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Regulation of the Iron Regulatory Proteins by Reactive Nitrogen and Oxygen Species

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Iron regulatory proteins 1 and 2 (IRP1 and IRP2) are RNA binding proteins that posttranscriptionally regulate the expression of mRNAs coding for proteins involved in the maintenance of iron and energy homeostasis. The RNA binding activities of the IRPs are regulated by changes in cellular iron. Thus, the IRPs are considered iron sensors and the principle regulators of cellular iron homeostasis. The mechanisms governing iron regulation of the IRPs are well described. Recently, however, much attention has focused on the regulation of IRPs by reactive nitrogen and oxygen species (RNS, ROS). Here we focus on summarizing the iron-regulated RNA binding activities of the IRPs, as well as the recent findings of IRP regulation by RNS and ROS. The recent observations that changes in oxygen tension regulate both IRP1 and IRP2 RNA binding activities will be addressed in light of ROS regulation of the IRPs.

Iron regulatory proteins Reactive nitrogen species Reactive oxygen species

MAMMALIAN IRON HOMEOSTASIS

Iron is required by cells for survival and growth. This metal plays a role in a variety of biological processes and serves as a cofactor for many enzymes. Iron is also required for the synthesis of heme and, therefore, for the activity of all hemoproteins. Free iron is toxic due to its ability to catalyze the generation of free radicals that oxidize proteins and DNA and initiate peroxidation of lipid membranes. In humans, the accumulation of excess cellular iron can result in cirrhosis, arthritis, cardiomyopathy, diabetes mellitus, and increased risk of cancer and heart disease.

Cells balance their need for iron with the toxicity of iron by regulating both iron uptake and storage. Cells accumulate iron by transferrin (Tf)-dependent and Tf-independent mechanisms (2,3,17,79). The Tf-dependent process involves the binding of transferrin-Fe^{3+} to the transferrin receptor (TfR), which is then endocytosed into cells where iron is released. Tf-independent mechanisms involve the uptake of iron by the proton-coupled metal transporter, DMT1/Nramp2/DCT1 (38), and by the iron transporter, SF1 (40). Iron taken up by cells enters a labile pool consisting of low molecular weight iron complexes (52). Although the exact nature of this pool is unknown, it appears that iron is chelated to ligands such as citrate, ascorbate, amino acids, and nucleotides. This pool is usually small due to iron's ability to catalyze free radical formation. Increases in the iron pool result in the induction of ferritin, which stores iron in a form unavailable to catalyze free radical formation, and in the destabilization of the TfR mRNA, resulting in decreased iron uptake. The coordinate regulation of ferritin and TfR by iron provides a mechanism by which cells balance their requirement for iron with the toxicity of iron.

REGULATION OF IRON HOMEOSTASIS BY IRON REGULATORY PROTEINS

Iron homeostasis is regulated by the iron regulatory proteins (IRPs) 1 and 2. IRPs are cytosolic RNA binding proteins that posttranscriptionally regulate the expression of proteins involved in iron uptake, storage, and utilization (47,57,80,81). IRPs bind with high affinity to RNA stem-loops, known as iron-

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responsive elements (IREs). Structural studies have shown that IREs consist of a conserved six-membered loop and an unconserved base paired stem containing either a bulge-C or an internal loop/bulge UGC/C (1,34,88). IREs are located in the 5′-untranslated (UTR) regions of mRNAs encoding the iron storage protein, ferritin; the heme biosynthetic enzyme, erythroid-aminolevulinate synthase; and the Krebs cycle enzymes, mitochondrial (m)-aconitase and *Drospophila* succinate dehydrogenase. The binding of IRPs to the 5′ IREs inhibits translation of these mRNAs by preventing 43S ribosome binding (65). Five IREs are located in the 3′ UTR of the TfR mRNA, where IRP binding stabilizes this mRNA and protects it from endonuclease attack (6). One IRE is located in the 3′ UTR of the cation transporter mRNA, DMT1 (38).

