While molecular mechanisms for iron entry and storage within cells have been elucidated, no system to mediate iron efflux has been heretofore identified. We now describe an ATP requiring iron transporter in mammalian cells. $^{55}$Fe is transported into microsomal vesicles in a Mg-ATP-dependent fashion. The transporter is specific for ferrous iron, is temperature- and time-dependent, and detected only with hydrolyzable nucleotides. It differs from all known ATPases and appears to be a P-type ATPase. The Fe-ATPase is localized together with heme oxygenase-1 to microsomal membranes with both proteins greatly enriched in the spleen. Iron treatment markedly induces ATP-dependent iron transport in RAW 264.7 macrophage cells with an initial phase that is resistant to cycloheximide and actinomycin D and a later phase that is inhibited by these agents. Iron release, elicited in intact rats by glycerol-induced rhabdomyolysis, induces ATP-dependent iron transport in the kidney. Mice with genomic deletion of heme oxygenase-1 have selective tissue iron accumulation and display augmented ATP-dependent iron transport in those tissues that accumulate iron.

Living cells require iron for many critical biological functions, including cellular respiration and DNA synthesis. Iron’s physiologic importance derives from its ability to exist in multiple valence states and therefore participate in reactions requiring single electron transfers. Pathophysiologically, electron transfer by iron can generate toxic free radicals, such as the hydroxyl radical (1, 2). Various mechanisms regulate cellular iron to ensure adequate supplies for cellular physiology while avoiding toxic iron excess. Many of the molecular details for iron accumulation and storage in cells are known. Iron circulates in the blood in the ferric form (Fe$^{3+}$) bound tightly to transferrin (3, 4). In most physiologic situations, iron enters cells upon the binding of diferric transferrin to the transferrin receptor followed by receptor-mediated endocytosis. Acidification of the lumen of the endocytic vesicle by the H$^{+}$-ATPase releases iron from transferrin, whereupon iron is transported into the cytoplasm by the divalent cation transporter (5). Excess cellular iron can be stored by ferritin (4). Transferrin receptor and ferritin levels are reciprocally controlled by iron regulatory proteins (IRPs)$^1$ that bind to specific sequences known as iron regulatory elements (IREs) in the mRNAs of these proteins (3, 4, 6, 7).

In contrast, molecular details of a cellular iron efflux pathway are lacking, even though physiologic evidence indicates that bodily stores of iron are conserved and re-utilized. Thus, the human daily dietary requirement for iron is only 1 mg, despite evidence that tissues mobilize 20–30 times that amount to support red blood cell synthesis by the bone marrow (8). Iron is released from hemoglobin and other heme proteins by heme oxygenase-1 (HO1), an inducible enzyme associated with the endoplasmic reticulum, which cleaves the heme ring giving rise to biliverdin, which is rapidly reduced to bilirubin, carbon monoxide, and ferrous iron (9). In mice with targeted genenic deletion of HO1, tissue stores of iron are elevated, while serum iron levels are low (10, 11). Recently, we demonstrated that expression of HO1 is linked to cellular iron efflux, demonstrating a role for HO1 in cellular iron mobilization (12). Since HO1 is an enzyme that catalyzes the breakdown of cytosolic heme but is not a transport protein, the mechanism by which HO1 activity is linked to cellular iron mobilization remains obscure. We reasoned that a membrane-associated transporter, like the calcium ATPase, might be the molecular link between cytosolic heme catabolism by HO1 and cellular iron release. We now report the identification and characterization of an Fe-ATPase associated with microsomal membranes that is co-distributed in tissues with HO1. Iron induces ATP-dependent iron transport in a macrophage cell line and in $^{1/2}$HO1 mice, and glycerol-induced rhabdomyolysis induces ATP-dependent iron transport in the kidney.

**EXPERIMENTAL PROCEDURES**

**Materials**—$^{55}$FeCl$_3$ (39.7 Ci/g) was obtained from NEN Life Science Products and used without altering the specific activity. Unless otherwise indicated all other chemicals were from Sigma.

