Secretion of Ferritin by Rat Hepatoma Cells and Its Regulation by Inflammatory Cytokines and Iron

By Tue N. Tran, Sharon K. Eubanks, Kenneth J. Schaffer, Cecilia Y.J. Zhou, and Maria C. Linder

The possibility that serum ferritin is a secreted protein and an acute phase reactant regulated by inflammatory hormones and iron was examined in a hepatic cell line that secretes plasma proteins. Differentiated rat hepatoma cells released albumin and ferritin into the medium, as determined by rocket immunoelectrophoresis and isolation of ferritin by standard procedures plus immunofinity chromatography, following labeling with radioactive amino acid. Administration of interleukin-1-β (IL-1) or tumor necrosis factor-α (TNF) doubled the amounts of ferritin released into the medium over 24 and 48 hours. Together, the cytokines had more than an additive effect. Albumin secretion was diminished by IL-1, but not TNF. Iron, administered as an iron dextran complex or as a 1:1 chelate with nitrilotriacetate (Fe-NTA), also enhanced ferritin release, but had no effect on albumin. Intracellular ferritin concentrations did not change significantly with cytokine treatment, but increased in response to iron. With or without treatments, release of ferritin and albumin from cells into the medium was inhibited by brefeldin A, an inhibitor of Golgi function. The effect of each of the cytokines and of iron on ferritin and albumin was also blocked by dichlorofuranosylbenzimidazole (DRB), an inhibitor of transcription. The stimulatory effect of Fe-NTA on ferritin secretion was diminished by TNF, and this was partially counteracted by IL-1, indicating additional regulatory complexity. These results show for the first time that hepatic cells secrete ferritin, that this ferritin secretion is regulated by iron and inflammatory cytokines, and that the mechanisms of regulation differ from those for intracellular ferritin. The results would explain why serum ferritin increases in inflammation or when iron flux is enhanced.

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Levels of serum ferritin also increase in response to these two kinds of stimuli, and it seemed possible to us that some of the increase in ferritin biosynthesis observed in liver cell extracts might reflect an increase in the rate of synthesis of ferritin destined for the serum. Indeed, we observed that inflammation did not increase the concentration of ferritin (or ferritin iron) in liver cells, suggesting that the increased ferritin synthesized might at least in part have been secreted. Although we had observed an increase in the proportion of ferritin L and H mRNA associated with the ER-bound polyribosomes after induction of turpentine inflammation in rats23,24 (as would be expected if ferritin were increasingly secreted during inflammation), no one had previously shown that ferritin is actually secreted by hepatic cells. In fact, Cairo et al23,24 were unable to show sequestration of H or L ferritin subunits in ER vesicles during in vitro translation in the presence of canine microsomes. Also, H and L ferritin mRNAs contain no sequence coding for a traditional signal peptide. Based on ongoing studies,21 we believe these apparent contradictions are the result of serum and intracellular ferritins being encoded by homologous, but different mRNAs; that both kinds of proteins interact (but interact differently) with antibodies against intracellular ferritin; and that serum ferritin mRNAs can hybridize with specific probes for intracellular ferritin L and H, depending upon the conditions used. The studies reported here establish for the first time that a form of ferritin is actually secreted by hepatic cells and that, contrary to what occurs with intracellular ferritin in hepatic cells, production and secretion of this ferritin is under transcriptional regulation by inflammatory hormones and iron.