Mammalian IRP1 shares ~30% identity with the [4Fe-4S] containing enzyme, m-aconitase. Aconitase is a Krebs cycle enzyme that interconverts citrate and isocitrate via the intermediate *cis*-aconitate, and its activity depends on the presence of a [4Fe-4S] cluster. Aconitase contains four domains surrounding an active-site cleft where substrate is bound (4,36). Three iron atoms within the cluster are liganded to three cysteines within this cleft, whereas the fourth iron, Fe_a, is solvent exposed and has a free coordination site that binds substrate. The 18 active-site residues in m-aconitase, including the three cysteines that serve as ligands for the [4Fe-4S] cluster, are conserved in IRP1. IRP1 exhibits aconitase activity and is identical to a previously described c-aconitase (46).

IRP1 RNA binding activity is regulated by cellular iron levels. Iron posttranslationally converts the apo-RNA binding form into the active [4Fe-4S] c-aconitase form without changes in IRP1 protein or mRNA levels (Fig. 1). RNA binding and c-aconitase activities are mutually exclusive. RNA binding activity of IRP1 is enhanced by phosphorylation (84). The process of cluster assembly for the aconitases is not understood; however, a genetic screen in yeast revealed three proteins that are involved in assembly and maturation of mitochondrial Fe-S-containing proteins (90). The [4Fe-4S] cluster can be disassembled by nitric oxide ($NO^{\bullet}$) and ROS in vivo and in vitro (Fig. 1). Although the function of c-aconitase is unknown, the conservation of this activity suggests that it has a functional role in iron and/or energy homeostasis. IRP2 shares ~60% identity with IRP1; however, unlike IRP1, IRP2 does not have detectable aconitase activity and does not appear to contain a [4Fe-4S] cluster. In addition, IRP2 is regulated by iron-induced proteolysis by the proteasome (39,51). Degradation is mediated by an iron-dependent oxidation mechanism that requires a unique 73-amino acid degradation domain containing three essential cysteines (39,50,51). Phosphorylation has also been shown to enhance IRP2 RNA binding activity (86).

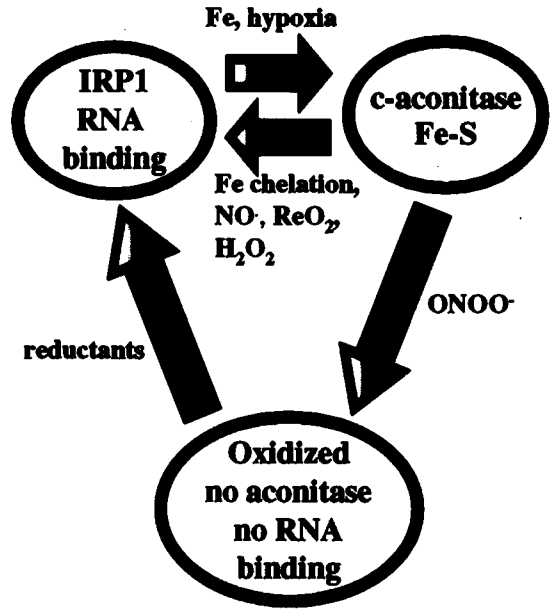

FIG. 1. Model for the regulation of IRP1 by iron, ROS, and RNS. IRP1 interconverts between a RNA binding form and a [4Fe-4S] c-aconitase form. Iron or hypoxia converts the apo-RNA binding form into the [4Fe-4S] aconitase form. $NO^{\bullet}$, H_2O_2, iron chelation, or reoxygenation (ReO_2) results in the formation of the IRP1 RNA binding form. $ONNO^-$ results in the disassembly of the [4Fe-4S] cluster and oxidation of cysteines required for RNA binding. This results in the formation of an oxidized protein, lacking RNA binding and c-aconitase activities. Addition of reductants to oxidized IRP1 restores RNA binding activity.

