Iron-regulatory proteins: molecular biology and pathophysiological implications

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Iron is required for key cellular functions, and there is a strong link between iron metabolism and important metabolic processes, such as cell growth, apoptosis and inflammation. Diseases that are directly or indirectly related to iron metabolism represent major health problems. Iron-regulatory proteins (IRPs) 1 and 2 are key controllers of vertebrate iron metabolism and post-transcriptionally regulate expression of the major iron homeostasis genes. Here we discuss how dysregulation of the IRP system can result from both iron-related and unrelated effectors and explain how this can have important pathological consequences in several human disorders.

Iron is essential for life but can also catalyse the formation of reactive oxygen species (ROS) that can lead to cell damage. Therefore, iron must be safely bound by specialised proteins to keep ‘free’ iron levels as low as possible. To achieve this, the control of genes encoding proteins involved in iron uptake, utilisation, storage and export must be tightly coordinated. This challenging task is mainly performed by the iron-regulatory proteins (IRPs), which control intracellular iron metabolism, and hepcidin, which regulates body iron homeostasis (Ref. 1). Detailed reviews of IRP structure and regulation have recently been published elsewhere (Refs 2, 3, 4, 5, 6, 7, 8); here, we focus on the role of IRPs in maintaining intracellular iron homeostasis and the pathophysiological implications caused by alterations in this function.

The cloning of genes encoding the H and L subunits of the iron-storage protein ferritin led to the identification of iron-responsive elements (IREs) in the 5’ untranslated regions (UTRs). IREs were found to control gene expression in response to changes in the iron level (Refs 9, 10). Cytosolic proteins that specifically recognise and bind IREs (IRP1 and IRP2) were later identified (Refs 11, 12), and subsequently the identification of five IRE motifs in the 3’ UTR of the mRNA for transferrin receptor 1 (TFR1) (approved gene symbol TFRC) (Ref. 13), which controls cellular iron uptake, indicated that IRPs might be the common regulators of genes involved in iron homeostasis. Indeed, several genes of iron metabolism are now known to be controlled post-transcriptionally through the IRE–IRP regulatory network (see below).

Soon after its identification, it was recognised that IRP1 (approved gene symbol ACO1) is a ‘moonlighting’ protein that can perform two entirely different functions: when present as an apoform, it is able to bind IRE and control gene expression; however, IRP1 can also assemble
a [4Fe–4S] cluster and become the cytosolic counterpart of mitochondrial aconitase. The switch between the two forms is mainly regulated by the availability of iron in the so-called labile iron pool (LIP), a pool of metabolically available iron whose nature is difficult to characterise, but whose importance is widely recognised (Ref. 14). Therefore, this characteristic makes IRP1 both a sensor of iron levels within the cell and a regulator of cellular iron homeostasis (reviewed by Refs 2, 3, 4, 5, 6, 7, 8).

Two bands were evident in the first published example of an IRP–IRE bandshift assay (Ref. 11), and cloning of the human cDNA led to the discovery of the second IRP, which was very similar to IRP1 (Ref. 15). However, the role of IRP2 (approved gene symbol IREB2) was somewhat neglected until it was demonstrated that it regulates cellular iron homeostasis (Ref. 16) and is itself regulated by specific pathophysiological stimuli (Ref. 4).

**Cellular iron metabolism**

The fine adjustment of intracellular iron levels is mainly achieved by means of a divergent but coordinated regulation of the iron-storage protein ferritin and the iron-uptake protein TfR1. Although transcriptional regulation of the ferritin heavy and light subunits (H-ferritin and L-ferritin, respectively) (see Refs 4, 17 for reviews) and of the TFRC gene (Refs 18, 19) in response to iron has been described, these two proteins are mainly controlled at the post-transcriptional level through the IRE–IRP system. Over the past decade it has been shown that other genes involved in iron uptake, release and utilisation are also controlled by the IRE–IRP regulatory network (see below).

IRP1 and IRP2 are cytoplasmic proteins belonging to the aconitase superfamily and regulate intracellular iron metabolism by binding with high affinity and specificity to conserved IREs in the UTRs of mRNA (Fig. 1) (Refs 2, 3, 4, 5, 6, 7, 8). In spite of the predominantly cytosolic localisation of IRPs, microscopic and biochemical approaches identified a fraction (10%) of IRP1 (but not IRP2) that is associated in a phosphorylation-dependent manner with Golgi and endoplasmic reticulum membranes, although the role of membrane-bound IRP1 is still undefined (Ref. 20). Under conditions of iron deficiency, IRPs actively bind to IREs and stabilise TfR1 mRNA while also decreasing translation of ferritin mRNA, eventually increasing the uptake and availability of iron within the cell. Conversely, high iron levels decrease IRE-binding activity, leading to efficient translation of ferritin mRNA and decreased stability of TfR1 mRNA, favouring iron sequestration over uptake (Fig. 1).