MATERIALS AND METHODS

Cell culturing and treatments. Rat hepatoma cells (H4-II-E-C3) obtained from the American Type Culture Collection (Rockville, MD) were grown from 3 to 4 days on Dulbecco’s Modified Eagle’s Medium (Sigma Chemical Co, St Louis, MO), either with 20% horse serum and 5% fetal calf serum (for the leucine incorporation studies), or with 5% iron-supplemented bobby calf serum and 15% horse serum. This was followed by 24 to 72 hours in the same medium or in serum-free, protein-free hybridoma medium (S2772, from Sigma). During the last 48 hours, murine IL-1β (1 to 20 ng/mL), murine tumor necrosis factor (TNF)-α (0.5 to 10 ng/mL) (both cytokines from Sigma), and/or iron were added to some of the cultures. The iron added was either in the form of the 1:1 molar complex of Fe(III)-nitrilotriacetate (Fe-NTA) or in the form of an iron-dextran complex (Imferon, Merrell National Laboratories, Cincinnati, OH). At the end of each experiment, the medium was aspirated off for analysis of albumin and ferritin in the medium, and for LDH activity.25 Cells were washed, released by 2 minutes incubation with 0.1% trypsin, transferred to centrifuge tubes with sterile isotonic saline, and collected by centrifugation. They were then either frozen at −85°C or homogenized directly, for determination of protein content and cell number. The cell line was found to have a protein content of 0.7 ± 0.2 mg per 10^6 cells (mean ± standard deviation [SD] for five determinations), which is close to the 0.8 mg value for adult rat liver cells.26 Nonfrozen cell homogenates (in 20 mmol/L K phosphate, pH 7.0, containing 0.02% NaN₃) were assayed for ferritin after heating to 70°C for 10 minutes and centrifuging off coagulated proteins. Cell number was determined by counting orcein-stained nuclei in a hemocytometer, as previously described.22 Protein was determined by the Bradford assay using the reagent and protocol of BioRad (Richmond, CA). In one study, 1-4,5,6-H-leucine (specific activity 58 Ci/mmol; Amersham, Arlington Heights, IL) was added to cells during culturing, at a rate of 2 μCi per flask, to determine whether ferritin synthesized by the cells was released into the medium. Radioactive samples were counted in a Tri-Carb Liquid Scintillation Analyser (Model 2200 CA, Packard, Canberra, Australia), after addition of scintillation fluid (Fluorodyne; National Diagnostics, Manville, NJ). In some cases, brefeldin A (from Sigma) was added to the cultures at the same time as IL-1, TNF, or iron, at doses of 1 to 4 μg/mL to inhibit Golgi function.25 5,6-Dichloroindolylciodazol (DRB; from Sigma) was added to some flasks (4 ng/mL) to inhibit transcription.28

Isolation of ferritin from the medium. Ferritin was isolated from the medium by a modification of the procedure of Cragg et al29 for serum ferritin, as described elsewhere.28 Medium was brought to pH 4.8 with 0.1 mmol/L Na acetate (pH 4.8), and 0.1 N acetic acid and heated to 70°C for 10 minutes, then cooled on ice. After centrifugation, the precipitate was discarded, and the heating and centrifugation procedure repeated. The final supernatant, brought back to pH 6.8, was treated with granular ammonium sulfate to 50% saturation overnight at 4°C, and the ferritin-containing precipitate, dissolved in a minimal amount of K phosphate buffer (10 mmol/L, pH 7.0, containing 0.02% NaN₃), was applied to immunoaffinity chromatography, using a gel prepared with the gamma globulin fraction of antiserum raised in a rabbit against horse spleen ferritin (the injected ferritin only had H and L subunits, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS PAGE]). After incubation of the extract with the affinity gel for 1 hour or more, at room temperature, nonbinding proteins were eluted by extensive washing of the gel in a column, until the 280 nm absorbance of effluent was less than 0.20. The ferritin was then eluted with 3 mmol/L K thiocyanate in the same buffer. The protein eluting was pooled and dialyzed into the nonionophane containing phosphate buffer (with 0.02% NaN₃). Protein determinations were by the Bradford procedure, using the protocol and reagent from BioRad and bovine serum albumin as the standard.