The functional role of two IRPs in the regulation of iron homeostasis is not known. IRP1 and IRP2 can specifically bind to unique subsets of synthetic IREs (12,45), and appropriately regulate RNAs containing these IREs in vivo (64). IRP1 and IRP2 bind naturally occurring IREs with different affinities. For example, IRP2 binds IREs containing the UGC/C-bulge (ferritin IRE) with higher affinity than C-bulge IREs (TfR-B, aconitase, and aminolevulinate synthase IREs) (56). These data suggest that IRP1 and IRP2 may bind and regulate different IRE mRNAs in vivo. The distinctive roles of IRP1 and IRP2 in iron homeostasis is further questioned by the finding of a cell line lacking IRP1, indicating the importance of IRP2 in IRE regulation (85). Identification of novel IRE mRNAs with specificity for IRP1 or IRP2 will expand our knowledge of the repertoire of the IRP network.

REGULATION OF IRPs BY $NO^{\bullet}$

Nitric oxide ($NO^{\bullet}$) is a reactive nitrogen species that is synthesized by nitric oxide synthases (NOSs)

(67). NO• serves as a ubiquitous second messenger and modulates a myriad of physiological functions, including neuronal signaling, vascular tone, and antibacterial activity of macrophages. NO• could be cytotoxic due to its ability to react with transition metals and sulfhydryls of proteins, leading to their inactivation (89). NO• produced by activated macrophages can kill tumor cells and combat invading microorganisms (68). When activated macrophages are co-cultured with tumor cells, the production of macrophage NO• results in the inactivation of mitochondrial Fe-S-containing enzymes, leading to inhibition of respiration and subsequent tumor cell death (20,21).

The affinity of NO• for Fe-S clusters, and the inactivation of m-aconitase by NO• led to the notion that NO• might also modulate IRP1 activity (18,19). When macrophages (22,77,97), fibroblasts (70,73), hepatoma cells (75), and erythroleukemia cells (78,97) are stimulated with cytokines to produce NO• or treated with NO•-generating chemicals, c-aconitase was inactivated and converted into its IRP1 RNA binding form. These data supported a model whereby NO• modulated IRP1/c-aconitase activity; however, questions arose concerning whether inactivation in vivo was due to a direct effect by NO• or by a NO•-derived species such as peroxynitrite ($ONOO^-$). $ONOO^-$ is the reaction product of superoxide anion ($O_2^{\bullet-}$) and NO•. Two studies reported that $ONOO^-$, but not NO•, inactivated aconitase (14,44). Recent studies by Gardner and colleagues refuted these data, demonstrating that the NO•-mediated inactivation of *Escherichia coli* aconitase in vivo was due to *S*-nitrosylation of the [4Fe-4S] center, which occurred independently of $ONOO^-$ (28). These data were supported by an electron paramagnetic resonance study showing that NO• reacts directly with the [4Fe-4S] cluster of m-aconitase and c-aconitase, resulting in cluster disassembly and the formation of a dintrosyl–iron–dithiol complex (58). $ONOO^-$ can inactivate c-aconitase in cell extracts; however, in contrast to NO•, $ONOO^-$ does not activate IRP1 RNA binding (8). In addition to disrupting the [4Fe-4S] cluster, $ONOO^-$ oxidizes critical cysteines required for RNA binding (8). The physiological role of $ONOO^-$ in IRP1 regulation in vivo is unclear. Whether NO• modulates IRP1 activity by *S*-nitrosylation of thiol groups in IRP1 as suggested by Ponka and colleagues remains to be determined (78).

Conflicting data regarding IRP2 regulation by NO• have been reported. IRP2 RNA binding was activated in J774 macrophages (96,97), fibroblasts (73), and in the liver during inflammation (13), but not in rat hepatoma cells (75). In contrast, other studies using J774 (77) and RAW264.7 macrophages (7) stimulated with cytokines to produce NO•, inactivation of IRP2 RNA binding was observed. In J774 macrophages, the addition of the inducible NOS inhibitor, N^G-monomethyl-L-arginine monoacetate or the iron chelator, deferioxamine, prevented the decrease in IRP2 RNA binding activity, indicating roles for both NO• and iron in IRP2 inactivation (77). Iron can be released from activated macrophages co-cultured with tumor cells, suggesting that NO• may alter iron levels at least in some cell types (48). In RAW264.7 macrophages, the decrease in IRP2 RNA binding activity was independent of NO•, suggesting that cytokines may modulate IRP2 activity by other pathways (7).

