Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7536–7540, August 1992
Biochemistry

Reciprocal control of RNA-binding and aconitase activity in the regulation of the iron-responsive element binding protein: Role of the iron-sulfur cluster

DAVID J. HAILE, TRACEY A. ROUAULT, CAREEN K. TANG, JEAN CHIN, JOE B. HARFORD, AND RICHARD D. KLAUSNER*

Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

Communicated by Igor B. Dawid, May 8, 1992

ABSTRACT **Several mechanisms of posttranscriptional gene regulation are involved in regulation of the expression of essential proteins of iron metabolism. Coordinate regulation of ferritin and transferrin receptor expression is produced by binding of a cytosolic protein, the iron-responsive element binding protein (IRE-BP) to specific stem–loop structures present in target RNAs. The affinity of this protein for its cognate RNA is regulated by the cell in response to changes in iron availability. The IRE-BP demonstrates a striking level of amino acid sequence identity to the iron-sulfur (Fe-S) protein mitochondrial aconitase. Moreover, the recombinant IRE-BP has aconitase function. The lability of the Fe-S cluster in mitochondrial aconitase has led us to propose that the mechanism by which iron levels are sensed by the IRE-BP involves changes in an Fe-S cluster in the IRE-BP. In this study, we demonstrate that procedures aimed at altering the IRE-BP Fe-S cluster *in vitro* reciprocally alter the RNA binding and aconitase activity of the IRE-BP. The changes in the RNA binding of the protein produced *in vitro* appear to match the previously described alterations of the protein in response to iron availability in the cell. Furthermore, iron manipulation of cells correlates with the activation or inactivation of the IRE-BP aconitase activity. The results are consistent with a model for the posttranslational regulation of the IRE-BP in which the Fe-S cluster is altered in response to the availability of intracellular iron and this, in turn, regulates the RNA-binding activity.**

Studies of the posttranscriptional control of critical proteins of iron metabolism have suggested models in which modulation of metabolic pathways occurs through regulation of the fate of cytoplasmic mRNAs. This regulation is based on the presence in certain transcripts of specific recognition structures called iron-responsive elements (IREs) (1–4). IREs were first identified in the 5′ untranslated region (UTR) of mRNAs encoding the iron storage/sequestration protein ferritin (2–4). A functional IRE is a moderately stable stem–loop structure containing approximately 26–35 nucleotides with a 6-membered loop and an unpaired cytosine 6 nucleotides 5′ of the first loop nucleotide. The 5′ IRE found as a single copy in the mRNA of all sequenced ferritin cDNAs is responsible for the iron-dependent regulation of the translation of ferritin (5). IREs were then identified in the 3′ UTR of the mRNA encoding the human transferrin receptor (TfR) (6, 7). More recently, IREs have been observed in the 5′ ends of the mRNAs encoding the erythroid form of δ-aminolevulinate synthase (8, 9) and mitochondrial aconitase (9).

IREs provide the binding site for a cytosolic protein called the IRE-binding protein (IRE-BP) (10, 11). This protein has also been referred to as the ferritin repressor protein (FRP) (12) and the iron regulatory factor (IRF) (13). The interaction between the IRE-BP and IRE-containing mRNAs is regulated by iron availability (4, 7, 10, 11, 14, 15). When cells are given excess iron, the cytosolic IRE-BP isolated from those cells does not bind IREs with high affinity. In contrast, the IRE-BP assayed from the cytosol of cells deprived of iron demonstrates a high affinity (K_d < 100 pM) for IREs. The switch between high and low RNA-binding activity does not require protein synthesis and thus represents a posttranslational modification of the protein (14, 15). The inactive protein can be activated to bind RNA with high affinity in the presence of high concentrations of reducing agents, leading us to propose that a redox-sensitive sulfhydryl switch distinguishes the two binding states (14, 15). However, while this manipulation increased RNA binding *in vitro*, the protein differed from the high-affinity RNA-binding protein observed in lysates from iron-depleted cells in one critical respect; the change in RNA binding achieved *in vitro* was dependent on the continued presence of reducing agents. Reversion to low-affinity RNA binding was seen when the reducing agent was removed by dialysis or chromatography (ref. 16; D.J.H., unpublished observations).