IRP1 is the cytoplasmic counterpart of mitochondrial aconitase, the enzyme that converts citrate to isocitrate through a cis-aconitate intermediate in the tricarboxylic acid cycle by means of a catalytic [4Fe–4S] cluster (Ref. 21). In iron-replete cells the cluster is assembled and IRP1 displays aconitase activity; in iron-depleted cells, no cluster is formed and apo-IRP1 functions as an RNA-binding protein (Fig. 2). IRE binding is also controlled by the redox state of cysteine residues involved in cluster coordination (Refs 22, 23). Therefore, it has been proposed that a reversible switch between a cluster-containing holoprotein and a cluster-deficient apoprotein allows aconitase/IRP1 to constantly sense iron levels and to adapt them to cell requirements without changes in protein levels. However, apo-IRP1 is also subject to iron-dependent degradation (Refs 24, 25) (Fig. 2), and crystallographic studies did not predict the direct insertion of the cluster in the apo-IRP1 bound to mRNA (Ref. 26). Therefore, the idea that a single molecule can reversibly assume both forms perhaps needs to be revised; IRP1 exists predominantly in an aconitase form (Refs 27, 28, 29) and de novo formation of either IRP1 or aconitase can be stimulated, depending on iron scarcity or availability, respectively.

In response to increased iron levels, apo-IRP1 is more likely to be degraded than to assemble a [4Fe–4S] cluster, whereas the loss of all four iron atoms that convert cytoplasmic aconitase to the RNA-binding form can probably occur only in response to oxidative or nitrative stress (see below). In addition, it has been shown that protein-kinase-C-dependent phosphorylation controls both the RNA-binding and aconitase activities of IRP1 and influences the mechanisms of IRP1 regulation in response to iron (reviewed by Ref. 7) (Fig. 2).

IRP2 is highly homologous to IRP1 but lacks aconitase activity, probably because of its inability to assemble a [4Fe–4S] cluster. The protein accumulates in iron-deficient cells and...
is rapidly targeted for proteasomal degradation in iron-replete cells (Ref. 30) (Fig. 2). IRP2 binds consensus IRE sequences with an affinity and specificity similar to that of IRP1 (Refs 2, 5), but it has been shown to recognise an exclusive subset of IRE-like motifs (Refs 31, 32). Furthermore, growing evidence suggests that IRP2 may play a specific role independently of aconitase/IRP1. IRP2 is specifically modulated in response to stimuli and agents other than iron, such as hypoxia (Refs 33, 34) and oxidative stress characterised by enhanced production of ROS (Refs 35, 36, 37) or reactive nitrogen intermediates (RNIs) (Refs 38, 39) (Fig. 2). Moreover, IRP2 expression varies greatly between tissues (Refs 2, 40). IRP2 is more sensitive than IRP1 to variations of iron in the diet (Ref. 28), is expressed at high levels in most cell lines (Ref. 41) and, when abundantly or uniquely (Ref. 16) expressed, can act as the major or only modulator of intracellular iron metabolism, as also indicated by studies in cells in which either IRP1, IRP2 or both were knocked down (Ref. 43).