The discrepancy among these studies is unclear. Recent studies have shown that in addition to iron-mediated oxidative damage (50), IRP2 activity is sensitive to oxidants. For example, IRP2 RNA binding is inactivated by 5′5′-dithiobis(2-nitrobenzoic) (DTNB) (75), $ONOO^-$ (7,8), and cobalt chloride (42). In contrast, under hypoxic conditions where ROS are expected to be altered, IRP2 RNA binding is activated (42). It is possible that cell growth conditions (density, medium, and serum) vary among the studies, resulting in subtle differences in the relative concentrations of iron and/or ROS, which can modulate IRP2 RNA binding activity. One recent study showed that in macrophages grown in L-arginine-depleted medium, inducible NOS generates both NO• and $O_2^{\bullet-}$, which react to form $ONOO^-$, revealing the importance of culture conditions on ROS/RNS formation (99).

What effect does NO• regulation of IRP1 and IRP2 have on target IRE mRNAs? Activation of IRP1 and IRP2 RNA binding correlated with decreased ferritin synthesis in macrophages (96,97), hepatoma cells (75), and fibroblasts (73), and increased TfR mRNA levels in erthroleukemia cells (70,78). In experiments where IRP2 activity decreased, ferritin synthesis increased and TfR mRNA levels decreased (77). The contributions of IRP1 and IRP2 in the regulation of ferritin, TfR, and other IRE mRNAs remain to be determined.

The physiological significance of NO• regulation of IRPs is unclear. Cairo and colleagues proposed that the increase in ferritin synthesis in J774 macrophages producing NO• is consistent with in vivo models of inflammation where iron is retained in reticuloendothelial cells (77). Regulation of IRP1 and IRP2 by NO• may be important in the anemia of chronic disease where the sequestration of iron by macrophages limits iron for hematopoiesis (18,77).

REACTIVE OXYGEN SPECIES

The $O_2^{\bullet-}$ anion is a ubiquitous and naturally produced reactive oxygen metabolite (25). There are sev-

eral sources leading to the production of cytosolic $O_2^{\bullet-}$ including the NADP(H) oxidase (41) and xanthine oxidase systems (63). A significant amount of cellular $O_2^{\bullet-}$ production is derived from the incomplete reduction of O_2 by the mitochondrial electron transport machinery (15,23,93). It has been estimated that approximately 1–2% of the total cellular uptake of O_2 is not fully reduced during respiration (9,10). The potential toxicity of mitochondrial- and cytosolic-derived $O_2^{\bullet-}$ is countered by the superoxide dismutases (SODs) that dismutates $O_2^{\bullet-}$ to the less reactive and more membrane diffusible H_2O_2 molecule (25,26). The concerted activities of the SODs and the H_2O_2 enzymatic decomposing systems, catalase and glutathione peroxidase, result in a steady-state level of $\sim 10^{-10}$ M for $O_2^{\bullet-}$ and $\sim 10^{-8}$ M for H_2O_2 (9).

ROS levels increase during aging and pathophysiological conditions that can lead to significant cellular oxidative damage (5). Furthermore, ROS have important roles in regulating cellular gene expression. For instance, H_2O_2 has gained recognition as an important signaling molecule involved in the regulation of MAP kinase pathways, as well as mediating transcriptional regulation of the NF-κB and AP1 transcription factors (55,66,91). The IRPs represent a posttranscriptional gene regulatory system that also responds to ROS. Regulation of IRPs by ROS allows IRPs to sense signals from oxygen-derived species in addition to sensing iron. Although not yet fully elucidated, the convergence of these signals on IRPs presumably coordinates communication between iron, oxidative stress, and oxygen homeostasis. Understanding the respective contributions that these signals have on IRP regulation in vivo is an important and formidable task.