**Microsome Preparation**—For routine isolation of microsomes all manipulations were performed at 4 °C as follows. Tissues or cell cultures were harvested and homogenized with 15 strokes of a Dounce-Teflon homogenizer in five volumes of ice-cold homogenization buffer (0.28 M sucrose, 20 mM Hepes (pH 7.5), 2 mM ß-mercaptoethanol, protease inhibitors). The homogenate was centrifuged at 20,000 × g for 15 min to remove intact cells, nuclei, mitochondria, and other debris. The resulting supernatant was then centrifuged at 200,000 × g for 45 min to isolate the microsomal fraction. This second pellet was resuspended in homogenization buffer at a concentration of approximately 20 mg/ml protein, and samples were stored at −80 °C for up to one month prior to use.

**Differential Centrifugation**—Spleen tissue was homogenized as described above, and the homogenate was centrifuged at 1000 × g for 20

---

$^*$ This work was supported by United States Public Health Service Grant MH-18501 and Research Scientist Awards DA-00074 (to S. H. S.) and DA-05900 (to D. E. B.). The costs of publication of this article were stored by ferritin (4). Transferrin receptor and ferritin levels are reciprocally controlled by iron regulatory proteins (IRPs)$^1$ that bind to specific sequences known as iron regulatory elements (IREs) in the mRNAs of these proteins (3, 4, 6, 7).

$^1$ The abbreviations used are: IRP, iron regulatory protein; IRE, iron regulatory element; HO1, heme oxygenase-1; HO2, heme oxygenase-2; AD, actinomycin D; CHX, cycloheximide; ER, endoplasmic reticulum; ATP-ßS, adenosine 5′-(thiotriphosphate); AMP-PCP, adenosine 5′-(β,γ-methylene)triphosphate; AMP-PNP, adenosine 5′-(β,γ-iminooctophosphate).
ATP-dependent Iron Transport Assay— For routine experiments, microsomes were thawed on ice, aliquoted (10–100 μg of protein) into reaction buffer (140 mM potassium gluconate, 40 mM Hepes (pH 7.5), 2 mM ascorbate, 5 mM MgSO4, 4 mM Na4ATP, and 5 μM 55FeCl3) in a final volume of 115 μl and incubated at 30 °C. At the indicated times, 100 μl of this reaction mixture was rapidly filtered (vacuum manifold, 0.45-μm filter, Millipore) and washed under continuous vacuum with 25 ml of ice-cold wash buffer (140 mM potassium gluconate, 20 mM Hepes (pH 7.5), and 5 mM ATP) and stored (−80 °C). Marker enzymes for specific subcellular organelles were assayed as described previously (13).

**Experimental Procedures**—HO1 and heme oxygenase-2 (HO2) expression were analyzed by Western blot analysis using antibodies developed

![Figure 1](https://example.com/figure1.png)

**FIG. 1.** Transport of 55Fe in microsomes is time (A)- and temperature (B)-dependent. A, time dependence of iron accumulation in spleen microsomes, (○, −ATP; △, +4 mM ATP). Microsomes were prepared as described under “Experimental Procedures” and incubated in the presence of 55Fe for the indicated times. Then, microsomes were collected by rapid filtration and the associated 55Fe determined by liquid scintillation spectrometry. B, temperature dependence of iron accumulation. Temperature dependence of iron accumulation was measured after 20 min of incubation using spleen microsomes as in A and as described under “Experimental Procedures.” For both A and B the data shown are the means of triplicate determinations with S.E. as indicated by the error bars. In some cases, the error bars are contained within the symbols. The experiment was repeated at least five times with similar results.

<table>
<thead>
<tr>
<th>Substrate (4 mM)</th>
<th>Transport (% ATP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>100 ± 4.3</td>
</tr>
<tr>
<td>GTP</td>
<td>21.3 ± 1.2</td>
</tr>
<tr>
<td>CTP</td>
<td>51.8 ± 4.0</td>
</tr>
<tr>
<td>TTP</td>
<td>29.3 ± 4.2</td>
</tr>
<tr>
<td>ITP</td>
<td>45.4 ± 0.8</td>
</tr>
<tr>
<td>AMP-PNP</td>
<td>3.1 ± 1.7</td>
</tr>
<tr>
<td>AMP-PCP</td>
<td>1.2 ± 1.5</td>
</tr>
<tr>
<td>ATP-γS</td>
<td>7.1 ± 3.3</td>
</tr>
</tbody>
</table>

