Hypoxia Alters Iron-regulatory Protein-1 Binding Capacity and Modulates Cellular Iron Homeostasis in Human Hepatoma and Erythroleukemia Cells*

(Received for publication, August 11, 1998, and in revised form, November 12, 1998)

Ildiko Toth, Liping Yuan, Jack T. Rogers, Hayden Boyce, and Kenneth R. Bridges†

From the Joint Center for Sickle Cell and Thalassemic Disorders, Hematology Division, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts 02115

Ferritin and transferrin receptor expression is post-transcriptionally regulated by a conserved mRNA sequence termed the iron-responsive element (IRE), to which a transacting protein called the iron-regulatory protein (IRP) is bound. Our data demonstrate that hypoxia powerfully enhances IRE/IRP-1 binding in human cell lines. Using the human hepatoma cell line Hep3B as a model, we found that 16 h in a 1% oxygen atmosphere markedly increases IRE/IRP-1 binding as assessed by electromobility shift assay. Hypoxia also decreased cytosolic aconitase activity. The hypoxia-enhanced IRE/IRP-1 binding stabilized the transferrin receptor message, increased the cellular mRNA content by over 10-fold, and doubled surface receptor expression. Simultaneously, hypoxia suppressed ferritin mRNA translation. Hypoxia’s effect was most strikingly depicted by the absence of ferritin synthesis in cells challenged with inorganic iron. Our results contrast with previously reported data (Hanson, E. S., and Leibold, E. A. (1998) J. Biol. Chem. 273, 7588–7593) in which a 3% oxygen atmosphere reduced IRE/IRP-1 binding in rat hepatoma cells. We discuss some possible reasons for the differences. In aggregate with other investigations involving responses to hypoxia, iron, or nitric oxide, our data indicate that cellular iron metabolic responses are complex and that IRE/IRP-1 interactions vary between cell lines and perhaps between species.

Iron is a key element in cellular growth and metabolism. The element is part of the active site of many enzymes, often as a component of heme or as part of an iron-sulfur complex (1). As an enzyme prosthetic group, iron catalyzes redox reactions involving proteins, lipids, carbohydrates, and nucleic acids. The ability of the element to exist in either of two stable oxidation states (ferric, Fe3+; ferrous, Fe2+) is the key to its enzymatic activity. Unrestrained, however, iron can wreak havoc on cells through the production of reactive intermediates (2), including the deadly hydroxyl radical (OH•).

Cellular iron is closely regulated, in part through the actions of the transferrin receptor and ferritin, the proteins of cellular iron uptake and storage, respectively (3). Expression of these two proteins is largely regulated at the post-transcriptional level (4). A conserved 28-base sequence termed the iron-responsive element (IRE)1 exists in the ferritin 5′-UTR, whereas five such elements are located in the transferrin receptor 3′-UTR (5). The two cytoplasmic iron-regulatory proteins 1 and 2 (IRP-1 and IRP-2) recognize and bind to the IRE (6, 7). IRE/IRP-1 binding in the 5′-UTR blocks message translation. Such binding in the 3′-UTR stabilizes the message against enzymatic degradation (8).

Regulation of IRE/IRP-1 binding is a complex affair (9). Iron modulates IRP-1 binding to IRE elements by forming a 4Fe-4SH cluster within the protein. When the cluster is intact, IRP-1 cannot bind to the IRE and exists free in the cytosol (10). In this state, IRP-1 has aconitase activity. In the absence of iron, the 4Fe-4SH cluster collapses, aconitase activity is lost, and IRP-1 acquires IRE binding capacity. Therefore, IRP-1 is a dual function protein, serving alternatively as a cytoplasmic aconitase or as a transacting RNA-binding protein. In contrast to IRP-1, the IRP-2 molecule lacks aconitase activity and is susceptible to proteolytic degradation when it is free in the cytosol (11, 12).

Investigators have described a number of other factors that modify IRE/IRP-1 binding and ferritin synthesis such as H2O2 and nitric oxide (13, 14). Cytoplasmic IRP-1 appears to exist in equilibrium between forms that either possess or lack aconitase activity. Ascorbic acid shifts the equilibrium toward aconitase (+) IRP-1 (15). This creates a thermodynamic “sink” that favors greater IRP-1 release when iron interacts with the IRE/IRP-1 complex.