Immunoassays for ferritin and albumin. Immunoelectrophoresis was performed with a Bio-Phoresis system (BioRad), cooled to 4°C or 9°C. Antibodies were added to 1% agarose (Standard Low m; Bio-Rad) in 27 mmol/L Tricine Buffer IV, pH 8.6 (Bio-Rad) at about 55°C, just before pouring the plates. Samples (5 to 8 μL) were placed in wells on 10×10 cm, 1.5 mm thick agar plates, immersed in Brilliant Blue R Staining solution (Sigma) for a minimum of 15 minutes, washed in deionized water for 15 to 30 minutes and then pressed under several layers of filter paper and a weighted glass plate for 30 minutes. Glass plates were then soaked in 0.9% NaCl for 15 to 30 minutes and pressed as before. Pressed gels were dried completely with a hair dryer or in a gel drying apparatus, and were then immersed in Brilliant Blue R Staining solution (Sigma) for a minimum of 15 minutes. Gels were destained with two to three changes of 10% acetic acid until a clear background was obtained, then dried as before. Areas under each rocket were calculated from height width at half height. Concentrations of antigen in the samples were determined from standard curves made with the area values for the three or more standards run on every plate. Samples were assayed in duplicate or triplicate. In the case of albumin, cell medium was assayed directly using commercial goat antirat albumin antiserum (Cappel, Westchester, PA) and rat albumin (Sigma) as the standard. In the case of ferritin, conditioned media and cell homogenates were heated to 70°C at pH 4.8 and (only in the case of the media) concentrated 20-fold (by Centriicon-30; Amicon, Beverly, MA) be-
Fig 1. Rocket immunoelectrophoresis of ferritin (A) and albumin (B) in hepatoma cell medium. (A) Rockets obtained with concentrated samples of conditioned medium from hepatoma cells in comparison with standards (Stds) of horse spleen ferritin (HSpFt) (3 slots on far right). Samples (6 lanes on the left) are from individual flasks of cells grown for 48 hours in protein-free medium (see Materials and Methods). Standards contained 10, 6, and 2 μg/mL horse spleen ferritin protein (from left to right). (B) Samples of medium from cells that were and were not treated with the Golgi function inhibitor, brefeldin A (BFA; 4 μg/mL), in comparison with rat albumin standards (Stds; 3 lanes on far right). Lanes 1 to 3 and 7 to 9 from left were from untreated cells, lanes 4 to 6 and 10 to 12 were from BFA treated cells, as indicated.

Table 1. Synthesis of Ferritin by Rat Hepatoma Cells in Culture

<table>
<thead>
<tr>
<th></th>
<th>Cell Medium</th>
<th>Hepatoma Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>2,390</td>
<td>51</td>
</tr>
<tr>
<td>Amount (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 3H (cpm × 10^4)</td>
<td>3.49</td>
<td>21.2</td>
</tr>
<tr>
<td>Specific activity (cpm/mg)</td>
<td>1,460</td>
<td>419,000</td>
</tr>
<tr>
<td>Total ferritin isolated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount (μg)</td>
<td>546</td>
<td>199</td>
</tr>
<tr>
<td>Total 3H (cpm)</td>
<td>1,821</td>
<td>387</td>
</tr>
<tr>
<td>Specific activity (cpm/mg)</td>
<td>3,336</td>
<td>1,946</td>
</tr>
</tbody>
</table>

Ten flasks of cells were grown a total of 7 days in serum containing medium. Tracer 3H-leucine was added to the last change in medium, and the medium and cells were harvested 72 hours later. Ferritin was isolated from medium and cells, and the total radioactivity incorporated into the ferritin and total protein was measured.

RESULTS

Synthesis and release of ferritin by rat hepatoma cells. To determine whether ferritin was synthesized and released by rat hepatoma cells, we followed the incorporation of 3H-leucine into intracellular ferritin and that in the medium. Medium and washed cells from 10 culture flasks that had been incubated for 72 hours with the radioactive amino acid, and represented about 5 × 10^6 cells, were pooled, and the ferritin present in each was isolated by the procedure used for serum ferritin (see Materials and Methods). The last step in this procedure is immunoaffinity chromatography with antibody against intracellular ferritin. The ferritin bound to the antibody was quantitated and analyzed for radioactivity.

Table 1 shows that significant amounts of ferritin were
isolated from both the cells and the conditioned medium. In fact, there was considerably more ferritin in the medium than in the cells. Both portions of ferritin were radioactively labeled, indicating they had been synthesized by the hepatoma cells. The medium itself (with 20% horse serum and 5% fetal calf serum) contained negligible amounts of ferritin, as determined by our immunoassay (see Materials and Methods). Thus, virtually all of the ferritin recovered had been synthesized by the hepatoma cells.

Imunoassays for ferritin and albumin were developed and confirmed that ferritin was being released from the hepatoma cells even in the absence of potential stimuli (Fig 1A), and that the cells were behaving like liver cells by releasing albumin into the medium (Fig 1B). We also confirmed that the albumin was secreted via the usual Golgi-dependent mechanism, as treatment of the cells with brefeldin A (BFA) greatly reduced the amounts of albumin released into the medium (Fig 1B); (BFA causes a fusion of the Golgi apparatus with the ER, preventing normal exocytosis and protein secretion).