*Table I*

**Nucleotide specificity of 55Fe accumulation in spleen microsomes**

Spleen microsomes were prepared as described under “Experimental Procedures” and incubated for 20 min at 30 °C with 4 mM amounts of each of the nucleotides or nucleotide analogs indicated. Poorly hydrolyzable or nonhydrolyzable analogues such as AMP-PNP, AMP-PCP, and ATP-γS do not support 55Fe accumulation. The results shown are the means of triplicate determinations ± S.E. as indicated. This experiment has been repeated two times with similar results.
in our laboratory. Specific polyclonal antibodies to HO1 and HO2 were generated using recombinant proteins, prepared in Escherichia coli, as antigens. Briefly, cDNAs for human HO1 and HO2 were obtained by polymerase chain reaction using human liver cDNA (CLONTECH) as template. Then, human HO1 and HO2 cDNAs were subcloned into pGEX4T2 (Amersham Pharmacia Biotech), and the sequence was confirmed. Next, glutathione S-transferase-HO1 and glutathione S-transferase-HO2 were obtained following transformation of competent E. coli and purification of the fusion proteins using GSH-Sepharose (Amersham Pharmacia Biotech). The purified antigens were provided for injection into rabbits (Covance, Denver, PA), and the resulting sera were analyzed for specific antibodies. For routine studies, protein samples were fractionated using standard SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Fol-
lowing incubation in blocking buffer (PBS, 0.1% Tween, 5% nonfat dry milk), blots were incubated in blocking buffer supplemented with either anti-HO1 (1:2500) or anti-HO2 (1:10,000) for 1–18 h. After washing, the blots were incubated with secondary antibody (goat anti-rabbit IgG, 1:5000, Amersham Pharmacia Biotech) for 1 h and developed using chemiluminescence (Renaissance, NEN Life Science Products). Antibody specificity was confirmed by determining the molecular weight of candidate immunoreactive bands and examining tissues derived from mice with genomic deletions of HO1 and HO2 (11, 15). Specific antisera for HO1 and HO2 were obtained without evidence of cross-reacting proteins (data not shown).

RESULTS

Identification and Characterization of an Fe-ATPase—To identify a possible iron transporter, we employed \(^{55}\text{Fe}\) in attempts to measure ATP-dependent transport of iron into microsomal fractions from rat spleen. In initial experiments, we used rapid filtration onto nitrocellulose filters to monitor the \(^{55}\text{Fe}\) associated with microsomal fractions following various incubation times (see “Experimental Procedures”). These initial experiments were conducted in buffer containing magnesium and potassium chloride, conditions similar to those used for monitoring \(^{45}\text{Ca}^{2+}\) accumulation (17, 18). Under these conditions, nearly 80% of added \(^{55}\text{Fe}\) was trapped on the filters, and the addition of ATP did not increase apparent \(^{55}\text{Fe}\) accumulation. \(^{55}\text{FeCl}_3\) is poorly soluble and, in the presence of oxygen, is rapidly converted to Fe(OH)\(_3\), which forms large insoluble complexes (3, 19). Thus, we reasoned that, in the presence of KCl, the \(^{55}\text{Fe}\) associated with the filters may represent nonspecific iron precipitates, perhaps Fe(OH)\(_3\) complexes. Accordingly, we replaced KCl with potassium gluconate to avoid iron precipitation. In potassium gluconate buffer, \(^{55}\text{Fe}\) associated with the filters was dramatically reduced, indicating that iron did not precipitate. Yet, ATP failed to augment \(^{55}\text{Fe}\) accumulation. Since ferrous iron (Fe\(^{2+}\)) is the intracellular form of iron in cells (3, 20), we reasoned that a microsomal Fe-ATPase may require iron in the reduced form. Thus, we added ascorbate to the assay buffer, which reduces iron to the ferrous form. Under these conditions ATP stimulates apparent \(^{55}\text{Fe}\) transport. Since ATP requiring transporters often use ATP complexed with magnesium ion as a substrate, Mg\(^{2+}\) was included for these initial measurements. Omitting Mg\(^{2+}\) eliminates the effect of ATP on \(^{55}\text{Fe}\) accumulation suggesting that Mg-ATP is required for apparent iron transport.

