Iron regulates the Intracellular Degradation of Iron Regulatory Protein 2 by the Proteasome*

(Received for publication, April 17, 1995, and in revised form, June 28, 1995)

Bing Guo, John D. Phillips, Yang Yu, and Elizabeth A. Leibold†

From the Eccles Program in Human Molecular Biology and Genetics and the Department of Medicine, University of Utah, Salt Lake City, Utah 84112

Iron regulatory proteins (IRP1 and IRP2) are RNA-binding proteins that bind to specific structures, termed iron-responsive elements (IREs), that are located in the 5'- or 3'-untranslated regions of mRNAs that encode proteins involved in iron homeostasis. IRP1 and IRP2 RNA binding activities are regulated by iron; IRP1 and IRP2 bind IREs with high affinity in iron-depleted cells and with low affinity in iron-repleted cells. The decrease in IRP1 RNA binding activity occurs by a switch between apoprotein and 4Fe-4S forms, without changes in IRP1 levels, whereas the decrease in IRP2 RNA binding activity reflects a reduction in IRP2 levels. To determine the mechanism by which iron decreases IRP2 levels, we studied IRP2 regulation by iron in rat hepatoma and human HeLa cells. The iron-dependent decrease in IRP2 levels was not due to a decrease in the amount of IRP2 mRNA or to a decrease in the rate of IRP2 synthesis. Pulse-chase experiments demonstrated that iron resulted in a 3-fold increase in the degradation rate of IRP2. IRP2 degradation depends on protein synthesis, but not transcription, suggesting a requirement for a labile protein. IRP2 degradation is not prevented by lysosomal inhibitors or calpain II inhibitors, but is prevented by inhibitors that block proteasome function. These data suggest the involvement of the proteasome in iron-mediated IRP2 proteolysis.

Iron regulatory proteins (IRPs) are cytosolic RNA-binding proteins that regulate the post-transcriptional expression of genes that are involved in iron homeostasis (1–4). IRPs were formerly known as the iron-responsive element-binding protein (IRE-BP), the ferritin repressor protein (FRP), and the iron regulatory factor (IRF). IRPs bind with high affinity to RNA stem-loops known as iron-responsive elements (IREs). IREs are located in the 5'-untranslated regions of ferritin and erythroid δ-aminolevulinic acid synthase mRNAs where binding causes translational repression (5–7). Five IREs are located in the 3'-untranslated region of transferrin receptor mRNA (8, 9), and binding of the IRP stabilizes transferrin receptor mRNA (9, 10).

Two distinct IRPs have been cloned and characterized in mammalian cells and have been designated as IRP1 and IRP2. IRP1 has been cloned from a variety of mammalian species (5, 11, 12). IRP1 has a molecular mass of 98,000 Da and shares 30% amino acid identity with the 4Fe-4S enzyme, mitochondrial aconitase (13). The 18 active site residues in mitochondrial aconitase, including the 3 cysteines that serve as ligands for the 4Fe-4S cluster are conserved in IRP1 (13). In addition, IRP1 is an active cytosolic aconitase (14, 15). In iron-repleted cells, IRP1 exhibits aconitase activity and contains iron, but binds the IRE with low affinity. In contrast, in iron-depleted cells, IRP1 lacks aconitase activity and iron, but binds the IRE with high affinity. UV cross-linking studies have shown overlap between RNA binding and the aconitase active sites, indicating that RNA binding and aconitase activities are mutually exclusive (16, 17). Recent data indicated that aconitase activity is not necessary for iron regulation of IRP1, since substitution of an alanine for an active site serine does not prevent assembly and disassembly of the 4Fe-4S cluster (18).

IRP2 has been characterized in rat tissues by RNA band shift assays (19–22) and has been purified from rat liver and rat hepatoma cells (20). The partial amino acid sequence of rat IRP2 is similar to the predicted protein sequence encoded by a cDNA isolated from a human T cell library (11, 23), suggesting that this is the rat version of the human protein. A second IRP has been characterized from mouse tissues by RNA band shift analysis (24), which is presumed to be homologous to the rat and the human IRP2.