REGULATION OF IRPs BY ROS

Regulation of IRP1/c-Aconitase by H_2O_2

IRP1/c-aconitase activities are affected by H_2O_2 (8,62,69,71–73) and $O_2^{\bullet-}$ (33,71). A central feature that these species share is the ability to modulate the aconitase [4Fe-4S] cluster. The best understood example of ROS regulation of IRP1/c-aconitase is the pathway initiated by extracellular H_2O_2. Martins et al. (62) and Pantopoulos et al. (71) were the first to report that IRP1 RNA binding activity is regulated by oxidative stress when they demonstrated rapid activation of IRP1 RNA binding activity by H_2O_2. De novo protein synthesis is not required for activation, demonstrating that H_2O_2 operates by a posttranslational mechanism (71). Furthermore, the continuous presence of H_2O_2 for IRP1 activation is not required. Rather, brief exposure to H_2O_2 is sufficient to initiate a signaling pathway resulting in IRP1 activation (62,71). As would be predicted, IRP1 activation by H_2O_2 results in the repression of ferritin protein synthesis and the upregulation of TfR mRNA (71). The observation that H_2O_2 activates IRP1 has since been reproduced by other laboratories [(64); E. S. Hanson and E. A. Leibold, unpublished results].

The regulation of IRP1 by H_2O_2 is mediated by an unknown extracellular event that initiates a signaling cascade (71–73). Using cytosolic extracts, H_2O_2 was not able to activate RNA binding (62,71), indicating that H_2O_2 is not directly acting on IRP1. Recently, an in vitro assay has recapitulated H_2O_2 activation of IRP1 similar to that seen using intact cells. Both cytosolic and membrane fractions are required for H_2O_2 activation of IRP1, demonstrating activation requires a multicomponent system (69). Because H_2O_2 activation of IRP1 is concomitant with decreased c-aconitase activity, the proximal signal in IRP1 activation may involve [4Fe-4S] cluster disassembly (53,71). The signaling pathway and the precise mechanism for IRP1 activation by extracellular H_2O_2 have yet to be defined.

Regulation of Aconitase by $O_2^{\bullet-}$

Aconitase activity is dependent on the presence of a cubane [4Fe-4S] cluster. Oxidative destruction of the [4Fe-4S] cluster leads to enzyme inactivation. This property is not unique to aconitases because other [4Fe-4S]-containing enzymes undergo oxidatively induced inactivation as well (24). It is well established that aconitases from *E. coli* (24,30,31,44) and mammalian (32,33) sources are sensitive to inactivation by $O_2^{\bullet-}$. Both m- and c-aconitase activities are inactivated by $O_2^{\bullet-}$ (33,44). Superoxide-induced inactivation of aconitase results from the oxidation of the [4Fe-4S] cluster due to the loss of a labile, solvent-exposed iron atom (Fe_a) yielding a [3Fe-4S] cluster ($[4Fe\text{-}4S]^{+2} \Rightarrow [3Fe\text{-}4S]^{+1}$). Due to the continuous attack of the [4Fe-4S] cluster by steady-state levels of $O_2^{\bullet-}$, it has been estimated that ~15% of total cellular aconitase activity is inactivated at any given time under normal respiratory conditions (33). Inactivation is inversely proportional to $O_2^{\bullet-}$ concentration (29,30,32). Accordingly, overexpression of the mitochondrial Mn-SOD (33) or the cytosolic CuZn-SOD (92) results in increased aconitase activity. Furthermore, Mn-$SOD^{+/-}$ mice have a ~30% decrease in m-aconitase activity, but no change in c-aconitase activity (60,98). Therefore, it is predicted that pathophysiological situations resulting in increased $O_2^{\bullet-}$ levels will be associated with an increased rate of cluster disintegration. Conversely, decreased $O_2^{\bullet-}$ should stabilize the Fe-S cluster leading to increased aconitase

activity. The fate of $O_2^{\bullet-}$-inactivated aconitase is not unidirectional, because rapid reactivation was demonstrated in a lung cell line (33) and in *E. coli* (30). Thus, reintroduction of the fourth iron into the [3Fe-4S] form results in a cyclic process of inactivation–reactivation (27,32,33).