**Figure 1. Regulation of cellular iron homeostasis by the iron-regulatory proteins.** Under conditions of iron deficiency, iron-regulatory proteins IRP1 and IRP2 bind to the iron-responsive elements (IREs) located in either the 5’ or 3’ untranslated regions (UTRs) of the indicated mRNAs, thus repressing mRNA translation (a) or preventing mRNA degradation (b), respectively. Increased iron levels result in the loss of IRP affinity for IRE, causing increased translation of 5’ IRE-containing mRNAs (a, right) and degradation of 3’ IRE-containing mRNAs (b, right). The functional role of IRE in some mRNAs remains unclear. mRNAs containing non-canonical IREs are indicated with a question mark. Abbreviations: APP, amyloid precursor protein; CDC14A, cell division cycle 14 homologue A; DMT1, divalent metal transporter 1; eALAS, erythroid aminolevulinate synthase; HIF-2α, hypoxia-inducible factor 2α; mAconitase, mitochondrial aconitase; MRCKα, myotonic dystrophy-related CDC42-binding kinase α; SDH *Drosophila*, *Drosophila* succinate dehydrogenase.
Gene-targeting experiments have provided additional information about the respective role of the two IRPs. Early embryonic lethality in mice doubly deficient for both IRPs (Ref. 44) shows that the IRP–IRE regulatory system is essential. However, mice lacking either IRP1 or IRP2 are viable, thus indicating that the two IRPs can compensate for each other. Irp2−/− mice display mild microcytosis and compromised haematopoiesis with abnormal body iron distribution (Refs 45, 46, 47, 48) and either late-onset neurodegeneration (Refs 49, 50) or a mild neurological phenotype (Ref. 51) in different mouse lines; however, mice lacking IRP1 present with no detectable phenotypic abnormality (Ref. 52). Therefore, IRP2 seems to dominate the regulation of iron homeostasis in animal models under normal conditions. Extensive investigation of the comparative expression of the two IRPs in human tissues and blood cells suggested that IRP2 is also the key regulator of intracellular iron homeostasis in humans (Ref. 29). The above indications of the minor role of IRP1 have been challenged by recent results obtained in anaemic zebrafish, which showed that IRP1 regulates expression of IRE-containing mRNA for erythroid aminolevulinate synthase (eALAS) and hence has a role in haem synthesis (Ref. 53).

**Effecct of iron and other signals on the IRE–IRP regulatory system**

In addition to modulating ferritin and TfR1 levels, IRPs can regulate the mRNAs for other proteins. IRE structures have been detected in several mRNAs encoding proteins related to iron utilisation (eALAS; mitochondrial aconitase, approved gene symbol ACO2; *Drosophila* succinate dehydrogenase, gene symbol SDHB), uptake [divalent metal transporter 1 (DMT1), official symbol SLC11A2] and release (ferroportin, gene symbol SLC40A1) (Refs 2, 3, 4, 5, 6, 7, 8). All these mRNAs except that encoding one splicing form of DMT1 – which contains an IRE-like structure in its 3' UTR and is upregulated by iron deficiency (Ref. 54) – have one IRE in their 5' UTR and are therefore regulated at the translational level (Fig. 1), although the extent of regulation varies between different transcripts (Ref. 55). However, recent findings indicate that the influence of IRPs extends over a number of regulatory pathways not directly related to iron homeostasis. In particular, integrated strategies utilising a variety of approaches led to the

**IRE-containing mRNAs**

- Hypoxia and high iron levels (Fe2+) favour the formation of the [4Fe–4S] cluster-bearing cytosolic aconitase (cAconitase).
- RNIs, DOXol and administration of extracellular \( \text{H}_2\text{O}_2 \) favour cluster disassembly and the acquisition of RNA-binding activity. ROS cause loss of both aconitase and IRE-binding activities by inducing the formation of a degradation-prone intermediate. Phosphorylation favours [4Fe–4S]-cluster-independent regulation of IRP1 in response to iron.
- Increased iron levels and exposure to conditions favouring the formation of ROS and RNIs promote the degradation of IRP2 by the proteasome, whereas hypoxia leads to IRP2 stabilisation. Abbreviations: DOXol, doxorubicinol; IRE, iron-responsive element; IRP, iron-regulatory protein; RNI, reactive nitrogen intermediate; ROS, reactive oxygen species.

**Figure 2. Effect of iron and other signals on the IRE–IRP regulatory system.**

(a) Regulation of iron-regulatory protein IRP1. The conversion of IRP1 into different isoforms is controlled by several effectors. Hypoxia and high iron levels (Fe2+) favour the formation of the [4Fe–4S] cluster-bearing cytosolic aconitase (cAconitase). RNIs, DOXol and administration of extracellular \( \text{H}_2\text{O}_2 \) favour cluster disassembly and the acquisition of RNA-binding activity. ROS cause loss of both aconitase and IRE-binding activities by inducing the formation of a degradation-prone intermediate. Phosphorylation favours [4Fe–4S]-cluster-independent regulation of IRP1 in response to iron. (b) Regulation of IRP2. Increased iron levels and exposure to conditions favouring the formation of ROS and RNIs promote the degradation of IRP2 by the proteasome, whereas hypoxia leads to IRP2 stabilisation. Abbreviations: DOXol, doxorubicinol; IRE, iron-responsive element; IRP, iron-regulatory protein; RNI, reactive nitrogen intermediate; ROS, reactive oxygen species.
identification of novel IRE-containing genes (Fig. 1). Myotonic-dystrophy-related CDC42-binding kinase α (MRCKα) is a kinase that acts downstream of small GTPases known to be involved in cytoskeletal regulation and has an IRE in its 3’ UTR. This IRE may mediate a similar response to iron as TfR1, albeit of lower intensity (Ref. 56).