IRP2 contains similar biochemical properties to IRP1 in that it binds IREs with similar affinity (20, 24) and represses translation of IRE-containing mRNAs in vitro (20, 25). IRP1 and IRP2 differ in two aspects: first, unlike IRP1, IRP2 does not exhibit aconitase activity, indicating that aconitase activity is not necessary for regulation by iron (20), and second, that although iron results in a decrease in IRP1 and IRP2 RNA-binding activities, the amount of IRP1 remains constant (12, 26), whereas the amount of IRP2 protein is substantially reduced (20, 27).

These novel properties of IRP2 raised questions as to how intracellular iron regulates IRP2 RNA binding activity. To answer this question, we analyzed the regulation of IRP2 by iron in rat hepatoma cells and human HeLa cells. We found that the marked reduction in IRP2 RNA binding activity and protein levels in iron-treated cells is due to increased turnover of IRP2. IRP2 synthesis and IRP2 mRNAs levels are unaffected by iron treatment. The iron-mediated degradation of IRP2 requires protein synthesis, but not transcription, suggesting that the synthesis of a labile protein is required. We also demonstrate that the proteasome complex is required for iron-mediated degradation of IRP2. Regulation of IRP2 protein levels by iron occurs in a variety of cell types, indicating that iron-mediated degradation is a common pathway for regulating IRP2 RNA binding activity.
EXPERIMENTAL PROCEDURES

Materials—The proteasome inhibitor MG-132 (carbobenzoxyl-leucyl-leucyl-leucinal-H) was a gift from Myogenics, Inc., Cambridge, MA. Calpain inhibitor II and actinomycin D was purchased from Sigma. Antibodies generated against rat IRP1 and IRP2 were prepared as described (20).

Cell Culture—The rat hepatoma cell line FTO2B and human HeLa cells were grown at 37 °C in a 5% CO2 atmosphere in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum. For iron and iron chelation studies, cells were treated with either 50 μg/ml ferric ammonium citrate (FAC) or 200 μM desferrioxamine, respectively. For protease inhibition studies, cells were pretreated for 1 h with either 0.1 mM MG-132 or 0.1 mM calpain II inhibitor. To inhibit lysosomes, cells were pretreated for 1 h with either 20 mM ammonium chloride or 0.15 mM chloroquine prior to the addition of FAC.

Measurement of IRP2 Degradation Rates—Cells were preincubated in methionine-free media in the presence of 100 μCi/ml Tran35S-label (ICN Biomedicals) for 4 h, after which fresh media containing an excess of unlabeled methionine with or without 50 μg/ml FAC was added. At the indicated times, cells were lysed in Buffer A (20 mM HEPES, pH 7.5, 2 mM diithothreitol, 5% glycerol, 40 mM KCl) containing 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and an aliquot of each sample was used to determine protein concentration using the bicinchoninic acid protein assay (Pierce). Immunoprecipitation of IRP2 was carried out by incubating 30 μg of labeled extracts with 5 μl of rabbit anti-IRP2 antiserum for 3 h, followed by the addition of 20 μl of protein A-agarose suspension for 2 h. The immunocomplexes were washed with RIPA buffer (150 mM NaCl, 0.5% deoxycholate, 0.1% SDS, 1% Nonidet P-40, 50 mM Tris-HCl, pH 8.0), boiled in SDS sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 1% β-mercaptoethanol, 10% glycerol), and analyzed by 8% SDS-PAGE. The labeled protein was transferred to polyvinylidene difluoride (Millipore) membrane and subjected to autoradiography. The tp1/2 of IRP2 was determined by densitometric analysis of the labeled IRP2 bands.

Measurement of IRP2 Synthesis Rates—FTO2B cells were preincubated for 2.5 h in medium in the presence or the absence of 50 μg/ml FAC. The medium was replaced with methionine-free medium for 15 min in the absence or presence of FAC. Tran32P-label (100 μCi/ml) was added to the cells and then cells were harvested at 10, 20, 40, or 60 min after labeling. IRP2 was immunoprecipitated from each extract (100 μg) and analyzed by 8% SDS-PAGE as described above.