Regulation of IRP1 RNA Binding Activity During Hypoxia

One situation that may represent $O_2^{\bullet-}$ regulation of IRP1/c-aconitase is hypoxia. Hypoxia is an important regulator of gene expression (11). The best understood example of mammalian oxygen-regulated gene expression is that mediated by the transcription factor hypoxia-inducible factor-1 (HIF-1) (11,37,76,87), whereas relatively little information is available regarding posttranscriptional mechanisms (16,59). Because IRP1 is regulated by ROS, and because ROS production is ultimately dictated by O_2 concentration, we speculated that IRP1 may be regulated by changes in O_2 concentration. To investigate a potentially novel pathway for gene regulation during hypoxia, a study on the regulation of the IRPs by changes in O_2 concentration was undertaken.

We have recently reported that hypoxia (1–3% O_2) posttranslationally downregulates IRP1 while upregulating IRP2 RNA binding activity in a variety of cell types (Fig. 2) (42,43). Iron is required for IRP1 hypoxic inactivation implicating the Fe-S cluster in "sensing" changes in O_2. Decreased IRP1 RNA binding activity during hypoxia is accompanied by ~40% increase in c-aconitase activity (E. S. Hanson and E. A. Leibold, unpublished results). Because there are no detectable changes in IRP1 protein levels (43), and because inactivation is cycloheximide insensitive (E. S. Hanson and E. A. Leibold, unpublished data), it appears that the [4Fe-4S] cluster is stabilized during hypoxia. This indicates that decreased O_2 concentration promotes a posttranslational conversion from IRP1 RNA binding to its [4Fe-4S] aconitase form. How does the Fe-S cluster of IRP1 "sense" changes in O_2? At least two possibilities arise that are not mutually exclusive (Fig. 3). First, hypoxia may increase iron leaching from mitochondrial Fe-S clusters due to hypoxia-induced increases in mitochondrial $O_2^{\bullet-}$ production (23,61,93). Iron liberated in this manner could encourage c-aconitase [4Fe-4S] cluster formation at the expense of RNA binding activity. If this were the case, IRP2 activity would be predicted to decrease when in fact hypoxia increases IRP2 activity

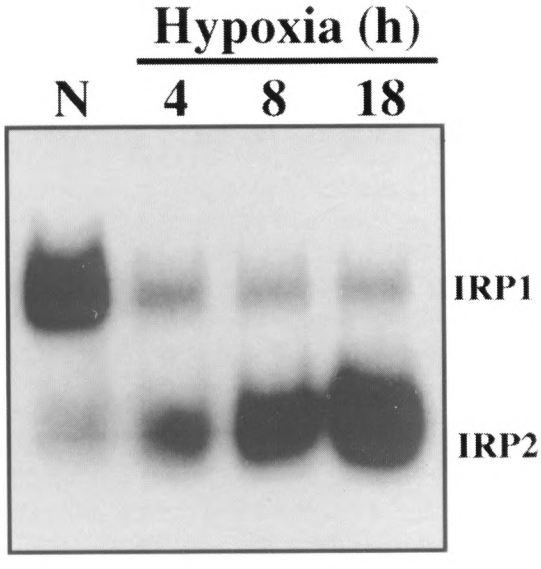

FIG. 2. Hypoxic regulation of IRP1 and IRP2 Hepa-1 cells. Mouse Hepa-1 c1c4 were exposed to normoxia (N) or hypoxia (1% O_2) for the indicated times. Bandshift analysis was performed by incubating cytosolic extracts (12 μg) with a ^{32}P-labeled iron responsive element RNA probe. The RNA–protein complexes were resolved on a 5% nondenaturing polyacrylamide gel, and the gel was exposed to film. IRP1–RNA and IRP2–RNA complexes are indicated.