The current work uses the human hepatoma cell line Hep3B to examine the effect of hypoxia on cellular iron metabolism. These cells mimic fetal hepatocytes in many respects and were the first cells shown to produce human erythropoietin in response to hypoxia (16). Their response to hypoxia has been studied extensively, making them an excellent model system. Hypoxia induces the expression of HIF-1 in Hep3B cells, a universal transcription factor that activates genes needed to adapt to low oxygen tensions (17, 18). The expression of HIF-1 (and its DNA binding capacity) increases as the oxygen tension falls, peaking at a 0.5–1% oxygen concentration (19). We now demonstrate that hypoxia promotes powerful IRE/IRP-1 binding in these cells that resists even iron-mediated dissociation. Hypoxia markedly alters the transcription of several genes (17), including those encoding erythropoietin (epo) and vascular endothelial growth factor (18–22). The promoters of these genes contain binding sites for HIF-1. Hypoxia changes gene transcription by altering HIF-1 binding (23). In distinction to these well-established effects of hypoxia, the effect on IRE/IRE-UTR stabilizes the message against enzymatic degradation (8).

‡ To whom all correspondence should be addressed: Joint Center for Sickle Cell and Thalassemic Disorders, BLI 327, 221 Longwood Ave., Boston, MA 02115. Tel.: 617-732-5842; Fax: 617-739-3324; E-mail: bridges@calvin.bwh.harvard.edu.

1 The abbreviations used are: IRE, iron-responsive element; epo, erythropoietin; IRP, iron-regulatory protein; HIF, hypoxia-inducible factor; TfR, transferrin receptor; UTR, untranslated region; bp, base pair(s).

* This work was supported by National Institutes of Health Grant HL 457940. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Hypoxia and IRE-1 in Human Cells

IRP-1 interaction is a post-transcriptional phenomenon. Hanson and Leibold (24) reported previously that exposing rat hepatoma cells to a 3% atmosphere decreased IRP-1 binding to the IRE but did not alter IRP-2 binding. We discuss possible reasons for the differences.

MATERIALS AND METHODS

Cell Culture—Human hepatoma Hep3B cells were maintained in α-minimum essential medium supplemented with 10% fetal bovine serum (Biowhittaker, Walkersville, MD), penicillin/streptomycin, and pyruvate (15). Cells at 75% confluence were exposed to hypoxia (1% O₂, 5% CO₂, and 94% N₂) at 37 °C in an Espe triple gas incubator (Taba- Espec Corp., Osaka, Japan) for different time periods (4 or 16 h) (23). Control cells were cultured under normoxic conditions (21% O₂, 5% CO₂, and 74% N₂) in a humidified Napco incubator. After hypoxia treatment, the cells were chilled, washed with cold phosphate-buffered saline to preserve the hypoxic response, harvested, and used immediately according to the experimental requirement.

K562 human erythroleukemia cells were maintained in RPM 1640 medium supplemented with 10% fetal bovine serum (Biowhittaker) and penicillin/streptomycin at a density of 5 × 10⁵ cells/ml. Hypoxia treatment was as outlined above.

Electromobility Shift Assay—IRE binding to RNA was assessed by electromobility shift assay as described previously (4, 15). Briefly, cytoplasmic cell extracts were prepared from Hep3B or K562 cells grown under conditions of hypoxia or normoxia in the presence or absence of 100 μM desferrioxamine or 10 μg/ml ferric ammonium citrate. An excess quantity of [³²P]UTP-labeled RNA transcript (from pSPT-fer, a 28-nucleotide fragment encoding the human H-ferritin IRE; a generous gift of Dr. Kühn (25)) was incubated at room temperature for 30 min with 3 μg of protein of fresh cytoplasmic cell lysate. RNase T1 (1 unit/reaction) and heparin (5 mg/ml) were added sequentially for 10 min each. IRE-IRP-1 complex was analyzed on a 6% nondenaturing polyacrylamide gel, as detailed previously (15).