RNA Band Shift Assays and Immunoblot Analysis—Cell lysates for RNA band shift assays and immunoblots were prepared by lysing cells in Buffer A containing 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride. The lysates were spun at 13,000 × g for 10 min, and protein concentration was quantitated using the bicinchoninic acid protein assay (Pierce). For RNA band shift assays using anti-IRP2 antisera to “supershift” the IRP2IRE complex from the lysates, 5 μl of anti-IRP2 antiserum generated against the 73-amino acid insertion not present in IRP1 and is therefore specific for IRP2. Fig. 1A shows that IRP1 and IRP2 RNA binding activities begin to decrease at 2.5 h after iron treatment and remain low during the 24-h time course. During this time, the amount of IRP1 remained constant (Fig. 1B, lanes 1–8), whereas the amount of IRP2 is reduced about 5-fold and reflected the decreases in RNA binding activity (Fig. 1B, lanes 9–16). To determine if the iron-mediated decrease in IRP2 levels occurs in other cell types, we measured RNA binding activity and protein levels for IRP1 and IRP2 in human HeLa cells treated with FAC for 1–24 h (Fig. 2). Since human IRP1 and IRP2 complexes comigrate on native polyacrylamide gels, we carried out supershift assays using anti-IRP2 antiserum in RNA band shift assays. We have previously demonstrated that anti-IRP2 antiserum does not interfere with RNA binding and results in a supershifted IRP2IRE complex (20). Treatment of cells with FAC caused RNA-binding activity of IRP1...
and IRP2 to decrease 2- and 5-fold, respectively (Fig. 2, A and C). Immunoblot analysis indicated that the amount of IRP1 remained constant during iron treatment; whereas the amount of IRP2 decreased 5-fold (Fig. 2, B and C). We have observed reductions in the amount of IRP2 in mouse 3T3 fibroblasts and transformed human primary embryonal 293 kidney cells treated with FAC for the same time course (data not shown). These data indicated that the decrease in IRP1 and IRP2 RNA binding activities induced by iron are mediated by different cellular processes and occurs in a variety of cell types.

Iron Does Not Affect Amounts of the IRP2 6.4-kb mRNA—The decrease in IRP2 protein levels induced by iron could reflect reduced mRNA levels due to changes in transcription or mRNA stability, or a decrease in the rate of translation of its mRNA, or an increased rate of IRP2 degradation. To determine if iron affects IRP2 mRNA levels, we quantified IRP2 mRNA in FTO2B cells treated with FAC for 0–24 h and desferrioxamine, an intracellular iron chelator for 16 h. Total RNA was transferred to a membrane and sequentially hybridized with a 32P-labeled IRP2 cDNA (A) or a 32P-labeled glyceraldehyde phosphate dehydrogenase cDNA (B) to control for gel loading. The size of the IRP2 transcripts is indicated by arrows. RNA molecular weight standards are from Life Technologies, Inc.

(Fig. 3A). The amount of the 3.7-kb transcript increases 2-fold in desferrioxamine-treated cells. Our previous studies have suggested that changes in the 3.7-kb levels may be due to increased utilization of an alternative polyadenylation site in iron-deprived cells; however, the significance of this result is unclear (29). These data indicate that the 5-fold reduction in IRP2 protein levels and RNA binding activity induced by iron is not due to alterations in the steady-state levels of the IRP2 mRNAs.

Iron Increases the Rate of IRP2 Degradation, but Has No Effect on the Rate of IRP2 Synthesis—The reduction in IRP2 levels by iron could be due to either an increase in IRP2 turnover, a decrease in IRP2 synthesis, or a combination of both. To test whether iron increases the degradation rate of IRP2, pulse-chase experiments were carried out in the presence or absence of FAC, labeled IRP2 was immunoprecipitated with anti-IRP2 followed by fractionation of the immunocomplexes by SDS-PAGE (Fig. 4A). IRP2 was identified by comparison with immunoprecipitated protein from a control extract with preimmune rabbit antisera (Fig. 4A, lanes 1 and 2). FAC treatment increased the rate of degradation of IRP2 approximately 3-fold (Fig. 4A, lanes 12–18). The half-life of IRP2 in the presence and in the absence of FAC was reduced from 4.5 h in control cells to 1.5 h in iron-treated cells (Fig. 4B).