**Effects of inflammatory cytokines on ferritin release and secretion.** Serum ferritin concentrations are elevated in inflammatory conditions, and we wondered whether serum ferritin might be an acute phase reactant responsive to certain cytokines. We, therefore, treated our cell line with IL-1 and TNF-α, and confirmed that ferritin was being released from the hepatoma cells even in the absence of potential stimuli (Fig 1A), and that the cells were behaving like liver cells by releasing albumin into the medium (Fig 1B). We also confirmed that the albumin was secreted via the usual Golgi-dependent mechanism, as treatment of the cells with brefeldin A (BFA) greatly reduced the amounts of albumin released into the medium (Fig 1B); (BFA causes a fusion of the Golgi apparatus with the ER, preventing normal exocytosis and protein secretion).

<table>
<thead>
<tr>
<th>Dose (ng/mL)</th>
<th>Ferritin Released</th>
<th>Albumin Released</th>
<th>Cell Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/mg cell prot.</td>
<td>µg/mg cell protein</td>
<td>µg/mg cell protein</td>
</tr>
<tr>
<td>Controls (12)</td>
<td>0</td>
<td>1.8 ± 0.3</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>IL-1 (12)</td>
<td>5.0, 10.0</td>
<td>4.8 ± 1.1*</td>
<td>19 ± 1*</td>
</tr>
<tr>
<td>TNF (12)</td>
<td>2.5, 5.0</td>
<td>5.7 ± 2.0*</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>IL-1 + TNF (16)</td>
<td>All combinations</td>
<td>9.5 ± 1.9*</td>
<td>19 ± 1*</td>
</tr>
</tbody>
</table>

* P < .01 or <.001 for difference from controls.
† P < .01 or <.001 for difference from IL-1 or TNF alone.

The data from several studies in which cells were treated with 5 to 10 ng/mL IL-1-β and/or 2.5 to 5 ng/mL TNF-α are summarized in Table 2. Lower doses were less effective; much higher doses induced cell damage, as shown by an increased release of LDH, particularly in the case of TNF (data not shown). All of the data are given as values per mg of cell protein, to correct for any differences in cell number among replicates and with treatments. There were no obvious effects of the cytokines on cell number, as determined by the amount of cell protein recovered per flask (data not shown).

IL-1 and TNF each increased ferritin release about 2.5-fold (Table 2). The administration of IL-1 and TNF together had a much greater effect than either cytokine alone, increasing the concentrations of ferritin in the medium more than five-fold. Overall, the effects of the two cytokines were additive, although in two of the four experiments, the effects were clearly synergistic. As the individual cytokines were each given at doses that had a maximal individual effect, one can conclude that the mechanisms used by these hormones to enhance ferritin release are not identical and that the mechanism of regulation used by one may enhance that of the other. Inflammation induces the release of both of these cytokines in vivo, so the effect of both together should be more representative of what can occur in vivo.

In vivo, during inflammation, it is also observed that the release of albumin from the liver is diminished. This phenomenon was also apparent in our hepatic cell culture system.
SECRETION OF FERRITIN BY HEPATOMA CELLS

Effects of iron on ferritin release and secretion. Treating the hepatoma cells with iron also greatly enhanced release of serum ferritin. The effects of various doses of the iron, given either in the form of a 1:1 molar complex or Fe(III) with nitrilotriacetate (Fe-NTA) or as a dextran complex (Fe-dextran; Imferon), are shown in Fig 3. Both forms of iron stimulated release of ferritin into the medium (Fig 3A), although the Fe-NTA was more effective, at a given dose (it is noteworthy that iron dextran was able to deliver iron even in the absence of RE cells, although the latter are thought to be involved in release of iron from this complex in vivo).

To verify that the effects of IL-1 and TNF were on ferritin secretion, we again used BFA. BFA markedly inhibited the effects of IL-1 and TNF on release of ferritin into the medium (Fig 2). Secretion of albumin was also inhibited (data not shown). In one single experiment, secretion of still another plasma protein (ceruloplasmin) was also monitored; BFA almost completely blocked release (data not shown). Thus, increased secretion rather than leakage accounted for the increased release of ferritin from hepatoma cells in response to stimulation by the cytokines.