The cDNAs encoding the human and murine IRE-BPs (17, 18) demonstrate a remarkable sequence similarity to mitochondrial aconitase. This similarity in primary sequence has suggested a model for a mechanism by which the IRE-BP might act as an iron sensor (19). Mitochondrial aconitase contains a single Fe-S cluster and, in its enzymatically active form, contains a [4Fe-4S] cluster. However, when purified, it readily loses one iron to become a [3Fe-4S] cluster and in this state has little, if any, enzymatic activity. Enzymatic activity can be readily restored by reloading the [3Fe-4S] cluster with iron *in vitro*. It is not known whether a similar cluster conversion occurs in the cell, but the reversible binding of iron in such Fe-S clusters would provide an intriguing mechanism for sensing levels of iron (20). The conservation of all structurally defined aconitase active-site residues in the IRE-BP led to the demonstration that IRE-BP is an aconitase (21, 22). We have now developed *in vitro* procedures that interconvert the RNA-binding states of the IRE-BP and demonstrate that the RNA-binding state of the protein and the functional aconitase state are mutually exclusive, both *in vitro* and in living cells. These studies provide evidence that Fe-S clusters can function as metal switches and can produce regulation of physiologically important RNA–protein interactions.

MATERIALS AND METHODS

Cells. All studies were done with either a human erythroleukemia cell line (K-562) or a mouse fibroblast cell line (B6).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IRE, iron-responsive element; IRE-BP, IRE-binding protein; TfR, transferrin receptor; DTT, dithiothreitol.

*To whom reprint requests should be addressed.

Cytosolic lysates were prepared as described (15). Hemin (Sigma) and desferrioxamine (CIBA Pharmaceutical) treatments of cells consisted of overnight incubation with the compounds at 50–100 μM (15).

RNA Synthesis and Gel Retardation Assay. Gel retardation assays and radiolabeled ferritin IRE RNA probe synthesis were performed essentially as described (15) except that an 8% acrylamide gel was used. Assay mixtures (20 μl) contained 5–20 μg of lysate protein and 0.25–1 ng of probe. Quantitation of the IRE-BP/RNA complex was done with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA).

Iron Unloading of IRE-BP in Lysates. Cytosolic lysate (20–100 μg) from hemin-treated K-562 cells in a 20-μl volume was treated with 100 mM dithiothreitol (DTT) in 100 mM Tris·HCl, pH 8.9/40 mM KCl for 10–20 min at room temperature. Subsequently, the samples were desalted on a G-50 spin column (Pharmacia) and 5–10 μg was used for the gel retardation assay to assess changes of binding of RNA to the IRE-BP in the lysate.

Iron Loading of IRE-BP in Lysates. Lysate (20–100 μg) in a 20-μl volume from desferrioxamine-treated cells was treated with 1 mM ferrous ammonium sulfate and 100 mM DTT in 25 mM Tris·HCl, pH 7.4/40 mM KCl for 10–20 min. The samples were desalted on a G-50 spin column prior to the gel retardation assay.

RNA-Binding and Aconitase Activity of Immunopurified Recombinant IRE-BP. The production of a Myc epitope-tagged, chimeric human–mouse IRE-BP has been described (22). The Myc-tagged IRE-BP (20 μg) was transfected into mouse B6 cells along with 0.5 μg of a thymidine kinase-encoding plasmid by previously described methods (7). A single cell line, termed human IRE-BP-1 (H/IRE-BP-1), that expressed the chimeric protein was selected. Cell lysate (800–1000 μg) was incubated with 10–20 μl of anti-Myc monoclonal ascites antibody (22, 23) for 30 min on ice in 5 ml of 25 mM Tris·HCl, pH 7.4/40 mM KCl/100 μM EDTA/1 mM sodium citrate. This was followed by 5 μl of affinity-purified rabbit anti-mouse antibody (Organon Teknika) and the incubation continued for an additional 30 min. Protein A-Sepharose (100 μl) was added, the sample was tumbled for 1 hr at 4°C and then centrifuged, and the pellet was washed twice with 100 mM Tris·HCl, pH 8.0. An aliquot was additionally washed in 25 mM Tris·HCl, pH 7.4/150 mM NaCl/1% (vol/vol) Triton X-100, and 1 ng of radiolabeled IRE was added in a 100-μl volume with 2-mercaptoethanol at a final concentration of 2% (vol/vol). After 5 min on ice the resin was washed twice in the buffer with 2-mercaptoethanol and the IRE-BP was quantitated by liquid scintillation counting. The number of moles of functional IRE-BP on the beads was estimated by assuming a 1:1 ratio of bound RNA to IRE-BP.