Conceivably, ATP may increase \(^{55}\text{Fe}\) binding to proteins rather than stimulate transmembrane \(^{55}\text{Fe}\) transport. However, ATP failed to increase \(^{55}\text{Fe}\) associated with cytoplasmic protein fractions or Triton X-100-solubilized microsomal membrane protein preparations (data not shown). If the apparent ATP-dependent \(^{55}\text{Fe}\) accumulation is due to an ATP-dependent transporter, then hydrolyzable nucleotide triphosphates should support iron accumulation, while nonhydrolyzable analogues should not. ATP, AMP, CTP, ITP, and GTP partially support iron accumulation (Table I). If the \(^{55}\text{Fe}\) accumulation reflects a membrane transporter rather than ATP-dependent \(^{55}\text{Fe}\) binding, it should have time and temperature dependence. \(^{55}\text{Fe}\) transport is time-dependent, with linear \(^{55}\text{Fe}\) accumulation for about 45 min at 30 °C (Fig. 1A). \(^{55}\text{Fe}\) transport is also temperature-dependent with maximal activity at 37 °C and a \(Q_{10}\) of 2.8 between 20 and 30 °C (Fig. 1B). Thus, the ATP-dependent \(^{55}\text{Fe}\) accumulation in microsomes does not simply reflect ATP-mediated binding of \(^{55}\text{Fe}\) to proteins. The requirement for hydrolyzable high energy phosphate bonds, and the magnesium, time and temperature.
dependence, suggest that an ATPase mediates $^{55}\text{Fe}$ transport across microsomal membranes.

As mentioned, in initial experiments, we found that $^{55}\text{Fe}$ transport is dependent upon ascorbate. We characterized the ascorbate requirement of $^{55}\text{Fe}$ transport and found maximal activity at 4 mM ascorbate (Fig. 2A). In addition, we examined the ATP and iron dependence. $^{55}\text{Fe}$ transport occurs at physiologic concentrations of ATP with maximal stimulation at 4 mM ATP and a decline in apparent transport at higher concentrations (Fig. 2B). $^{55}\text{Fe}$ transport is saturable with half-maximal transport at about 10 $\mu\text{M}$ iron (Fig. 2C). To evaluate whether $^{55}\text{Fe}$ transport activity is specific, we examined a variety of cations (Fig. 3). Minimal effects are observed with 0.1 mM barium, cadmium, calcium, cobalt, or manganese. Zinc ions produce 23% inhibition, while copper(II) inhibits transport about 70%.

To determine whether the $^{55}\text{Fe}$ transport reflects the activity of known ATPases, we evaluated a variety of ATPase-specific pharmacological agents (Table II). Thapsigargin, a potent inhibitor of microsomal Ca$^{2+}$-ATPases, fails to block $^{55}\text{Fe}$ transport. Bafilomycin, a selective high affinity inhibitor of the V-type ATPases, is similarly ineffective. Oligomycin and ouabain, inhibitors of the mitochondrial proton ATPase and the plasma membrane Na$^{+}$/K$^{+}$-ATPase, respectively, are also inactive. Orthovanadate, a known inhibitor of P-type ATPases, reduces $^{55}\text{Fe}$ transport by 65%. Thus, the iron transport activity appears to represent a novel ATP-dependent transport process with the properties of a P-type ATPase.

**Localization of the Fe-ATPase**—
Most of heme and iron turnover occurs in the reticuloendothelial system, primarily the macrophages of the spleen, where senescent red blood cells are phagocytosed, and the iron from the heme in hemoglobin is freed for re-utilization (3). We monitored $^{55}\text{Fe}$ transport in

![Fig. 5](http://www.jbc.org/) Iron-mediated induction of ATP-dependent iron transport in RAW 264.7 cells is biphasic (A) with inhibition of the late phase induction by cycloheximide and actinomycin D (B). A, time dependence of Fe-ATPase induction by FeSO$_4$. RAW 264.7 cells were cultured as described under “Experimental Procedures” and incubated with 0.5 mM FeSO$_4$ for the indicated times prior to preparation of microsomes from the cells. B, inhibition of iron-mediated induction of Fe-ATPase by cycloheximide (CHX) and actinomycin D (AD). RAW 264.7 cells were incubated with 0.5 mM FeSO$_4$ for 2 or 20 h as indicated. To examine the rapid phase induction, CHX (100 $\mu$g/ml) and AD (1 $\mu$g/ml) were added 30 min prior to the addition of FeSO$_4$ and cells were harvested after 2 h of iron treatment. To study the late phase induction AD was added 30 min prior to the addition of iron, while CHX was withheld due to its toxicity and added 16 h after initiating incubation with 0.5 mM FeSO$_4$ prior to harvesting the cells after 20 h of iron treatment. The data shown are the means of duplicate or triplicate determinations with standard error as indicated by the error bars. This experiment has been repeated two times with similar results.