Nuclear Run On Assay—Nuclei were isolated from control or experimentally manipulated cells using a STAT-30 kit from TEL-TES B, Inc. (Friedewood, TX), following the manufacturer's instructions. Twenty μg of RNA were separated on an agarose/formaldehyde gel and immobilized to Hybond-N nylon membrane (Amersham, Arlington Height, IL) using a 0.05 N fixation (26). The RNA was hybridized with a 600-bp pBS1 fragment of TfR cDNA labeled by the Random primer method according to the supplier's protocol. After hybridization and washing by the method of Church and Gilbert (27), the membrane was exposed to x-ray film for 2 days. The TfR signal was stripped off by boiling the membrane in 0.2% SDS for 40 min at 68 °C and exposed to x-ray film for 3 days.

Cytosolic Aconitase Assay—Cytosolic aconitase activity was assessed by the consumption of cis-aconitate as measured by the spectrophotometric absorbance at 240 nm (as detailed previously in Ref. 15). Briefly, mitochondrial-free lysates from Hep3B cells grown under conditions of hypoxia or normoxia (50 μg) were incubated with 200 μM cis-aconitate in 50 mM Tris-HCl buffer, pH 7.2, 100 mM NaCl, and 0.02% bovine serum albumin at room temperature in a volume of 1 ml. Specific activity (μM substrate converted/mg protein/min) was calculated as described by Emery-Goodman et al. (30).

Cytosolic Ferritin Content Determination—The steady-state level of cytosolic ferritin was determined using a Coat-A-Count Ferritin IRMA assay kit whose limit of ferritin detectability is 0.1 ng/ml (Diagnostic Products Corp., Los Angeles, CA). Briefly, 10⁶ cells/assay were harvested, washed, and solubilized in a detergent buffer (1% Triton X-100, 150 mM NaCl, 20 mM Tris-HCl, pH 7.5, and 0.2 mM phenylmethylsulfonyl fluoride). Ferritin was immunoprecipitated with monoclonal antibody to the L subunit and detected with a second antibody labeled with ¹²⁵I.
Hypoxia and IRE-1 in Human Cells

Hypoxia and IRE-1 in Human Cells

Fig. 2. A brief period of hypoxia modestly increases IRE/IRP-1 binding. Hep3B cells were treated as detailed in the Fig. 1 legend, except that the period of hypoxia was 4 h. The experiment was repeated several times, and one representative result is shown.

hypoxic cells exceeds that in the normoxic cells by at least 10-fold.

Consistent with a substantial body of previous information, IRE/IRP-1 binding rose dramatically when cells in a 21% O2 atmosphere were treated for 16 h with 100 μM desferrioxamine (D). By contrast, desferrioxamine treatment did not increase IRE/IRP-1 binding in cells in a 1% O2 atmosphere. Therefore, chelation of intracellular iron does not further enhance hypoxia-stimulated IRE/IRP-1 binding. This suggests that hypoxia promoted IRE/IRP-1 association to the maximum possible extent.

With each electromobility shift assay, a parallel incubation was performed that included 2-mercaptoethanol. This agent promotes maximal IRE/IRP-1 binding when added to the in vitro incubation mixture. As shown in Fig. 1, maximal IRE/IRP binding was equivalent for cells in a 21% O2 or 1% O2 atmosphere. Therefore, hypoxia increases the extent of IRE/IRP-1 binding but does not change the overall IRE/IRP-1 binding capacity of the cells.

The expected decrease in IRE/IRP-1 binding that occurred with the addition of iron in the form of ferric ammonium citrate to the normoxic cells was paralleled in the hypoxic cells. Therefore, hypoxia enhances IRE/IRP-1 binding without altering its physiological response to iron.

The 16-h period of hypoxia did not affect the viability of Hep3B cells as assessed by trypan blue staining. Hypoxia enhanced IRE/IRP-1 binding in cells exposed to 1% O2 for only 4 h (Fig. 2). The increase was only 2-fold over the baseline, suggesting that the metabolic changes that augment IRE/IRP-1 binding develop over time.

We also examined the effect of hypoxia on IRE/IRP-1 binding in K562 cells. Fig. 3 shows that 16 h of hypoxia enhanced IRE/IRP-1 binding by only 2-fold (as assessed by densitometric scanning). In contrast, incubating the cells with erythropoietin at a concentration of 50 units/ml boosted IRE/IRP-1 binding by 5-fold, consistent with the observations by other investigators (32). Therefore, K562 cells can shift IRE/IRP-1 binding characteristics in response to environmental stimuli. The response of Hep3B cells and K562 cells to hypoxia was qualitatively similar but differed in magnitude. The standard electromobility shift assay separates RNA/protein complexes by charge. The technique does not separate IRE/IRP-1 and IRE/IRP-2 in extracts derived from human hepatoma or erythroleukemia cells (32). Western blot analysis indicates that the level of IRP-2 protein is very low in these cell lines (data not shown). Supershift experiments were inconclusive.