It was possible that in addition to decreasing the half-life of IRP2, iron could also reduce its rate of synthesis. Measurements of synthesis rates in the presence of iron could be misleading, since it would not only measure synthesis, but would also measure degradation of newly synthesized protein. To determine if synthesis of IRP2 was affected by iron, we treated cells with FAC for 2.5 h, then quantified the amount of [35S]methionine incorporated into IRP2 during a short 1-h time course. Our turnover data indicated that in 1 h after iron treatment approximately 30% of labeled pre-existing IRP2 is degraded.

---

**Fig. 2.** Effect of iron treatment on RNA binding activity and protein levels of IRP1 and IRP2 in human HeLa cells. A, HeLa cells were grown in the presence (lanes 2–7) or absence (lane 1) of 50 \( \mu \text{g/ml} \) FAC. Equal amounts of protein (10 \( \mu \text{g} \)) were incubated with (lanes 9–15) or without (lanes 1–8) anti-IRP2 antisera for 5 min followed by the addition of \( ^{32}\text{P} \)-labeled IRE RNA. As a control, rabbit preimmune antisera was added to an extract from untreated cells (C/P1, lane 8). IRP2/IRE complexes are indicated by an asterisk. B, equal amounts of protein (50 \( \mu \text{g} \)) from extracts in A were subjected to immunoblot analysis using anti-IRP1 or anti-IRP2 antisera. C, the data in A and B were quantified by densitometry and plotted using untreated control as 100%.

**Fig. 3.** Effect of iron treatment on IRP2 mRNA levels in rat hepatoma cells. FTO2B cells were grown in the presence of 50 \( \mu \text{g/ml} \) FAC (Iron) for 1–24 h or 200 \( \mu \text{M} \) desferrioxamine (DF) for 16 h. Total RNA was isolated and analyzed on an 1% formaldehyde-agarose gel. C0 and C24, untreated cells harvested at 0 and 24 h, respectively. The RNA was transferred to a membrane and sequentially hybridized with a \( ^{32}\text{P} \)-labeled IRP2 cDNA (A) or a \( ^{32}\text{P} \)-labeled glyceraldehyde phosphate dehydrogenase cDNA (B) to control for gel loading. The size of the IRP2 transcripts are indicated by arrows. RNA molecular weight standards are from Life Technologies, Inc.
Labeled IRP2 was immunoprecipitated using anti-IRP2 antibodies at 10, 20, 40, and 60 min followed by fractionation of the immunocomplexes by SDS-PAGE (Fig. 5b). As a control, IRP2 was immunoprecipitated from extracts from untreated cells using preimmune rabbit serum (lanes 1 and 2). Labeled immunoprecipitated protein was analyzed by 8% SDS-PAGE. The positions of the molecular weight standards and IRP2 are indicated. B, the turnover data in A was quantified by densitometry, and the intensity of the IRP2 bands were plotted relative to the percent of radioactivity remaining after 0 h (lanes 3 and 4). These experiments were carried out three times, and one representative experiment is shown. Symbols: ■, no addition; ●, iron.

Fig. 4. Effect of iron on the rate of IRP2 degradation. A, FTO2B cells were pulse-labeled for 4 h with Tran35S-label and chased in medium containing an excess of unlabeled methionine in the absence (lanes 1–11) or the presence (lanes 12–18) of 50 μg/ml FAC for 0–8 h. IRP2 was immunoprecipitated using anti-IRP2 antisera (lanes 3–18). As a control, IRP2 was immunoprecipitated from extracts from untreated cells using preimmune rabbit serum (PI) (lanes 1 and 2). Labeled immunoprecipitated protein was analyzed by 8% SDS-PAGE. The positions of the molecular weight standards and IRP2 are indicated. B, the turnover data in A was quantified by densitometry, and the intensity of the IRP2 bands were plotted relative to the percent of radioactivity remaining after 0 h (lanes 3 and 4). These experiments were carried out three times, and one representative experiment is shown. Symbols: ■, no addition; ●, iron.