A functional IRE has been found in a differentially spliced mRNA isoform encoding CDC14A (cell division cycle 14 homologue A), a phosphatase involved in the regulation of critical cell cycle proteins that has been suggested to be a tumour suppressor (Ref. 57). Although it is not known how IRPs regulate CDC14A expression, this finding reveals interesting links between iron metabolism and cell cycle regulation, particularly in light of recent findings connecting iron-depletion-mediated growth suppression at the G1–S transition with a mechanism regulating cyclin D1 expression (Ref. 58).

A functional IRE has also been found in the mRNA for the hypoxia-inducible factor (HIF) 2α (gene symbol EPAS1), a transcription factor that is activated by lack of oxygen or iron (Ref. 59). This result, which suggests a negative feedback control of the HIF-mediated response under conditions of limited iron availability, may shed further light on the link between iron and oxygen homeostasis (see below).

When considering that brain iron homeostasis is known to be disrupted in several neurodegenerative disorders (Ref. 60), the reported presence of an IRE-like motif in the 5’ UTR in mRNA of α-synuclein (Ref. 61), a presynaptic protein that accumulates in several brain disorders including Parkinson disease, and APP (amyloid precursor protein) (Ref. 62), is particularly intriguing. However, it should be kept in mind that the sequences are not canonical IREs, and IRE-like motifs found in other mRNAs have turned out to be nonfunctional (Refs 63, 64). Often, the iron regulation of these IRE-containing mRNAs is much lower than those of ferritin and TfR1 (Refs 4, 6).

**Oxidative stress, inflammation, xenobiotics and hypoxia affect IRPs**

The regulation of IRPs under conditions of oxidative stress – that is, in the presence of an excess of ROS – has been the subject of intensive research and some debate. Early observations showed a rapid upregulation of IRP1 in cells exposed to exogenous H$_2$O$_2$ (reviewed by Refs 2, 3, 4, 38), a transient effect that is permissive to later induction of ferritin (Ref. 65) and that seems to be due to the pleiotropic effects of H$_2$O$_2$ on iron metabolism, rather than to a direct effect of oxidative stress on IRP1 activity (Ref. 66). However, studies in several types of cell line, as well as in vivo, have shown that a number of conditions or agents known to increase cellular levels of H$_2$O$_2$ and O$_2^-$ were able to induce reversible inactivation of IRP1 (Refs 35, 67, 68, 69, 70). This body of evidence has led to the conclusion that IRP1 downregulation, aimed at decreasing TfR1 levels while also increasing ferritin levels and hence diminishing the LIP, may be a common response to prevent enhanced formation of ROS (Fig. 2). In line with this, increased ferritin synthesis has been documented in a variety of cell types exposed to oxidative stimuli (Refs 4, 17, 38), whereas reduced oxidative damage has been observed in cells overexpressing H-ferritin (Refs 71, 72, 73). IRP2 is an additional target of ROS produced under conditions of oxidative stress. Available data suggest that IRP2 is highly sensitive to ROS-induced downregulation; this may be triggered by oxidative modifications of sensitive residues and could lead to ubiquitin-dependent proteasomal digestion (Ref. 30) (Fig. 2). Thus, IRP2 promptly undergoes inactivation in rat liver exposed to glutathione depletion or ischaemia–reperfusion (Refs 35, 68), as well as in cells exposed to menadione (Ref. 70). IRP2 is not downregulated in cells exposed to exogenous H$_2$O$_2$ (Refs 2, 3, 4, 38), confirming that extracellular H$_2$O$_2$ acts through mechanisms independently of oxidative stress.

On balance, there is solid evidence to conclude that both IRP1 and IRP2 serve as controllable targets of intracellularly produced ROS. This provides the cell with a regulatory loop to reduce the LIP and prevent further oxidative damage.