Hypoxia Decreases Cytosolic Aconitase Activity—Cytosolic aconitase activity and RNA binding capacity are mutually exclusive properties of IRP-1. The hypoxia-induced increase in IRE/IRP-1 binding should lessen the amount of free cytosolic IRP-1 protein. IRP-1 is an aconitase enzyme when it is free in the cytosol, but it lacks this activity when it is part of an IRE/IRP-1 complex. On this basis, we predicted that hypoxia would lower cytosolic aconitase activity.

K562 cells and Hep3B cells exposed to 1% O2 for 16 h were lyzed, and the 100,000 × g supernatant was collected. Cytochrome c oxidase activity was 1.5 unit in the pellet and was undetectable in the supernatant, indicating no contamination by this mitochondrial enzyme. The same should hold for mitochondrial aconitase (15). As shown in Table I, hypoxia markedly decreased cytosolic aconitase activity in both cell lines. The magnitude of the decline produced by hypoxia was similar to that seen with desferrioxamine treatment. The significant decrease in enzyme activity correlates well with the marked increase in the binding of IRP-1 to IREs. Although hypoxia shifts IRP-1 onto IRE-containing transcript, the aconitase that remains in the cytosol still shows iron-dependent regulation in a 1% oxygen atmosphere (Table I; Hep3B cells). Only IRP-1 has aconitase activity. Our electromobility shift assay can not distinguish between IRP-1 and IRP-2. Consequently, we can not assess the effect of hypoxia on IRP-2.

Hypoxia Increases Transferrin Receptor mRNA levels—IRE/IRP-1 binding stabilizes mRNAs with IREs in the 3′-UTR. Therefore, hypoxia should increase the steady-state level of the transferrin receptor transcript. To test this prediction, mRNA was isolated from Hep3B cells maintained at 1% O2 for 16 h.

Fig. 4 shows that hypoxia substantially increases transferrin receptor mRNA levels relative to control as assessed by Northern blot analysis (lane 1 versus lane 4). The magnitude of the increase in transferrin receptor mRNA produced by hypoxia is similar to that produced by desferrioxamine. In contrast to the striking increase in transferrin receptor message produced by 16 h of hypoxia, a 4-h period of oxygen deprivation had little, if any, effect on the level (data not shown). Fig. 4 also shows that 16 h of hypoxia did not affect the level of the ferritin L subunit transcript. Therefore, the hypoxia-induced increase in transferrin receptor message did not reflect a global change in mRNA metabolism.

Nuclear Run On Assay Indicates No Effect by Hypoxia on Transferrin Receptor Gene Transcription—In both prokaryotic
and eukaryotic organisms, hypoxia can induce transcriptional activity of physiologically important genes (for a review, see Ref. 17). Northern blot analysis (Fig. 4) showed an increase of greater than 10-fold in the steady-state level of Tfr mRNA in Hep3B cells exposed to hypoxia. Nuclear run on assay was performed to determine whether the observed increase was due to enhanced transcription. We predicted that the hypoxia-induced increase in transferrin receptor message represented stabilization of the message due to binding of the IRP-1 to the IREs in the 3′-UTR of the transcripts.

Nuclei were isolated from control Hep3B cells or from cells exposed to 1% O2 for 16 h. Hypoxia affected neither the cell viability nor yield of isolated nuclei. Fig. 5 shows no effect by hypoxia on the transcription of the transferrin receptor message. In contrast, hypoxia increased the synthesis of erythropoietin message, as expected (16). These data confirm that hypoxia increases Tfr mRNA levels in Hep3B cells by stabilizing the message.

**Hypoxia Increases Transferrin Receptor Surface Expression**—An increase in IRP binding to the IREs in the 3′-UTR of the transferrin receptor message raises the level of transferrin receptor mRNA and secondarily increases transferrin receptor expression. This phenomenon is seen most strikingly in cells treated with desferrioxamine. Because hypoxia mimics the effect of desferrioxamine on IRE/IRP-1 binding and transferrin receptor mRNA expression in these cells, we predicted that hypoxia would also increase transferrin receptor protein expression.