Aconitase activity was measured with the coupled aconitase/isocitrate dehydrogenase assay (24). The reaction components were 100 mM Tris·HCl (pH 8.0), 1 mM citrate, 1 mM $MgCl_2$, 1 mM NADP (Boehringer Mannheim), and isocitrate dehydrogenase (100 μg/ml; Boehringer Mannheim). The immunoprecipitated Myc–IRE-BP attached to Protein A-Sepharose was added to the aconitase assay components and the production of NADPH was followed by the change in absorbance at 340 nm.

RESULTS

Activation and Inactivation of the RNA-Binding Activity of IRE-BP *in Vitro*. The RNA-binding activity of IRE-BP is low if derived from cytosolic extracts of cells that have been treated with an iron source (hemin) and high if derived from cells treated with an iron chelator (desferrioxamine). These differences (Fig. 1) are stable and resistant to dialysis and column chromatography. While the RNA-binding activity of

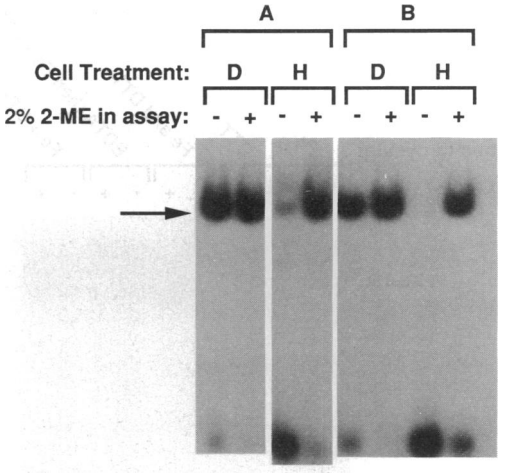

FIG. 1. Gel retardation assay of lysates from hemin- and desferrioxamine-treated cells. (*A*) Gel retardation assay using 5 μg of lysate from K-562 cells pretreated with either hemin (H) or desferrioxamine (D), assayed in the presence or absence of 2% 2-mercaptoethanol (2-ME). (*B*) Prior to the gel retardation assay, lysates from hemin- or desferrioxamine-treated cells were pretreated with 2% 2-ME for 5 min at room temperature and then put over a G-50 spin column to remove the reducing agent. Assays were then performed with or without 2% 2-ME as in *A*. These results indicate that activation with 2% 2-ME is dependent upon continued presence of reducing agent, whereas the hemin and desferrioxamine treatment of cells results in a difference that is stable to column chromatography.

IRE-BP derived from extracts of hemin-treated cells can be recovered if the RNA interaction is assessed in the presence of high concentrations of reducing agents (14–16), removing the reductant returns it to low RNA-binding activity (16) (Fig. 1). Thus, while the addition of reducing agents alone to the inactive IRE-BP activates RNA binding, it does not induce a stable change in the protein such as that which is produced within the iron-deprived cell. Because IRE-BP most likely contains an Fe-S cluster, we next focused on the possible role of alteration of this cluster as a basis for the stable differences between IRE-BP from iron-replete as compared to iron-deprived cells. One reasonable model might be that the iron-replete form of IRE-BP, which essentially does not bind RNA, contains a fully assembled [4Fe-4S] cluster. The [3Fe-4S] form of mitochondrial aconitase can be readily converted to the [4Fe-4S] form by the addition of iron salts in the presence of a reducing agent (20). When lysates derived from desferrioxamine-treated cells containing IRE-BP active for RNA binding were incubated with DTT and ferrous ammonium sulfate and then desalted, the IRE-binding activity was lowered (Fig. 2). Two percent (vol/vol) 2-mercaptoethanol will substitute for DTT in the iron-loading procedure, and DTT levels of 12.5 mM are sufficient for maximal inactivation of RNA-binding activity. The RNA-binding activity of the IRE-BP after iron loading resembled the activity of IRE-BP derived from lysates of cells grown in the presence of hemin. Binding activity can be recovered if assayed in the presence of 2-mercaptoethanol (Fig. 1). Treatment with either DTT or iron salts alone before desalting does not result in inactivation of RNA binding. Abrogation of the RNA-binding activity of the IRE-BP with DTT/Fe^{2+} is prevented by the inclusion of the iron chelator EDTA during the iron loading procedure. However, once the iron has been added to the protein, subsequent addition of EDTA, in the absence of reducing agents, fails to reactivate the protein. We also were able to inactivate the RNA binding by an alternative protocol that employed cysteine as a substitute for DTT. In this procedure, 2.5 mM cysteine (which will not itself transiently activate inactive IRE-BP) plus 40 μM iron reproduces the DTT/Fe^{2+}