**Table II**

Effects of pharmacological inhibitors of ATPases on ATP-dependent iron transport

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Control activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thapsigargin (1 $\mu$M)</td>
<td>93.7 ± 2.1</td>
</tr>
<tr>
<td>Bafilomycin (0.2 $\mu$M)</td>
<td>94.1 ± 3.8</td>
</tr>
<tr>
<td>Ouabain (1 $\mu$M)</td>
<td>90.3 ± 4.1</td>
</tr>
<tr>
<td>Oligomycin (1 $\mu$M)</td>
<td>104 ± 5.2</td>
</tr>
<tr>
<td>Orthovanadate (100 $\mu$M)</td>
<td>35.1 ± 2.3</td>
</tr>
</tbody>
</table>
microsomal fractions from a wide range of rat tissues (Fig. 4). ATP-dependent iron transport is highest in the spleen, almost 20 times higher than levels in any of the other tissues examined. Levels of ATP-dependent iron transport are similar in brain, lung, kidney, heart, intestine, testes, and liver. The high level of $^{55}$Fe transport in the spleen is paralleled by a comparable enrichment of HO1 protein (Fig. 4). In contrast, HO2 is distributed much differently with selective enrichment in the brain and testes (data not shown).

Since HO1 and ATP-dependent iron transport are co-distributed in tissues, we wondered whether they would have a similar subcellular localization. We conducted limited subcellular fractionation of spleen tissue (Table III). The microsome-enriched fraction (P3) contains $>$75% of total $^{55}$Fe transport activity. NADPH cytochrome c reductase activity, a marker for endoplasmic reticulum, is similarly enriched in the microsomal fraction (P3). Alkaline phosphatase, a enzyme marker for plasma membranes, is also enriched in the microsomal fraction, although the distribution of the ATP-dependent iron transport more closely matches that of NADPH cytochrome C reductase. The nuclear fraction (P1) contains $>$95% of detectable DNA, yet displays undetectable iron transport. As reported previously (21, 22), HO1 protein is comparably enriched in the microsomal fraction. The mitochondrial protein, cytochrome C oxidase, is concentrated 4–5-fold in P2. The proportion of ATP-dependent iron transport in P2 is similar to that of the endoplasmic reticulum marker (NADPH cytochrome c reductase). These findings are consistent with a localization of
the Fe-ATPase in endoplasmic reticulum membranes, together with HO1.

Fe-ATPase Induction by Iron Treatment—The catabolism of hemoglobin heme by HO1 to liberate iron in the spleen takes place in macrophages, which express HO1 whose activity is induced in response to heme or erythrocytes (23, 24). Accordingly, we examined $^{59}$Fe transport in RAW 264.7 cells, a mouse-derived macrophage cell line. Saturable, ATP-dependent, iron transport is readily demonstrable in microsomal fractions of these cells (data not shown). Incubating these cells overnight with 0.5 mM FeSO$_4$ elicits a 10-fold increase in iron transport (Fig. 5A), while treatment with 0.1 mM FeSO$_4$ produces a significant but smaller increase (data not shown). The augmented iron transport does not reflect an immediate effect of iron, as incubation of the cells with 0.5 mM iron just prior to homogenization has no effect on iron transport (data not shown). We examined the time dependence for induction of the ATP-dependent iron transport by exogenous iron. We observe a biphasic induction with a 2-fold increase in the first 1–2 h, followed by a plateau, and then a delayed and rapid five-fold induction after 18 h of iron treatment. To address the possible mechanism of iron’s induction of ATP-dependent iron transport in RAW 264.7 cells, we examined the effects of transcriptional and translational inhibitors. Inhibition of transcription with actinomycin D (AD), or translation with cycloheximide (CHX), does not block the initial 2-fold induction seen after 1–2 h of iron treatment (Fig. 5B). However, AD and CHX do prevent the delayed induction, reducing the observed iron transport to levels similar to that seen after 2 h of iron treatment (Fig. 5B).