Fig. 5. Effect of iron on the rate of IRP2 synthesis. A, FTO2B cells were grown in the presence (lanes 5–8) or the absence (lanes 1–4) of 50 μg/ml FAC for 2.5 h. The cells were incubated in methionine-free medium for 15 min with or without FAC. After 15 min, the cells were labeled with 100 μCi/ml Tran35S-label and were then harvested after 10, 20, 40, and 60 min. IRP2 was immunoprecipitated using anti-IRP2 antisera and analyzed by 8% SDS-PAGE. Molecular weight standards and IRP2 are indicated. PI, control 60-min lysate immunoprecipitated with preimmune serum. B, the synthesis data in A was quantified by densitometry and the integrated density of labeled IRP2 bands was plotted. These data represent the results from two experiments.

IRP2 RNA binding activity and IRP2 protein levels observed with iron treatment did not occur when protein synthesis was inhibited (Fig. 6A and B). The expected decrease in IRP1 RNA-binding activity in cells treated with cycloheximide and iron showed that cycloheximide did not interfere with iron uptake into cells. Identical results were obtained in FTO2B cells treated with the protein synthesis initiation inhibitor, emetine (data not shown).

We also determined if transcription is required for the degradation of IRP2 induced by iron. FTO2B cells were treated with the transcription inhibitor, actinomycin D alone, or in the presence or absence of FAC for 0, 1, 2.5, and 4 h, and IRP1 and IRP2 RNA binding activities and IRP2 levels were measured (Fig. 7A and B). Actinomycin D alone had no effect on IRP1 or IRP2 RNA binding activities (Fig. 7B) or protein levels (Fig. 7A). When cells were treated with FAC and actinomycin D, IRP1 and IRP2 RNA binding activities and IRP2 levels decreased, but not to the levels observed with iron alone. These data indicated that the iron-mediated degradation of IRP2 requires protein synthesis, but to a lesser extent transcription, suggesting that the synthesis of a labile protein is required for IRP2 degradation.

A Proteasome Inhibitor Blocks the Iron-mediated Degradation of IRP2 in FTO2B Cells—to identify the proteolytic system responsible for the iron-mediated degradation of IRP2, we tested whether proteosomal, lysosomal, and cysteine protease inhibitors prevented IRP2 iron-mediated degradation. The multicatalytic 26S proteasome complex catalyzes the degradation of proteins via either ubiquitin-dependent or ubiquitin-
In this paper we report the differential regulation of IRP1 and IRP2 by iron in mammalian cells. IRP1 exhibits two functions in cells dependent on iron levels: IRP1 with an 4Fe-4S cluster functions as a cytosolic aconitase converting citrate into isocitrate when iron is abundant and as a RNA binding apoprotein regulating the translation and stabilization of IRE-containing mRNAs when iron is scarce (18, 34–36). The switch between the 4Fe-4S form and the apoprotein forms occurs without changes in IRP1 levels (12, 26). By contrast, IRP2 lacks aconitase activity and functions solely as an RNA binding protein (20). Our results indicate that IRP2 is regulated by specific proteolysis induced by iron in a variety of cells types and that the proteasome is responsible for IRP2 degradation.