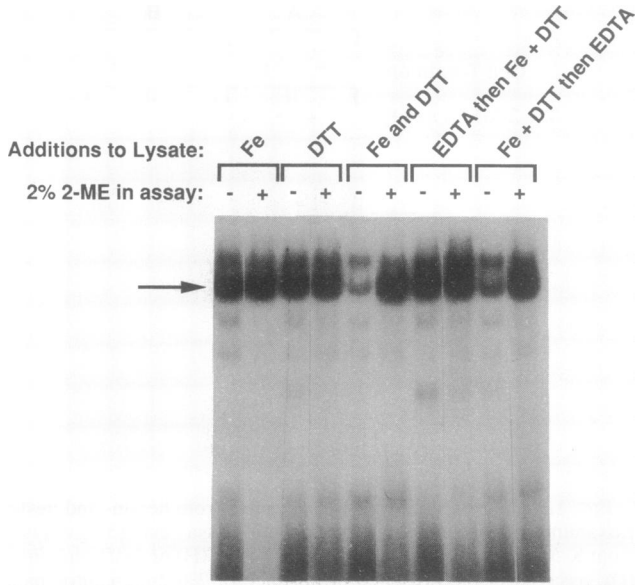

FIG. 2. Inactivation of IRE binding of IRE-BP in desferrioxamine-treated lysate by the addition of iron. Lysate (40 μg) from desferrioxamine-treated K-562 cells was incubated with 100 mM DTT and/or 1 mM ferrous ammonium sulfate as indicated. Approximately 5 μg of lysate from each treatment condition was assayed in the presence or absence of 2% 2-mercaptoethanol (2-ME) as indicated. EDTA added prior to DTT and iron prevent the inactivation of the RNA-binding activity. EDTA added after DTT and iron is without significant effect.

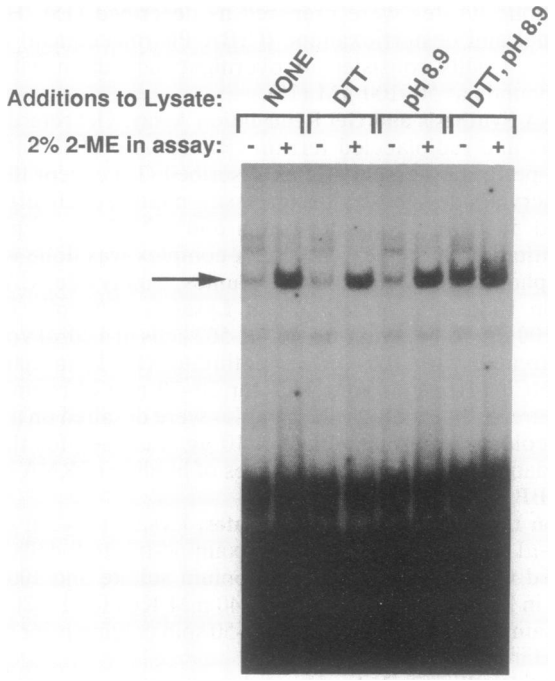

FIG. 3. Activation of RNA binding of the IRE-BP in lysate from hemin-treated cells. Lysate (40 μg) was incubated for 20 min at room temperature at pH 8.9, with 100 mM DTT, or the combination of high pH and DTT. All samples were desalted on a G-50 spin column and a portion of each sample was assayed by gel retardation assay with or without 2% 2-mercaptoethanol (2-ME).

effect. As in the DTT protocol, iron loading is blocked by the inclusion of EDTA.

The fact that procedures known to add iron to incomplete Fe-S clusters lead to loss of RNA-binding activity implies that the cluster may play a fundamental role in determining RNA-binding affinity. We therefore attempted to activate RNA binding of the IRE-BP found in lysates derived from hemin-treated cells. To accomplish this, lysates were treated with high concentrations of DTT at an alkaline pH (8.9) (Fig. 3). This treatment resulted in stable activation of RNA binding, even after removal of the reductant and neutralization. Neither the addition of reductant nor the alkaline pH alone stably activated the RNA-binding activity. This stable activation of RNA binding in the IRE-BP derived from hemin-treated cells can be contrasted with the reversible activation of RNA binding seen in the presence of high concentrations of reducing agents at neutral pH (Fig. 1). We further examined activation of inactive IRE-BP as a function of the concentration of DTT at pH 8.9. Increasing levels of activation were achieved with increasing amounts of DTT up to 50 mM. If the effects of this treatment on RNA binding activity were the result of labilizing the IRE-BP Fe-S cluster, we would predict that these effects would be antagonized by substrates for the enzyme, as substrate has been shown to enhance the stability of the Fe-S cluster of mitochondrial aconitase (20, 25). Inclusion of 1 mM citrate eliminated the activation of IRE-BP RNA-binding activity by DTT/high pH. As predicted from studies with mitochondrial aconitase, the same protection was afforded by two other ligands that have been shown to stabilize the aconitase fourth iron (20), isocitrate and tricarballylate (data not shown). The same cluster loading procedure can be used to inactivate the RNA-binding activity of the hemin-derived IRE-BP after activation of the RNA binding by the alkaline DTT protocol described above. Thus, the alterations in RNA-binding properties produced by these *in vitro* manipulations are stable to desalting procedures but are reversible when the protocols described above for inducing stable changes are applied.