To ascertain the influence of increased iron in intact animals on the ATP-dependent iron transport, we utilized HO1$^{-/-}$ mice in which accumulation of non-heme iron has been demonstrated (11, 12). ATP-dependent iron transport in kidney microsomes from 40-week-old HO1$^{-/-}$ mice is increased 5–8-fold compared with levels in heterozygotes that are similar to wild-type animals (Fig. 6A). In liver microsomes from 40-week-old HO1$^{-/-}$ mice, the ATP-dependent iron transport is increased >10-fold. The elevated ATP-dependent iron transport is likely due to increased tissue iron levels, since we do not detect an increase in iron transport in microsomes from the kidney and liver of young (10-week-old) HO1$^{-/-}$ mice whose tissue iron levels are not yet elevated (11) (data not shown). ATP-dependent iron transport in brain microsomes is not increased even in 40-week-old HO1$^{-/-}$ animals, presumably because iron does not accumulate in the brains of the mutant mice (11) (Fig. 6A). In contrast to the marked augmentation of ATP-dependent iron transport in kidney and liver of HO1$^{-/-}$ animals, microsomes from the spleen of HO1$^{-/-}$ mice display a >75% decline in $^{59}$Fe transport compared with heterozygote and wild-type specimens (Fig. 6A).

We reasoned that directly elevating cellular iron levels by increasing heme oxygenase activity in tissues might also induce ATP-dependent iron transport. Injection of glyceral into skeletal muscles of rodents causes rhodamine-123 labeling of the labile or intermediate iron pool (3) before being transported to the lumenal side of the ER by the Fe-ATPase. This fraction of the ER may also include transferrin and transferrin receptors as they are being recycled through the ER compartment back to the plasma membrane through exocytosis. Conceivably, within the lumen of the ER, Fe$^{3+}$ may also be oxidized to Fe$^{2+}$ bind transferrin, and be returned to the extracellular fluid with transferrin following exocytosis.

FIG. 7. ATP-dependent iron transport and HO1 are functionally coupled. HO1 is present on the endoplasmic reticulum (ER) membrane, where in conjunction with cytochrome P450 reductase (CPR) and biliverdin reductase (BVR), it catabolizes heme producing bilirubin, carbon monoxide, and Fe$^{2+}$. Released iron is transiently part of the labile or intermediate iron pool (3) before being transported to the luminal side of the ER by the Fe-ATPase. This fraction of the ER may also include transferrin and transferrin receptors as they are being recycled through the ER compartment back to the plasma membrane through exocytosis. Conceivably, within the lumen of the ER, Fe$^{2+}$ may also be oxidized to Fe$^{3+}$ bind transferrin, and be returned to the extracellular fluid with transferrin following exocytosis.

DISCUSSION

In the present study we report the identification and characterization of an Fe-ATPase in mammalian tissues. The iron transport we observe appears to be mediated by an Fe-ATPase, since it is dependent upon hydrolyzable nucleotide triphosphates, magnesium, time, and temperature. Inhibition by orthovanadate suggests that the Fe-ATPase may be a P-type ATPase, mediating transmembrane transport through a mechanism that involves transient phosphorylation of an aspartate residue (26–28). The Fe-ATPase we describe appears to be a novel entity, as its activity is not affected by specific inhibitors of the other known ATPases. Conceivably, the ATP-dependent iron transport may reflect transport mediated by a copper ATPase encoded by the Wilson’s or Menke’s disease genes (29). However, neither of these genes are significantly expressed in the spleen (30, 31). Meneghini and associates (32) have described nuclear transport of iron in the liver that differs from the Fe-ATPase described here. The transporter they identified utilizes the ferric form of iron (32), which does not support the ATP-dependent iron transport described in this report. Moreover, under our experimental conditions, we do not detect any iron transport in nuclear fractions. Thus, the Fe-ATPase described herein represents a new mammalian transport mechanism.

The total transport activity of the Fe-ATPase is less than that of other P-type ATPases. We measured about 10 pmol/min/mg protein in the spleen, whereas the Ca-ATPase is substantially more active with activities of 115 protein and 234 nmol/min/mg protein in platelet and cerebellar membranes respectively (33). The copper ATPases are the only other metal transporting P-type ATPases described in mammals and direct biochemical measurements of their activity are lacking. While ATP-dependent iron transport is low, this may reflect the low levels of free iron compared with other ions in cells.