Our data suggest a mechanism for the iron-mediated degradation of IRP2. When intracellular iron is scarce, IRP2 binds IREs with high affinity. An increase in intracellular iron results in the induction of a labile protein that is required for
IRP2 degradation. Although we do not know the identity and function of this protein, it is possible that it is a targeting protein that binds IRP2 via the 73-amino acid domain, marking it for degradation. Iron could also cause the assembly of an 4Fe-4S cluster in IRP2, similar to the cluster in IRP1. Rat IRP2 contains the 3 conserved cysteines that coordinate the 4Fe-4S cluster in IRP1 (27, 29). In addition, the presence of 4 cysteines and 1 histidine in the 73-amino acid insertion of IRP2 suggests that this region might also participate in iron binding (29). Preliminary data suggests that in vitro reconstitution of IRP2 with iron results in loss in RNA binding activity.3 Thus, according to our model, cluster assembly would lead to a conformational change in IRP2 and subsequent loss in RNA binding activity. IRP2 would then be recognized by the targeting protein and rapidly degraded by the proteasome. Finally, our data indicate that the decrease IRP2 RNA binding activity mediated by iron is also prevented when IRP2 proteolysis is blocked either by MG-132 or by cycloheximide. One possibility to explain these data is that the putative Fe-S cluster is unstable in IRP2 and is disassembled during extract purification, leading to the generation of an apoprotein containing RNA binding activity.

The 26 S proteasome contains subunits which are important in the degradation of ubiquitin-conjugated proteins (30, 31). We have not detected higher molecular weight IRP2 complexes by gel electrophoresis, which might suggest that ubiquitination of IRP2 is not processed by the proteasome. However, since ubiquitin-conjugated proteins are very labile, they are generally difficult to detect. The 26 S proteasome also degrades non-ubiquitinated proteins (31, 37). The signals required for targeting non-ubiquitinated proteins to the proteasome are poorly understood; however, it is possible that the putative targeting protein discussed above could mark IRP2 for degradation by the proteasome.

Although we cannot eliminate the possibility that MG-132 may affect other unknown proteases and enzymatic activities in cells, the utilization of these inhibitors both in vitro and in vivo have demonstrated the specificity and effectiveness of these compounds against the proteasome (33). Our data suggested that MG-132 may increase cellular iron levels, perhaps by blocking the degradation of iron transporter proteins. Peptide-aldehyde inhibitors have been used to demonstrate the role of the proteasome in the generation of peptides presented on the major histocompatibility class I molecules (33) and in the proteolytic processing of the transcription factor NF-κB1 (32).

The structural determinants required for IRP2 iron-mediated degradation are unknown. IRP2 does not contain PEST regions (sequences rich in proline, glutamine, serine, and threonine) which are commonly found in proteins that are rapidly degraded (38). However, IRP2, the 73-amino acid insertion, contains a site that is susceptible to proteolysis during purification and results in the production of an 83,000-Da proteolytic polypeptide (20). The cleavage site has the sequence SQ-LENTP and is not a known protease cleavage sequence. Whether proteolysis at this site represents a physiological mechanism for iron-mediated degradation or whether the 73-amino acid insertion is a determinant required for degradation remains to be determined.

The biological relevance of two IRPs in cells is unclear. Both IRP1 and IRP2 bind IREs with high affinity (20, 24, 27) and function as translational repressors of IRE-containing RNAs in vitro (20). First, it is possible that IRP2 binds to a subset of IRE-containing mRNAs containing slightly different sequences. A recent study using in vitro synthesized IREs demonstrated that mouse IRP2 has a preference for specific IRE sequences, suggesting that IRP2 may bind to specific IRE-containing mRNAs in vivo (39). Second, since IRP2 is present in the highest amounts in skeletal muscle and heart, this suggests that IRP2 may regulate muscle-specific mRNAs (29). Third, IRP2 RNA binding activity is decreased in the livers of rats treated with chemicals to induce oxidative stress (22) and increased in regenerating rat livers (21), suggesting that IRP2 is regulated under a variety of physiological states. It is unclear whether these effects are due to alterations in intracellular iron levels or to stimuli other than iron.

A recent study suggested that iron-mediated regulation of IRP2 degradation may be cell-specific (27) and may affect other unknown proteases in cells, the utilization of these inhibitors both in vitro and in vivo have demonstrated the specificity and effectiveness of these compounds against the proteasome (33). Our data suggested that MG-132 may increase cellular iron levels, perhaps by blocking the degradation of iron transporter proteins. Peptide-aldehyde inhibitors have been used to demonstrate the role of the proteasome in the generation of peptides presented on the major histocompatibility class I molecules (33) and in the proteolytic processing of the transcription factor NF-κB1 (32).