Iron Manipulation Reciprocally Affects Aconitase Enzymatic Activity and RNA-Binding Activity of IRE-BP Both *in Vitro* and in Living Cells. If the *in vitro* manipulations described above accurately reflect *in vivo* alterations in the iron content of the putative Fe-S cluster, then iron manipulations, either of cells or of the isolated protein, should result in predictable alterations in the aconitase activity of the IRE-BP. To examine this issue, we constructed a stable mouse B6 cell line that expressed the human IRE-BP (H/IRE-BP-1). The RNA-binding activity of the human protein can be readily observed in this line and shown to be regulated by growth of the cells in the presence of either desferrioxamine or hemin in a manner that is indistinguishable from the regulation of the endogenous IRE-BP (Fig. 3). The human protein can be specifically immunoprecipitated from lysates by virtue of an added Myc epitope tag, and the purified protein can then be assayed for aconitase activity (22). Western blotting of total cell lysates revealed that the total amount of human IRE-BP was sometimes up to 2-fold higher in lysates of desferrioxamine-treated cells than in lysates of hemin-treated cells, but total amounts were never more significantly disparate (data not shown). Little aconitase activity was produced by IRE-BP purified from cells treated with desferrioxamine prior to lysis. However, *in vitro* treatment with either of the iron loading protocols that inactivate RNA binding of the IRE-BP successfully activated the aconitase enzyme activity. More importantly, we could directly assess whether the IRE-BP isolated from hemin-treated cells possessed aconitase activity in the absence of any further *in vitro* manipulations. The IRE-BP from hemin-treated cells had ≈10 times greater aconitase activity than the IRE-BP from desferrioxamine-treated cells (Fig. 4*B*). The number of moles of IRE-BP was determined by measuring the amount of radiolabeled IRE specifically bound to the immunoprecipitated protein after transient activation of RNA binding with 2% 2-mercaptoethanol. Measurement of total IRE-BP, assuming a 1:1 stoichiometry of RNA to protein, allowed us to