Our findings suggest that the ATP-dependent iron transport we have described physiologically regulates cellular iron homeostasis. RAW 264.7 cells manifest significantly augmented ATP-dependent iron transport when treated with iron. In HO1$^{-/-}$ mice, wherein iron accumulates in the liver and kidney, ATP-dependent iron transport is strikingly augmented. In the...
spleen of these mice there is a dramatic reduction in ATP-dependent iron transport, possibly as a result of anemia in these mice (11) leading to a decrease in total erythrocyte turnover. Glycerol-induced rhabdomyolysis, which leads to increased heme levels, HO1 induction, and iron liberation in the kidney, concurrently induces ATP-dependent iron transport. Thus, in cultured cells and animal models, cellular iron accumulation is associated with elevations in ATP-dependent iron transport, implying that, physiologically, the Fe-ATPase responds to iron.

Our findings suggest that the regulatory mechanisms determining expression and induction of ATP-dependent iron transport may be complex. We observe a biphasic induction of ATP-dependent iron transport in RAW 264.7 cells that may reflect different underlying mechanisms. Rapid induction (1–2 h following treatment with iron) may reflect post-translational modifications as the initial induction is not blocked by CHX or AD. A more pronounced induction 16–20 h after iron treatment is blocked by incubation with CHX and AD. Even when added to the cells 16 h after the iron, CHX blocks the delayed induction. Other genes related to cellular iron homeostasis, such as transferrin receptor and ferritin, are regulated by IRPs (4, 6, 34). Thus, with iron deficiency, IRPs prevent the translation of ferritin mRNA and stabilize transferrin receptor mRNA. IRPs also regulate the heme-synthesizing enzyme Δ-aminolevulinate synthase as well as the divalent cation transporter-1 (5, 35, 36). Our results suggest that a rapid, perhaps post-translational, regulatory mechanism mediates the 2-fold induction of ATP-dependent iron transport seen in the first 1–2 h, and a second, translational (e.g. IRE-mediated) or transcriptional regulatory mechanism underlies the delayed induction of ATP-dependent iron transport after 18 h of iron exposure. Since both CHX and AD block iron-mediated induction of ATP-dependent iron transport, we cannot exclude an IRE-mediated mechanism.

A variety of evidence suggests that HO1 is functionally coupled to the Fe-ATPase and iron mobilization (11, 12). HO1 transfection stimulates iron egress from cells, which is markedly diminished in cells from HO1−/− mice (12). The ATP-dependent iron transport is more than 20-fold enriched in spleen compared with other tissues, closely resembling the distribution of HO1. Increases in cellular iron, both in vitro and in vivo, induce HO1 and the Fe-ATPase in parallel. HO1 and the Fe-ATPase are similarly enriched in microsomal fractions, where they may co-localize on endoplasmic reticulum membranes. Accordingly, we suggest that HO1 and the Fe-ATPase act in concert (Fig. 7). As heme is degraded by HO1, the freed iron is transported by the Fe-ATPase to the lumenal side of the endoplasmic reticulum for subsequent exocytosis (Fig. 7). Iron may bind transferrin in the lumen of the ER if the pH is neutral or following exocytosis. Transferrin is important for physiologic iron uptake, although alternate pathways enable cells to acquire iron. Interestingly, transferrin may be required for cellular iron release. Thus, in a perfused organ model, transferrin was required for 59Fe release from the liver (37). In our own studies, monitoring 59Fe release from HEK-293 cells, we find that cells take up 59Fe in the absence of transferrin, although 59Fe is released only in the presence of transferrin. Together these findings suggest that a portion of the endoplasmic reticulum may be devoted to the regulation of iron uptake, heme turnover, and iron efflux with specific roles for transferrin receptor, HO1, and the Fe-ATPase in close association.

REFERENCES


2 C. D. Ferris and S. H. Snyder, unpublished observations.
A Mammalian Iron ATPase Induced by Iron
David E. Barañano, Herman Wolosker, Byoung-Il Bae, Roxanne K. Barrow, Solomon H. Snyder and Christopher D. Ferris

doi: 10.1074/jbc.275.20.15166

Access the most updated version of this article at http://www.jbc.org/content/275/20/15166

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 35 references, 12 of which can be accessed free at http://www.jbc.org/content/275/20/15166.full.html#ref-list-1