalian cells is greatly improving. A second family of zinc transporters has also been identified in mammals. This family is called the CDF (cation diffusion facilitator) family, and like the ZIP proteins, members of this group have been implicated in zinc transport in organisms of all phylogenetic levels (10, 23). One mammalian CDF protein, ZnT-1, is a zinc efflux protein that transports zinc out of the cell (22). ZnT-1 may play a role in removing excess zinc from cells and may also serve to transport zinc across the basolateral membrane of the intestinal enterocyte during zinc absorption (24, 25). A second member of the CDF family, ZnT-2, compartmentalizes intracellular zinc in the late endosome of the cell (26, 27). This zinc sequestration reduces the toxicity of intracellular zinc. Cellular zinc status is likely to be controlled by regulation of many of these transporters. Expression of both ZnT-1 and ZnT-2 has been shown to be induced in zinc-treated cells (28) or in animals fed zinc-rich diets (24, 29). The zinc-responsive transcription factor MTF-1 was found to regulate ZnT-1 (28), and it seems likely that MTF-1 also regulates ZnT-2 expression. Thus, zinc treatment increases the cell’s capacity to both export and sequester excess zinc. It is not yet clear if the uptake transporters are also regulated in response to zinc status. Many ZIP genes in yeast and plants are expressed at higher levels under zinc-limiting conditions (1, 7, 9). In yeast, this up-regulation was shown to occur at a transcriptional level and is mediated by the zinc-responsive Zap1 transcription factor (30). It is therefore intriguing that hZIP1 mRNA levels decrease in prostate-derived PC-3 cells treated with higher than normal levels of zinc (17). We are currently examining the regulation of hZIP1 and hZIP2 in response to zinc availability to determine if such a mechanism contributes to mammalian zinc homeostasis. Furthermore, the potential roles of the other human ZIP genes remain to be addressed.

Acknowledgments—We thank Louise Barnett, Jessica Wagner, and Jon Broomhead for technical assistance and the members of the Eide laboratory for many helpful discussions.

REFERENCES
1. Zhao, H., and Eide, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2454–2458
2. Zhao, H., and Eide, D. (1996) J. Biol. Chem. 271, 32938–32940
3. Eide, D., Broderius, M., Fett, J., and Guerinot, M. L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5624–5628
4. Korshunova, Y. O., Eide, D., Clark, W. G., Guerinot, M. L., and Pakrasi, H. B. (1999) Plant Mol. Biol. 40, 57–44
5. Rogers, E. E., Eide, D. J., and Guerinot, M. L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12356–12360
6. Guerinot, M. L. (2000) Biochim. Biophys. Acta 1465, 190–198
7. Grotz, N., Fox, T., Connolly, E., Park, W., Guerinot, M. L., and Eide, D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7220–7224
8. Pence, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L., Lasat, M. M., Garvin, D. F., Eide, D., and Kochian, L. V. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4956–4960
9. MacDiarmid, C. W., Gaither, L. A., and Eide, D. (2000) EMBO J. 19, 2845–2855
10. Gaither, L. A., and Eide, D. J. (2001) Biometals, in press
11. Eng, B. H., Guerinot, M. L., Eide, D., and Saier, M. H. (1998) J. Membr. Biol. 166, 1–7
12. Gaither, L. A., and Eide, D. J. (2000) J. Biol. Chem. 275, 5560–5564
13. Cleland, W. W. (1979) Methods Enzymol. 43, 103–138
14. Feinberg, A. P., and Vogelstein, B. (1984) Anal. Biochem. 137, 266–267
15. Zucker, M., Matthews, D. H., and Turner, D. H. (1998) in RNA Biochemistry and Bio/Technology (Barciszewski, J., and Clark, B. F. C., eds) Kluwer Academic Publishers, Norwell, MA
16. Branch, A. D. (1998) Trends Biochem. Sci. 23, 45–50
17. Costello, L. C., Liu, Y., Zou, J., and Franklin, R. B. (1999) J. Biol. Chem. 274, 17499–17504
18. Magnuson, G. R., Puvathingal, J. M., and Ray, W. J. (1987) J. Biol. Chem. 262, 11140–11148
19. Lioumi, M., Ferguson, C. A., Sharpe, P. T., Freeman, T., Marenholz, I., Mischke, D., Heizmann, C., and Ragoussis, J. (1999) Genomics 62, 272–280
20. Yamaguchi, S. (1995) Kobunshi Gakkaishi Zasshi 62, 78–93
21. Reyes, J. G. (1996) J. Biol. Chem. 271, C401–C410
22. Palmiter, R. D., and Findley, S. D. (1995) EMBO J. 14, 639–649
23. Paulsen, I. T., and Saier, M. H. (1997) J. Membr. Biol. 156, 99–103
24. McMahon, R. J., and Cousins, R. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 4841–4846
25. McMahon, R. J., and Cousins, R. J. (1998) J. Nutr. 128, 667–670
26. Palmiter, R. D., Cole, T. B., and Findley, S. D. (1996) EMBO J. 15, 1784–1791
27. Kobayashi, T., Beuchat, M., Lindsay, M., Frias, S., Palmiter, R. D., Sakuraba, H., Parton, R. G., and Gruneberg, J. (1999) Nat. Cell Biol. 1, 113–118
28. Langmade, S. J., Ravindra, R., Daniels, P. J., and Andrews, G. K. (2000) J. Biol. Chem. 275, 34803–34809
29. Liuzzi, J. P., Blanchard, R. K., and Cousins, R. J. (2001) J. Nutr. 131, 46–52
30. Zhao, H., and Eide, D. J. (1997) Mol. Cell. Biol. 17, 5044–5052

hZIP1 Mediates Zinc Uptake in Human Cells
The Human ZIP1 Transporter Mediates Zinc Uptake in Human K562 Erythroleukemia Cells
L. Alex Gaither and David J. Eide

J. Biol. Chem. 2001, 276:22258-22264.
doi: 10.1074/jbc.M101772200 originally published online April 11, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M101772200

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 28 references, 15 of which can be accessed free at http://www.jbc.org/content/276/25/22258.full.html#ref-list-1