Fig. 6 shows that surface expression of transferrin receptors doubles in Hep3B cells exposed to hypoxia for 16 h (2.5 × 10^4 versus 4.9 × 10^4 receptors/cell). Scatchard analysis of the data shows no change in ligand/receptor affinity (K_d = 8.6 nM). Binding studies also show that the total transferrin binding capacity of the cells increased by more than 2-fold (5 versus 13.7 ng/10^6 cells). The increase in transferrin receptor message produced by 16 h of hypoxia is functionally significant in that it substantially raises transferrin receptor surface expression. In contrast, a 4-h period of hypoxia had no effect on transferrin receptor expression (data not shown).

**Hypoxia Decreases Cellular Ferritin Synthesis and Content**—A second functional consequence of IRE/IRP-1 binding is impaired translation of messages containing an IRE element in the 5′-UTR. For example, desferrioxamine chelates intracellular iron, increases IRE/IRP-1 binding, and blocks translation of the ferritin message. We predicted that the increase in IRE/IRP binding produced by hypoxia would also block translation of the ferritin message.

We therefore assessed ferritin synthesis in control cells and in cells exposed to hypoxia for 16 h. Fig. 7 is a polycrylamide gel electrophoresis of [35S]methionine-labeled ferritin immunoprecipitated with polyclonal anti-ferritin antibody. Ferritin synthesis is diminished modestly in the hypoxic cells relative to controls. Iron normally increases ferritin synthesis by dissociating the IRP from the IRE. Fig. 7 shows that this occurred in control cells. However, hypoxic cells did not increase ferritin synthesis in response to iron. This indicates that the hypoxia-induced IRE/IRP-1 binding is very tight and resists dissociation in response to iron.

Reduced ferritin synthesis in hypoxic cells should reduce the cellular ferritin content. We assessed the ferritin content of control cells and hypoxic cells using a commercially available radioimmunoassay kit. Table II shows that the ferritin content of hypoxic cells is about one-third that of the control. Also consistent with the previous ferritin biosynthesis studies, the
Hypoxia and IRE-1 in Human Cells

FIG. 6. Hypoxia increases cell surface expression of the transferrin receptor. Hep3B cells (5 × 10⁶) were exposed to hypoxia for 16 h. Cell surface ¹²⁵I-labeled transferrin binding was measured as described under “Materials and Methods.” Briefly, cells were incubated in 25 mM HEPES, pH 7.4, 150 mM NaCl, and 1 mg/ml bovine serum albumin with the indicated concentrations of ¹²⁵I-labeled transferrin (0.5–50 nM) with or without 100-fold excess cold transferrin for 1 h at 4 °C. After the binding, the buffer was removed, the cells were solubilized in situ, and the radioactivity was measured by gamma counting. The number of binding sites/cell (after correcting for nonspecific binding) are as follows: control, 2.5 × 10⁷; and hypoxia-treated cells, 4.9 × 10⁷. The dissociation constant (Kd) was 8.6 nM for both cell populations. The binding studies were repeated several times, and the result of one representative experiment is shown.

FIG. 7. Hypoxia reduces baseline ferritin synthesis and blocks iron-stimulated ferritin synthesis. Hep3B cells (5 × 10⁶) at 75% confluence were treated with 10 μg/ml ferric ammonium citrate (Fe) for 16 h under conditions of normoxia (21% oxygen) or hypoxia (1% oxygen). Control and iron-treated cells were washed with phosphate-buffered saline and metabolically labeled with [³²P]methionine in a methionine-free medium for 2 h. The cells were washed and solubilized in a buffer containing 1% Triton X-100, 0.15 mM NaCl, 0.02 M Tris-HCl buffer, pH 7.5, and 0.2 mM phenylmethylsulfonyl fluoride. The newly synthesized, labeled ferritin molecules were immunoprecipitated with human ferritin antibody, and the complex was separated on a 15% SDS-phosphate-urea gel. The experiments were repeated several times, and one representative result is shown.

H-ferritin-L-ferritin

21% O₂ 1% O₂
C Fe C Fe

Ferritin content of control cells treated with 10 mg/ml ferric ammonium citrate was 10-fold greater than the baseline. In striking contrast, iron salt increased the ferritin content of hypoxic cells by only about 20%.