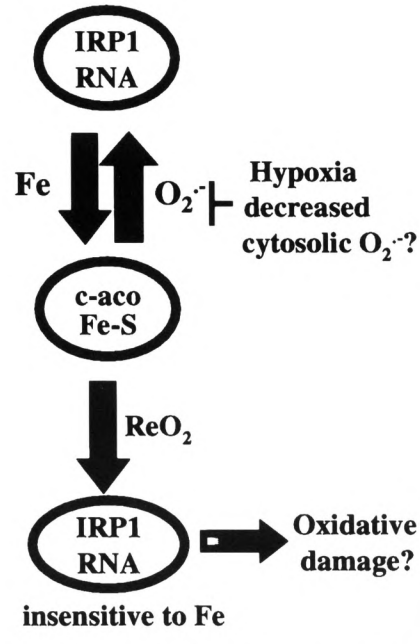

FIG. 3. Model depicting oxygen regulation of IRP1. During normoxia IRP1 interconverts between its RNA binding form and its [4Fe-4S] c-aconitase form. This interconversion is dependent on the relative levels of iron and $O_2^{\bullet-}$. Hypoxia decreases RNA binding activity (see Fig. 2) and increases c-aconitase activity. The model suggests that hypoxic regulation of IRP1 could be due to increased iron and/or decreased cytosolic $O_2^{\bullet-}$, either of which would lead to stabilization of the [4Fe-4S] cluster at the expense of RNA binding activity. Reoxygenation (ReO_2) activates IRP1 to a constitutively active RNA binding form. The dysregulated form of IRP1 (shaded) is refractory to iron downregulation. By adversely affecting iron levels, it is possible that this form of IRP1 may contribute to ReO_2-induced oxidative damage.

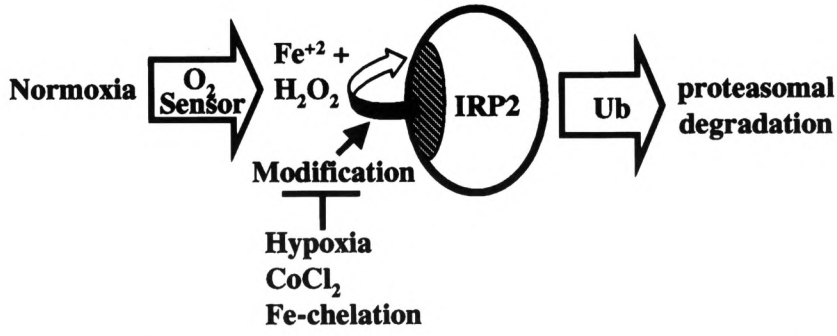

FIG. 4. Model for the oxygen regulation of IRP2. Normoxic degradation of IRP2 by the proteasome is dependent on the 73-amino acid degradation domain that contains three essential cysteines required to sense Fe^{2+} (hashed) (51). In this model, IRP2 stability is dependent on Fe^{2+} and H_2O_2, which are required for oxidative modification leading to ubiquitination (Ub) and proteasomal degradation (50). Hypoxia, iron chelation, and $CoCl_2$ increase IRP2 protein levels by stabilization. Hypoxia may act through an unknown O_2 sensor that lowers cytosolic H_2O_2 resulting in decreased IRP2 oxidation and degradation. $CoCl_2$ mimics hypoxia by possibly altering the activity of an O_2 sensor or by competing with iron at an iron binding site on the degradation domain.

(Fig. 2). Thus, it appears that any increase in cellular iron during hypoxia cannot be the only factor in the hypoxic regulation of IRPs. A second possibility for the hypoxic stabilization of c-aconitase may involve decreased cytosolic $O_2^{\bullet-}$ production during hypoxia. A decrease in cytosolic $O_2^{\bullet-}$ would slow the rate of $O_2^{\bullet-}$-mediated cluster disassembly. Because aconitase inactivation has been used as a marker for $O_2^{\bullet-}$ (25,30,74), this latter scenario would suggest that the increase in mitochondrial generated $O_2^{\bullet-}$ that accompanies hypoxia is not accessible to the cytosol (23,93). This result indicates that $O_2^{\bullet-}$ is compartmentalized, which is consistent with data demonstrating that Mn-SOD$^{+/-}$ mice display a decrease in m-aconitase but not c-aconitase (98). It should be noted, however, that there are contradicting reports regarding cellular compartmentalization of $O_2^{\bullet-}$ (15,33,93).