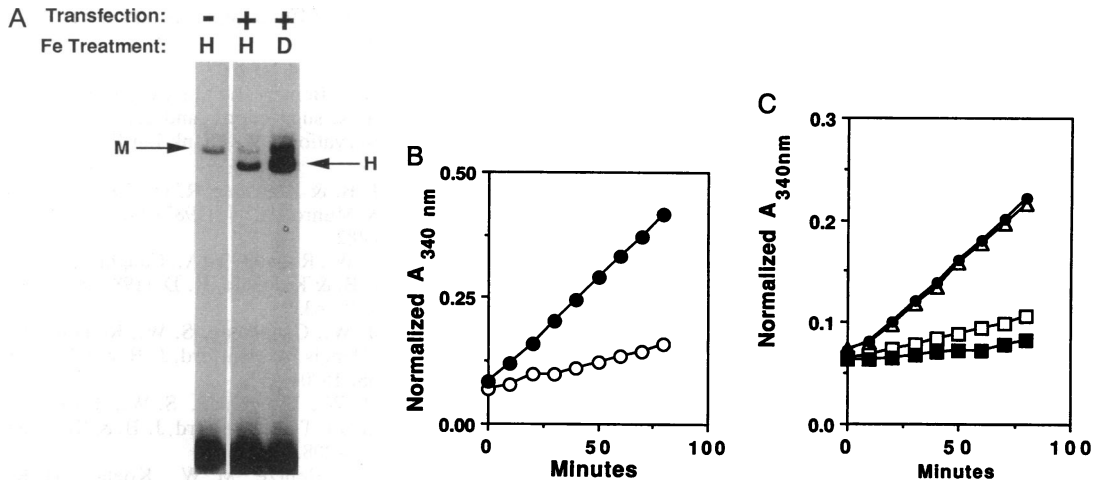

FIG. 4. (*A*) Recombinant IRE-BP expressed in a mouse cell line exhibits iron-dependent regulation of IRE binding. Cytosolic lysate (5 μg) from hemin (H)- or desferrioxamine (D)-treated H/IRE-BP-1 cells was assayed for RNA binding by gel retardation. (*B*) Representative experiment showing that IRE-BP from hemin-treated cells has more aconitase activity than IRE-BP from desferrioxamine-treated cells. IRE-BP from H/IRE-BP-1 cells that had been treated with either hemin (●) or desferrioxamine (○) was immunoprecipitated with anti-Myc. Aconitase activity was assayed on the immunoprecipitated material. The amount of IRE-BP was quantitated by binding of radiolabeled IRE to the IRE-BP bound via anti-Myc to protein A-Sepharose resin. The amount of IRE-BP on the resin was calculated on an assumption of 1 mol of RNA bound per mol of protein. The recovery of IRE-BP on the protein A-Sepharose resin varied between 6.5 and 16.4 ng for the experiments shown in this and *C*. Data on the ordinate were normalized to the sample with the highest recovery for the plots shown. Control reactions done with immunoprecipitates from B6 cells treated with hemin or desferrioxamine showed no activity. The calculated specific activity for aconitase activity in lysates from hemin-treated H/IRE-BP-1 cells was 55 ± 16 units/mg of IRE-BP (n = 3) and in lysates from desferrioxamine-treated H/IRE-BP-1 cells was 4 ± 3 units/mg of IRE-BP (n = 3). (*C*) *In vitro* manipulations that modulate RNA-binding activity also modulate aconitase activity in a reciprocal fashion. Lysates from desferrioxamine-treated H/IRE-BP-1 cells were treated with 100 mM DTT and 1 mM ferrous ammonium sulfate in the presence (□) or absence (△) of 5 mM EDTA. Lysates from hemin-treated H/IRE-BP-1 cells were treated with 100 mM DTT at pH 8.9 in the presence (●) or absence (■) of 1 mM citrate. The IRE-BP was immunoprecipitated with anti-Myc and then assayed for aconitase activity.

calculate a specific aconitase activity of the IRE-BP derived from hemin-treated cells of approximately 55 ± 16 units/mg of protein (n = 3). This compares to a specific activity of about 15 units/mg for the [4Fe-4S] form of mitochondrial aconitase (20), thus demonstrating that the IRE-BP has a specific activity that is comparable to that which has been calculated for the mitochondrial aconitase. These data indicate that *in vivo* iron manipulations of RNA-binding activity correlate with a reciprocal change in the aconitase activity of the IRE-BP.

Immunoaffinity-purified IRE-BP obtained from lysates from the H/IRE-BP-1 cells was used to assess whether the *in vitro* iron manipulations were effective at altering the IRE-BP aconitase activity (Fig. 4*C*). When the immunopurified IRE-BP from cells treated with hemin was subjected to the DTT/high pH treatment that activates RNA binding, the IRE-BP no longer had aconitase activity, consistent with disruption of the cluster. This loss of enzyme activity was prevented by inclusion of 1 mM citrate and could be recovered by the iron loading protocols. Conversely, when the IRE-BP derived from desferrioxamine-treated cells was subjected to the iron loading protocol that inactivates RNA binding, the IRE-BP developed full aconitase activity that was resistant to the subsequent addition of EDTA. If, however, EDTA was included during the iron loading, activation of enzymatic activity was prevented. Thus, as in the cell, there is a reciprocal relationship between RNA binding and aconitase enzyme activity of the purified protein for all of the *in vitro* manipulations described in this paper.

DISCUSSION

The regulated fates of mRNAs encoding proteins of iron metabolism have provided an excellent system for the study of regulation at the level of mRNA (1). Ferritin expression is translationally regulated, and TfR expression is regulated at the level of mRNA turnover. The recognition that the trans-acting factor in this system, IRE-BP, is a cytosolic aconitase, homologous to the well-described mitochondrial aconitase, has suggested a possible model for aspects of how this protein might sense changes in iron levels within the cytosol (19, 22). Formulation of this model was stimulated by the observation that, *in vitro*, the cubane Fe-S cluster of aconitase can interconvert between a [3Fe-4S] and a [4Fe-4S] state (26). Whether or not such an interconversion for the mitochondrial enzyme or any other Fe-S cluster protein ever occurs *in vivo* is a question that has not been previously explored.