DISCUSSION

The interplay of IRE/IRP-1 binding in the post-transcriptional regulation of ferritin and transferrin receptor synthesis was first described in the context of iron-mediated changes (1, 5). In the presence of iron, the reactive center of the IRP-1 protein forms a 4Fe-4SH structure that confers aconitase activity to IRP-1, the first of the two presently known IRP proteins to be described. In the absence of iron, the active site collapses, abolishing aconitase activity. This change is counterbalanced by the acquisition of RNA binding capacity with the IRE sequence as the binding target.

Factors other than iron modulate IRE/IRP-1 interaction. Nitric oxide promotes IRP-1 binding to the IRE (33, 34). The likely mechanism is an attack by the free radical on the iron in the 4Fe-4SH cluster. The result is a marked reduction in ferritin synthesis, along with a rise in transferrin receptor expression. Ascorbic acid also strikingly alters the synthesis profile of ferritin and the transferrin receptor. Ascorbate alone does not change ferritin synthesis (15, 26). However, the vitamin strikingly increases ferritin synthesis in response to iron. Ascorbate does not directly alter the IRE/IRP-1 interaction but rather shifts free IRP-1 between states with or without aconitase activity (15). The result is that a larger fraction of IRP-1 dissociates from the ferritin message in response to iron.

The present report details our experience with the effects of hypoxia on cellular iron metabolism using two human cell lines, K562 and Hep3B. We focused on the latter because of the extensive studies of hypoxia in the expression of e-p and HIF-1-mediated gene responses (20–23). Electromobility shift assay shows that IRE/IRP-1 binding increases substantially in cells maintained for 16 h in a 1% O₂ atmosphere. The phenomenon occurred in both Hep3B and K562 cells, although the magnitude was much greater in the former. The electromobility shift assay involves the addition of radiolabeled RNA probe (in this case, IRE) to cytosol isolated from control and hypoxia-treated cells. The increase in IRE/IRP-1 binding in this assay means that the change in the IRP produced by the in vivo manipulation of cellular oxygen status persists after the cells have been lysed.

Several readouts of cell activity indicate that hypoxia enhances IRE/IRP-1 binding in living cells as well. Messages with IRE elements in the 3'-UTR resist enzymatic digestion once IRPs bind to the IREs (5). The striking increase in transferrin receptor message levels in cells exposed to hypoxia attests to IRE/IRP-1 binding in vivo.

The decrease in ferritin biosynthesis in hypoxic cells is further indication of significantly enhanced in vivo IRE/IRP-1 binding. Attachment of IRP-1 to IRE elements in the 5'-UTR blocks the translation of the transcript. Hypoxia decreases ferritin translation without altering message levels. The effect is most prominent in hypoxic cells that are simultaneously exposed to iron. Hypoxia completely abrogates the iron-mediated enhancement of ferritin synthesis.

IRE/IRP-1 binding as a biochemical event alters the biology of the cell. Transferrin receptor expression doubles in Hep3B cells exposed to hypoxia for 16 h. Scatchard analysis shows an identical transferrin binding affinity for control and hypoxic
cells. The increase in transferrin surface binding therefore reflects an increase in surface expression of transferrin receptors rather than a change in the binding characteristics of a fixed number of receptors.

Hanson and Leibold (24) examined rat hepatoma cells in a 3% oxygen atmosphere and found a time-dependent decrease in IRE/IRP-1 binding. These cells also contain substantial quantities of IRP-2, whose binding to the IRE was not affected by hypoxia. Several possibilities could account for the divergent results between their experiments and our experiments.

The most apparent difference is that they used a rodent cell line, whereas our cell lines were derived from humans. One difference in this respect is that their cell line prominently expresses both IRP-1 and IRP-2, whereas our cell lines produce less IRP-2. The metabolic machinery may be set differently in cells from the two species with respect to the hypoxia response owing to this difference in IRP-1 and IRP-2 expression. Another example of a cell-specific response occurs in rat oligodendroglial cells that increase ferritin production under hypoxic conditions, whereas astrocytes and neurons do not (35). Interestingly, mouse peritoneal macrophages that are of similar derivation as oligodendroglial cells also show decreased IRE/IRP binding and increased ferritin synthesis with hypoxia (36).