Hypoxic inactivation of IRP1 is reversible because upon reexposure to normoxia RNA binding ensues (Fig. 3) (43). Importantly, reactivated IRP1 is modified to a dysregulated form since iron does not downregulate its RNA binding activity. The mechanism for IRP1 dysregulation is not known; however, one possibility may involve some form of oxidative protein damage that precludes [4Fe-4S] cluster formation. Interestingly, the pro-oxidant condition associated with aged house flies is responsible for oxidative damage and inactivation of m-aconitase (100). Whether this bears any relationship to reoxygenation-induced IRP1 activation is not known. Furthermore, it will be of interest to determine if mammalian IRP1/c-aconitase is altered during aging in mammalian cells. A second possibility is that IRP1 may be phosphorylated during reoxygenation, which could preclude Fe-S assembly (84). Finally, ROS produced during reoxygenation may disassemble the Fe-S cluster faster than cluster assembly. Regardless of the mechanism, the inability of IRP1 to sense iron during reoxygenation is predicted to result in aberrant iron homeostasis, and may be an important contributor to reoxygenation-induced cell injury, an event known to be exacerbated by increased iron.

Hypoxic Activation of IRP2

IRP2 RNA binding activity is significantly upregulated during hypoxia (Fig. 2). The increase in RNA binding activity parallels an equal increase in IRP2 protein levels (42). Hypoxic activation of IRP2 has several intriguing similarities to the well-studied HIF-1α subunit of HIF-1. HIF-1 is a heterodimeric transcription factor (HIF-1α/β) composed of two helix–loop–helix PAS family proteins (37,76,94). HIF-1 activates the transcription of many genes during hypoxia and therefore is believed to be an important mediator of cellar adaptation to hypoxia. Whereas HIF-1β levels do not change during hypoxia, both HIF-1α and IRP2 are activated during hypoxia by a mechanism involving protein stabilization (42,49,82). Iron chelation and $CoCl_2$ mimic hypoxia by increasing HIF-1α (54,95) and IRP2 protein levels (39,42,83). Although $CoCl_2$ increases IRP2 levels, IRP2 is unable to bind RNA in bandshift analysis unless first reduced with DTT. Thus, $CoCl_2$ has the dual effect of inducing IRP2 accumulation while at the same time reversibly inactivating RNA binding. Whether redox regulation of IRP2 occurs in vivo under more physiological conditions is not known.

It has been suggested that $CoCl_2$ and iron chelation mimic hypoxia by inactivation of a cytosolic hemoprotein that functions as an O_2 sensor (11,35,37). Such an O_2 sensor could modulate a signal during low O_2 conditions that could result in IRP2 and HIF-1α stabilization. However, such a cytosolic O_2 sens-

ing protein has not yet been definitively identified. Mechanistic studies have demonstrated that IRP2 is oxidized by metal-catalyzed oxidation mechanism requiring Fe^{2+} and O_2. In turn, oxidized IRP2 results in a good substrate for ubiquitination and proteasomal degradation (Fig. 4) (50). Based on this finding, we suggest a model whereby altering the rate of IRP2 metal-catalyzed oxidation by hypoxia and $CoCl_2$ activates IRP2 by a mechanism involving protein stabilization (Fig. 4). $CoCl_2$ could also compete for iron binding at the degradation domain, thus blocking IRP2 iron sensing.

It should be noted that recent data indicate that the mitochondria may function as an O_2 sensor that initiates signaling to HIF-1α by increasing rather than decreasing H_2O_2 levels (15,23,93). Elucidating the source(s) and defining the precise signaling pathway(s) for IRP2 and HIF-1α hypoxic stabilization is an important next step in understanding hypoxic signaling and gene regulation.

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