Our data show that the inactive RNA-binding protein isolated from iron-replete cells appears to be a fully active aconitase, whereas the protein derived from iron-starved cells has little aconitase activity. When the latter protein is loaded with iron *in vitro*, RNA-binding activity is lost and aconitase activity is recovered. These data provide evidence that the cell alters the Fe-S cluster, which, in turn, regulates the IRE-binding activity of this protein. The evidence that the Fe-S cluster is the locus of regulation of this protein leaves unanswered some questions about the nature of the cluster alteration. That the form of the protein which cannot bind RNA is a fully active aconitase strongly suggests that this form of the protein contains a fully assembled [4Fe-4S] cluster. The status of the cluster components in the protein derived from desferrioxamine-treated cells is not yet defined. Treatment of IRE-BP from either hemin- or desferrioxamine-treated cells with concentrations of ferricyanide known to completely remove Fe from Fe-S clusters (20) demonstrates that the apoprotein is fully capable of RNA binding (data not shown). Thus, while the status of the IRE-BP cluster within iron-starved cells remains to be determined, the ability of the apoprotein to bind to RNA suggests that the physiological switch may be between a [4Fe-4S] cluster in iron-replete cells and the apoprotein in iron-deprived cells. Thus, the content of iron and sulfide of the physiological RNA-binding form of

the IRE-BP remains to be determined. That aconitase activity of the IRE-BP from iron-starved cells can be recovered by the addition of iron without the addition of exogenous sulfur suggests that the RNA-binding form of the protein may already contain sulfur (25).

The relationship between alterations in the Fe-S cluster and the ability of the protein to bind RNA must be further defined and related to previous observations that led to the idea that the RNA-binding state could be regulated by a "sulfhydryl switch" (14). This idea was based on two types of observations: (*i*) the protein inactive for RNA binding in extracts from iron-treated cells was activated *in vitro* to bind RNA by the addition of reducing agents and (*ii*) the active, RNA-binding protein could be reversibly inactivated by oxidation and irreversibly inactivated for RNA binding by *N*-ethylmaleimide, a sulfhydryl-alkylating reagent, whereas the inactive binding protein was insensitive to alkylation. Both the observations concerning the Fe-S cluster reported here and the previous results can be described by a model in which the protein is proposed to exist in either of two conformations: "open," in which it is competent for high-affinity RNA binding but has no aconitase activity, and "closed," in which it is competent for enzymatic activity but does not bind RNA. The conformation is determined by the Fe-S cluster, with a replete [4Fe-4S] cluster favoring the closed state. Reduction alone may allow the protein to enter the open state, regardless of the Fe-S cluster status. Whether the reductant, in this situation, is acting on the cluster, the protein, or both remains to be determined, although it is attractive to imagine that only certain oxidation states of the cluster can maintain the "closed" protein conformation. Reductant alone does not, however, change the underlying composition of the cluster, since removal of the reducing agent results in the reversion of the protein to its inactive RNA-binding state. The alkylation-sensitive site presumably represents one or more sulfhydryls that are exposed only in the open state. We can speculate about the relationship between a fully assembled cluster and a protein conformational change based upon the structure of the mitochondrial aconitase (21), in which the fourth protein domain is connected to the first three domains by a hinge/linker. Residues of the fourth domain interact with substrate, which in turn interacts with the Fe-S cluster substrate and the other domains of the protein (21). In the absence of the cluster and substrate, the interaction between the fourth domain and the rest of the protein may be weakened, allowing the exposure of an RNA binding site that may include the active-site cleft.

We need to understand the mechanism by which the cluster is actually altered within the cell and whether the regulatory mechanism requires that the protein have enzymatic activity. Whatever the details of the events that lead to and take place after the loading or release of iron, we believe that a physiologically labile Fe-S cluster provides the key to the ability of this protein to be altered in response to available iron levels. Such a cluster-based model provides a powerful mechanism for sensing environmental changes. It has been suggested that another Fe-S cluster-containing protein, ferredoxin I of *Azotobacter*, may be a DNA-binding protein that is regulated by iron (27). It has also been proposed that, in *Escherichia coli*, the aconitase Fe-S cluster provides a switch for sensing oxidative stress (28), suggesting that this inorganic complex may represent a very ancient molecular sensing device. These observations on the regulation of the IRE-BP point to an important regulatory role for Fe-S clusters in addition to the multiple known functions already ascribed to Fe-S clusters (26, 29, 30).

We thank Helmut Beinert and Mary Claire Kennedy for numerous helpful discussions, suggestions, and generous communications of unpublished observations. We thank Ian Paul for his assistance.