Pleiotropic cell and tissue responses to a particular stimulus may be due in part to the fact that IRP-1 and IRP-2 have distinct binding characteristics for IRE structures with variations in the base sequences of the loop structure (37). Differences in loops and bulge/loops between IRE isotypes produce dramatic differences in the relative binding affinity of IRP-1 and IRP-2 (38). IRP-2 has the greatest variation in interactions with IRE isotypes, raising the possibility that IRP-2 contributes substantially to differences in IRE-dependent regulation in vivo. IRP-1 and IRP-2 function independently as translational repressors in vivo (39). This fact is driven home strikingly by a cell line that expresses no IRP-1 and yet responds appropriately to all iron-mediated stimuli solely through IRP-2 (40). The differences between IRP-1 and IRP-2 may allow fine tuning to a host of stimuli.

The relative expression of IRP-1 and IRP-2 varies greatly between species and between tissues in a single species (37, 41). These differences have likely contributed to some of the conflicting results in the literature concerning changes in cellular iron metabolism in response to various stimuli. A case in point involves nitric oxide effects on cellular iron homeostasis. One group of investigators reported that the effect of nitric oxide is slow and analogous to desferrioxamine (42), whereas another group found the changes to be rapid (43). The explanation for the divergent reports may be the fact that in some cells, such as macrophages, differences in relative expression of IRP-1 and IRP-2 bring the differences in their binding specificities into greater relief (44).

Clearly, the cellular response to hypoxia is both important and complex. Genes are activated, enzyme levels change, and the local generation of free radicals is altered. This work raises the possibility that the relative expression of IRP-1 and IRP-2 also contributes to the adaptation of particular cells to hypoxia. More work is needed to tease apart the many variables in these systems and to understand this important biological response.

Acknowledgments—We are grateful to Drs. H. Franklin Bunn, Mark A. Goldberg, and Gabor Lazar for valuable input over the course of this study and to Dr. L.C. Kühn for the generous gift of pSPT IRE probe, Dr. Eric Huang for the epo cDNA probe, and Dr. Mark O. Showers for the recombinant human ePO.