The structural determinants required for IRP2 iron-mediated degradation are unknown. IRP2 does not contain PEST regions (sequences rich in proline, glutamine, serine, and threonine) which are commonly found in proteins that are rapidly degraded (38). However, IRP2, the 73-amino acid insertion, contains a site that is susceptible to proteolysis during purification and results in the production of an 83,000-Da proteolytic polypeptide (20). The cleavage site has the sequence SQ-LENTP and is not a known protease cleavage sequence. Whether proteolysis at this site represents a physiological mechanism for iron-mediated degradation or whether the 73-amino acid insertion is a determinant required for degradation remains to be determined.

The biological relevance of two IRPs in cells is unclear. Both IRP1 and IRP2 bind IREs with high affinity (20, 24, 27) and function as translational repressors of IRE-containing RNAs in vitro (20). First, it is possible that IRP2 binds to a subset of IRE-containing mRNAs containing slightly different sequences. A recent study using in vitro synthesized IREs demonstrated that mouse IRP2 has a preference for specific IRE sequences, suggesting that IRP2 may bind to specific IRE-containing mRNAs in vivo (39). Second, since IRP2 is present in the highest amounts in skeletal muscle and heart, this suggests that IRP2 may regulate muscle-specific mRNAs (29). Third, IRP2 RNA binding activity is decreased in the livers of rats treated with chemicals to induce oxidative stress (22) and increased in regenerating rat livers (21), suggesting that IRP2 is regulated under a variety of physiological states. It is unclear whether these effects are due to alterations in intracellular iron levels or to stimuli other than iron.

A recent study suggested that iron-mediated regulation of IRP2 degradation may be cell-specific (27). A c-myc-tagged recombinant IRP2 expressed in HeLa cells treated with iron or hemin for 16 h resulted in a decrease in RNA binding activity, but no change in the amount of protein. Our experiments analyzing the iron-mediated regulation of endogenous IRP2 in HeLa cells treated with iron for up to 24 h showed a steady decrease in RNA binding activity and protein levels up to 6 h, after which RNA binding activity and protein levels gradually increased. The half-life of recombinant IRP2 expressed in RD-4 cells was greater than 24 h in desferrioxamine-treated cells and 6 h in iron-treated cells (27). By contrast, our data indicated that the half-life of endogenous IRP2 in untreated FTO2B cells was 6 h and 1.5 h in iron-treated cells. The discrepancies between these studies may reflect differences in experimental design due to use of overexpressed protein or to different cell growth conditions.

The regulation of gene expression by specific proteolysis provides a way by which cells can change the concentration of specific proteins depending on the metabolic state of the cell. The iron-dependent regulation of IRP2 turnover may be similar to the mechanisms regulating the mammalian enzyme ornithine decarboxylase. Ornithine decarboxylase is the first enzyme in the polyamine biosynthesis pathway and is degraded when intracellular polyamine levels increase (40). Polyamines

---

3 J. D. Phillips and E. A. Leibold, unpublished data.
induce antizyme, a protein which binds with high affinity to ornithine decarboxylase (41, 42) and targets ornithine decarboxylase for degradation by the proteasome (37). Thus, it is possible that IRP2, like ornithine decarboxylase, may utilize other proteins that specify its degradation during changes in intracellular iron levels. The characterization of the other components responsible for IRP2 iron-mediated degradation will provide a clearer understanding of the mechanism by which IRP2 is targeted and degraded by the proteasome.

Acknowledgments—We thank Dennis Winge, Liz Wyckoff, Andy Sewell, and members of the laboratory for insightful comments during the course of this work and for critical readings of the manuscripts.

REFERENCES
Iron Regulates the Intracellular Degradation of Iron Regulatory Protein 2 by the Proteasome
Bing Guo, John D. Phillips, Yang Yu and Elizabeth A. Leibold

doi: 10.1074/jbc.270.37.21645

Access the most updated version of this article at http://www.jbc.org/content/270/37/21645

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 42 references, 27 of which can be accessed free at http://www.jbc.org/content/270/37/21645.full.html#ref-list-1