1. Harford, J. B. & Klausner, R. D. (1990) *Enzyme* **44**, 17–27.
2. Aziz, N. & Munro, H. N. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8478–8482.
3. Hentze, M. W., Rouault, T. A., Caughman, S. W., Dancis, A., Harford, J. B. & Klausner, R. D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6335–6339.
4. Hentze, M. W., Caughman, S. W., Rouault, T. A., Barriocanal, J. G., Dancis, A., Harford, J. B. & Klausner, R. D. (1987) *Science* **238**, 1570–1573.
5. Hentze, M. W., Caughman, S. W., Casey, J. L., Koeller, D. M., Rouault, T. A., Harford, J. B. & Klausner, R. D. (1988) *Gene* **72**, 201–208.
6. Casey, J. L., Hentze, M. W., Koeller, D. K., Caughman, S. W., Rouault, T. A., Klausner, R. D. & Harford, J. B. (1988) *Science* **240**, 924–928.
7. Casey, J. L., DiJeso, B., Rao, K., Klausner, R. D. & Harford, J. B. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1787–1791.
8. Cox, T. C., Bawden, M. J., Martin, A. & May, B. K. (1991) *EMBO J.* **10**, 1891–1902.
9. Dandekar, T., Stripecke, R., Gary, N. K., Goossen, B., Constable, A., Johansson, H. E. & Hentze, M. W. (1991) *EMBO J.* **10**, 1903–1910.
10. Leibold, E. A. & Munro, H. N. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2171–2175.
11. Rouault, T. A., Hentze, M. W., Caughman, S. W., Harford, J. B. & Klausner, R. D. (1988) *Science* **241**, 1207–1210.
12. Brown, P. H., Daniels-McQueen, S., Walden, W. E., Patino, M. M., Gaffield, L., Bielser, D. & Thach, R. E. (1989) *J. Biol. Chem.* **264**, 13383–13386.
13. Mullner, E. W., Neupart, B. & Kuhn, L. C. (1989) *Cell* **58**, 373–382.
14. Hentze, M. W., Rouault, T. A., Harford, J. B. & Klausner, R. D. (1989) *Science* **244**, 357–359.
15. Haile, D. H., Hentze, M. W., Rouault, T. A., Harford, J. B. & Klausner, R. D. (1989) *Mol. Cell. Biol.* **9**, 5055–5061.
16. Barton, H. A., Eisenstein, R. S., Bomford, A. & Munro, H. N. (1990) *J. Biol. Chem.* **265**, 7000–7013.
17. Rouault, T. A., Tang, C. K., Kaptain, S., Burgess, W. H., Haile, D. J., Samaniego, F., McBride, D. W., Harford, J. B. & Klausner, R. D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7958–7962.
18. Philpott, C., Rouault, T. A. & Klausner, R. D. (1991) *Nucleic Acids Res.* **19**, 6333.
19. Rouault, T. A., Stout, C. D., Kaptain, S., Harford, J. B. & Klausner, R. D. (1991) *Cell* **64**, 881–883.
20. Kennedy, M. C., Emptage, M. H., Dreyer, J. & Beinert, H. (1983) *J. Biol. Chem.* **258**, 11098–11105.
21. Lauble, H., Kennedy, M. C., Beinert, H. & Stout, C. D. (1992) *Biochemistry* **31**, 2735–2748.
22. Kaptain, S., Downey, W. E., Tang, C. K., Philpott, C., Haile, D., Orloff, D. G., Harford, J. B., Rouault, T. A. & Klausner, R. D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10109–10113.
23. Pelham, H. R. B. (1988) *EMBO J.* **7**, 913–918.
24. Rose, I. A. & O'Connell, E. L. (1967) *J. Biol. Chem.* **242**, 1870–1879.
25. Kennedy, M. C. & Beinert, H. (1988) *J. Biol. Chem.* **263**, 8194–8198.
26. Beinert, H. & Kennedy, M. C. (1989) *Eur. J. Biochem.* **186**, 5–15.
27. Thomson, A. J. (1991) *FEBS Lett.* **285**, 230–236.
28. Gardner, P. R. & Fridovich, I. (1991) *J. Biol. Chem.* **266**, 19328–19333.
29. George, S. J., Armstrong, F. A., Hatchikian, E. C. & Thomson, A. J. (1991) *Biochem. J.* **264**, 275–284.
30. Beinert, H. (1990) *FASEB J.* **4**, 2483–2491.