Effects of iron on ferritin release and secretion. The effects of iron on ferritin concentration in the medium (A) and in the hepatoma cells (B) after 48 hours, with and without (control) treatments with different doses of iron (during that period) in the form of Fe-dextran (Imferon; 1, 4, 6, and 15 μg Fe/mL) or the 1:1 complex of Fe(III) with nitrilotriacetate (Fe-NTA; 1, 4, 6, 10, and 20 μg Fe/mL). Data are means ± SD for 3 to 6 determinations (flasks), as microgram of protein per milligram of cell protein. Asterisk (*) indicates increases that are statistically significant (P < .01) by t-test, in comparison with the control. (C) LDH activity in the medium from cells untreated (control) or treated with 4, 10, 15, or 20 μg/mL doses of iron (Fe-NTA), brefeldin A (4 μg/mL) for 48 hours. After the control, under successive bars (from left to right) are the results at a given iron dose (for example, 4 μg Fe/mL) followed by the same, but also treated with BFA. The only significant change in LDH was with the highest dose of Fe-NTA (asterisk; P < .01).

In one experiment, secretion of albumin and another plasma protein (ceruloplasmin) was also monitored; BFA almost completely blocked release (data not shown). Thus, increased secretion rather than leakage accounted for the increased release of ferritin from hepatoma cells in response to stimulation by the cytokines.

Effects of iron on ferritin release and secretion. The effects of iron on ferritin concentration in the medium (A) and in the hepatoma cells (B) after 48 hours, with and without (control) treatments with different doses of iron (during that period) in the form of Fe-dextran (Imferon; 1, 4, 6, and 15 μg Fe/mL) or the 1:1 complex of Fe(III) with nitrilotriacetate (Fe-NTA; 1, 4, 6, 10, and 20 μg Fe/mL). Data are means ± SD for 3 to 6 determinations (flasks), as microgram of protein per milligram of cell protein. Asterisk (*) indicates increases that are statistically significant (P < .01) by t-test, in comparison with the control. (C) LDH activity in the medium from cells untreated (control) or treated with 4, 10, 15, or 20 μg/mL doses of iron (Fe-NTA), brefeldin A (4 μg/mL) for 48 hours. After the control, under successive bars (from left to right) are the results at a given iron dose (for example, 4 μg Fe/mL) followed by the same, but also treated with BFA. The only significant change in LDH was with the highest dose of Fe-NTA (asterisk; P < .01).

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actions between iron and cytokines. Iron stimulates synthesis of intracellular ferritin primarily by altering the rate of translation of existing mRNA. 6,8-10 and iron stimulation of intracellular ferritin biosynthesis is not altered by inhibitors of transcription, such as actinomycin D. 5 Similarly, it has been reported that IL-1 stimulates synthesis of intracellular ferritin in hepatic cells by enhancing translation 28,29 and without changing levels of ferritin mRNA. 23,24,28 We wondered whether regulation of serum ferritin secretion might be different and require enhanced transcription. To test this possibility, we used dichlororibofuranosylbenzimidazole (DRB), which inhibits transcription by binding to RNA polymerase II. 44 It is not as toxic as actinomycin D and can be used in more long-term experiments. In this case, we added it to 4-day cultures in protein-free medium, at the same time as the cytokines or iron, and cells and media were harvested 24 hours later. The data in Table 4 show that without DRB, there was the usual enhancement of ferritin secretion in response to IL-1, TNF or Fe-NTA, iron again being the most effective at the doses applied. Simultaneous treatment with DRB almost completely blocked the stimulatory effects of each factor. The same was true for albumin secretion. There was no increase in LDH release or a reduction in the number of cells per flask with DRB treatment (data not shown), indicating that the treatment was not toxic to the cells over the 24-hour period examined. Thus, the stimulatory effects of the two cytokines and iron appear to involve increased transcription of the mRNA for secreted (serum) ferritin and/or of factors that control this process.

Finally, potential interactions between the cytokines and iron were examined. Figure 4 shows that at the concentration of 10 ng/mL, iron (as the NTA complex) had a stimulatory effect greater than that produced by IL-1 and TNF, together. Iron increased ferritin secretion more than sevenfold, while TNF plus IL-1 caused a sixfold enhancement. Unexpectedly, secretion was significantly diminished when TNF was given with the iron. However, IL-1 given along with the Fe and TNF appeared to prevent this inhibition, and all three factors together had the same effect as IL-1 plus TNF, without the iron. Clearly, some complex interactions of stimulatory cascades induced by these factors are occurring that will need to be dissected out.