REFERENCES

1.  Kühn, L. C., and Hentze, M. W. (1992) J. Inorg. Biochem. 47, 183–195
2.  Morris, C. J., Earl, J. R., Trenam, C. W., and Blake, D. R. (1995) Int. J. Biochem. Cell Biol. 27, 109–122
3.  Hentze, M. W., and Kühn, L. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8175–8182
4.  Leibold, E. A., and Munro, H. N. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2171–2175
5.  Kühn, E. A., Rouault, T. A., and Harford, J. B. (1993) Cell 72, 19–28
6.  Haile, D. J., Rouault, T. A., Harford, J. B., Kennedy, M. C., Blondin, G. A., Beinert, H., and Klausner, R. D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11735–11739
7.  Anderson, B., Seiser, C., and Kühn, L. C. (1993) J. Biol. Chem. 268, 27327–27334
8.  Theil, E. C. (1990) J. Biol. Chem. 265, 4771–4774
9.  Rouault, T. A., Hentze, M. W., Harford, J. B., and Klausner, R. D. (1988) Science 241, 1207–1210
10.  Hirling, H., Henderson, B., and Kühn, L. C. (1994) EMBO J. 13, 453–461
11.  Guo, B., Yu, Y., and Leibold, E. A. (1994) J. Biol. Chem. 269, 24252–24260
12.  Samaniego, F., Chinn, J., Iwai, K., Rouault, T. A., and Klausner, R. D. (1994) J. Biol. Chem. 269, 30904–30910
13.  Pantopoulos, K., and Hentze, M. W. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1267–1271
14.  Heis, G., Goossens, B., Doppler, W., Fuchs, D., Pantopoulos, K., Wernher-Felmayer, G., Wachtler, H., and Hentze, M. W. (1993) EMBO J. 12, 3631–3635
15.  Toto, I., and Bridges, K. R. (1995) J. Biol. Chem. 270, 19540–19544
16.  Golberg, A. M., Glass, G. A., Cunningham, J. M., and Bunn, H. F. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7972–7976
17.  Bunn, H. F., and Peyton, R. O. (1990) Physiol. Rev. 70, 839–885
18.  Wang, G. L., and Semenza, G. L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4304–4308
19.  Elber, B., L. Firth, J. D., and Ratcliffe, P. J. (1995) J. Biol. Chem. 270, 29083–29089
20.  Huang, L. E., Arany, Z., Livingston, D. M., and Bunn, H. F. (1996) J. Biol. Chem. 271, 32253–32259
21.  Semenza, G. L., Roth, P. H., Fang, H. M., and Wang, G. L. (1985) J. Biol. Chem. 260, 25377–25383
22.  Golberg, M. A., Dunnung, S. P., and Bunn, H. F. (1988) Science 242, 1412–1415
23.  Levy, A. P., Levy, N. S., and Goldberg, M. A. (1996) J. Biol. Chem. 271, 2746–2753
24.  Hanson, E. S., and Leibold, E. A. (1998) J. Biol. Chem. 273, 7588–7593
25.  Müller, E. W., Neupert, B., and Kühn, L. C. (1989) Cell 58, 373–382
26.  Toto, I., Rogers, J. T., McPhee, J. A., Elliott, S. M., Abramson, S. L., and Bridges, K. R. (1995) J. Biol. Chem. 270, 2846–2852
27.  Church, M. G., and Gilbert, W. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 1991–1995
28.  Constanzo, F., Columbo, M., Staemglin, S., Sanzota, C., Marone, M., Frank, R., Delius, H., and Cortese, R. (1986) Nucleic Acids Res. 14, 721–736
29.  Greenberg, M. E., and Ziff, E. B. (1984) Nature 313, 433–438
30.  Emery-Goodman, A., Hirling, H., Scarpellino, L., Henderson, B., and Kühn, L. C. (1993) Nucleic Acids Res. 21, 1457–1461
31.  Schonhorn, J. E., Akompong, T., and Wessling-Resnick, M. (1991) J. Biol. Chem. 264, 3398–3403
32.  Weiss, G., Houston, T., Kastner, S., Jöhrer, K., Grunewald, B., and Brock, J.
33. Drapier, J. C., Hirling, H., Wietzerbin, J., Kaldy, P., and Kühn, L. C. EMBO J. 12, 3643–3649.
34. Weiss, G., Goossen, W. D., Fuchs, D., Pantopoulos, K., Werner-Felmayer, G., and Hentze, M. W. (1993) EMBO J. 12, 3651–3657.
35. Qi, Y., and Dawson, G. (1994) Neurochemistry 63, 1485–1490.
36. Kuriyama-Matsumura, K., Sato, K., Yamaguchi, M., and Bonnai, S. (1998) Biochem. Biophys. Res. Commun. 249, 241–246.
37. Henderson, B. R., Menotti, E., and Kühn, L. C. (1996) J. Biol. Chem. 271, 4900–4908.
38. Ke, Y., Wu, J., Leibold, E. A., Walden, W. E., and Theil, E. C. (1998) J. Biol. Chem. 273, 23637–23640.
39. Menotti, E., Henderson, B. R., and Kühn, L. C. (1998) J. Biol. Chem. 273, 1821–1824.
40. Schalinske, K. L., Blemings, K. P., Steffen, D. W., Chen, O. S., and Eisenstein, R. S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10681–10686.
41. Guo, B., Brown, F. M., Phillips, J. D., Yu, Y., and Leibold, E. A. (1995) J. Biol. Chem. 270, 16529–16535.
42. Bouton, C., Raveau, M., and Drapier, J. C. (1996) J. Biol. Chem. 271, 2300–2306.
43. Pantopoulos, K., Weiss, G., and Hentze, M. W. (1996) Mol. Cell. Biol. 16, 3781–3788.
44. Bouton, C., Oliveira, L., and Drapier, J.-C. (1998) J. Biol. Chem. 273, 9403–9408.
Hypoxia Alters Iron-regulatory Protein-1 Binding Capacity and Modulates Cellular Iron Homeostasis in Human Hepatoma and Erythroleukemia Cells
Idiko Toth, Liping Yuan, Jack T. Rogers, Hayden Boyce and Kenneth R. Bridges

J. Biol. Chem. 1999, 274:4467-4473.
doi: 10.1074/jbc.274.7.4467

Access the most updated version of this article at http://www.jbc.org/content/274/7/4467

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 44 references, 30 of which can be accessed free at http://www.jbc.org/content/274/7/4467.full.html#ref-list-1