**DISCUSSION**

We have shown for the first time that cells of hepatic origin secrete ferritin; that secretion is stimulated by inflammatory hormones; that two cytokines together have much more of a stimulatory effect than one alone; and that transcription is required for this hepatic cell response. We have also shown for the first time that iron enhances ferritin secretion, again by a mechanism involving transcription, and...
that there is a complex interaction between the inflammatory cytokines and iron in regulating secretion. Although indirect evidence strongly suggested that serum ferritin is at least in part a secreted protein, actual secretion of ferritin has never before been demonstrated for cells from a major organ of the body, such as liver.

Ferritin concentrations in human serum have been measured in many different physiologic states and are routinely used to assess body iron stores. As indicated earlier, concentrations of ferritin in serum increase in a variety of conditions that include cancer, liver disease, inflammation, iron overload, or iron treatment. In the present studies, we have attempted to model two of these conditions using cultured, differentiated hepatic cells, with apparently positive results.

Our cells acted like hepatocytes. They synthesized and secreted albumin, and the secretion of albumin was slowed by treatment with IL-1. This reflects the in vivo situation, in which there is decreased albumin secretion during inflammation. To monitor ferritin release, we used immunoassays based on antibodies to intracellular ferritin, as is the case for clinical assays of ferritin. The assay showed that hepatic cells release ferritin, and that the release was greatly stimulated by a combination of cytokines that circulate during inflammation, and also by iron. BFA inhibited ferritin release, indicating that the ferritin was being secreted rather than leaked from the hepatic cells by the usual Golgi-mediated mechanism (BFA being a specific inhibitor of Golgi function). BFA inhibited not just ferritin, but also albumin and ceruloplasmin secretion. Proteins using this secretory mechanism are synthesized on ER-bound polyribosomes and have coding for a signal peptide sequence in their mRNAs (which is not the case for intracellular H and L ferritins).

The response to IL-1 + TNF can explain why serum ferritin concentrations increase in vivo during inflammation and indicates that serum ferritin can legitimately be added to the list of acute phase reactant proteins. The response to iron can explain why serum ferritin concentrations increase in vivo, when iron is given to treat iron deficiency, or in iron overload, when the flux of iron in and out of cells is high. Our results also indicate that liver cells are a significant source of serum ferritin.

Our results for secreted ferritin should not be confused with those for intracellular ferritin, which appears to be a different protein. Synthesis of intracellular ferritin by liver, or expression of ferritin H mRNA in adipocytes, muscle cells, and myoblasts, as well as in fibroblasts and monocytes is stimulated by the same cytokines we have been studying. In most cells it is also stimulated by iron. However, our studies show that the mechanisms underlying synthesis and secretion of serum ferritin (at least by hepatic cells) differ from those for intracellular ferritin. In hepatic cells, stimulation of intracellular ferritin biosynthesis by iron occurs primarily at the translational level via a change in the use of existing mRNA, and with little or no change in rate of transcription or concentration of ferritin mRNA. In the case of iron, this involves release of iron response proteins (IRP1 and IRP2) from a stem loop structure in the 5′-untranslated region (5′UTR) of the ferritin H and L mRNA. In the case of IL-1, this may also involve binding of regulatory proteins to a specific nucleotide sequence in the 5′UTR. We have now shown that for secreted (serum) ferritin the situation is quite different. We found that stimulation of serum ferritin secretion by hepatic cells, induced by iron or IL-1, was markedly inhibited by DRB and is thus primarily or exclusively under transcriptional control. This is supported by our observations of a 10-fold to 13-fold increase in mRNA for serum ferritin in these hepatic cells and liver, as determined with our partial cDNA clones. All these findings are consistent with the concept that serum ferritin is the product of separate mRNAs and separate genes. The details of the regulatory mechanisms involved and the intriguing interactions between the mechanisms for iron and the cytokines will be worked out when full-sized clones of the serum ferritin subunit cDNAs (and the genes for these messages) become available.
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