IRPs may also be direct or indirect targets of RNIs (Fig. 2); in fact, iron–sulphur clusters have long been recognised as molecular targets of nitric oxide (‘NO). Therefore, several studies have addressed whether endogenously generated or exogenous ‘NO may influence iron metabolism by attacking the [4Fe–4S] cluster of cytoplasmic aconitate/IRP1. Several reports have shown that ‘NO does increase
IRP1 activity, although the precise mechanism(s) of such activation remain unclear: some investigators have suggested that NO attacks the [4Fe–4S] cluster of cytoplasmic aconitase, inducing its disassembly to form IRP1 (reviewed by Refs 2, 3, 4, 38, 74), whereas others suggest that NO acts by inducing cellular iron release and decrease of the LIP (Refs 2, 3, 4, 38, 74), although these two events may not be mutually exclusive. Other RNIs, such as the nitrosonium ion (NO$^+$), which nitrosylates thiol groups of proteins, may have other effects. In fact, it has been reported that treatment of human erythroleukaemia K562 cells with an NO$^+$ donor decreased the RNA-binding activity of both IRP1 and IRP2 (reviewed by Ref. 75). Whereas IRP1 is susceptible to activation under defined conditions, IRP2 is almost invariably inactivated by RNIs, which causes redox modifications of the residues that are exposed by the [4Fe–4S] cluster-free IRP2 followed by proteasome-mediated protein degradation (Refs 2, 3, 4, 38, 74, 76) (Fig. 2).

From a pathophysiological viewpoint, RNI-induced inactivation of IRP2 may be of greater relevance than a concomitant activation of IRP1-binding activity because IRP2 is highly expressed in macrophages, which are key cells of inflammatory processes. IRP2 downregulation may therefore account for the enhanced ferritin synthesis and reduced TfR1 mRNA content observed in cytokine-stimulated macrophages producing NO or ONOO$^-$ (Refs 42, 76). The described downregulation of IRP1 protein levels may contribute to the maintenance of such responses (Ref. 77). Total IRP activity (IRP1 plus IRP2) of human monocytes and macrophages tends to rise shortly after treatment with cytokines or NO donors, but then decreases markedly accompanied by an enhanced ferritin content (Ref. 78), similarly to the downregulation of IRP2 in mouse macrophage lines (Ref. 42). The effects of RNIs on IRPs may therefore explain the iron-sequestration pattern that characterises macrophages under inflammatory conditions.

The cardiotoxicity induced by doxorubicin (DOX), an antitumour anthracycline that also causes a severe form of chronic cardiomyopathy, provides an excellent model to investigate whether IRPs may also be the target of xenobiotics (Refs 79, 80). Three independent studies have shown that the combined action of an alcohol metabolite of DOX (DOXol), which delocalises iron from the [4Fe–4S] cluster of cytoplasmic aconitase, and O$_2^-$ and H$_2$O$_2$ derived from redox activation of DOX, eventually converts IRP1 into a ‘null’ protein, which lacks both aconitase and RNA-binding activities (Refs 81, 82, 83) (Fig. 2). According to another study (Ref. 84), the formation of the null protein is independent of the action of DOXol on the cluster and derives from the attack of anthracycline–iron complexes on aconitase/IRP1. IRP2 is not attacked by DOXol; only ROS seem to be active in this setting, triggering its degradation (Refs 36, 37).

What are the pathological consequences of the actions of DOXol and ROS on IRP1 and IRP2? The decline of IRP2 that occurs in response to ROS formation might serve as a protective stratagem to facilitate the translation of ferritin mRNA, and to sequester free iron before it converts O$_2^-$ and H$_2$O$_2$ into more-potent oxidants. In fact, DOX treatment increases L-ferritin and H-ferritin synthesis in H9c2 cardiomyocytes (Ref. 37) and in mouse hearts (Ref. 85), with a prominent upregulation of H-ferritin, which has been proposed to have an antioxidant function (Refs 17, 86). IRP1 deficiency does not alter DOX-induced iron metabolism changes nor DOX-induced oxidative damage of the myocardium, showing that high cardiac IRP1 activity in DOX-treated animals does not counteract the potential cardio-protective effect of IRP2 downmodulation (Ref. 85). However, the conversion of aconitase/IRP1 into a null protein, by making cells unable to sense iron levels, may have a major role in inducing chronic cardiomyopathy, which coincides with the gradual conversion of DOX to DOXol.

The ability of DOX to modulate the RNA-binding activity of both IRP1 and IRP2, and hence the expression of IRE target genes, could be involved in both the antitumour and cardiotoxic properties of the drug, and has produced some important clinical results. Indeed, it has been shown that combining DOX with other antineoplastic drugs (e.g. taxanes) enhances chronic cardiotoxicity because conversion of DOX to DOXol is accelerated (Ref. 82). Conversely, anthracyclines with a lower level of formation of their alcohol metabolites exhibit reduced cardiotoxicity in...
preclinical settings, and have entered clinical trials to assess their efficacy and safety compared with DOX (Refs 82, 87).

IRPs are also regulated by oxygen tension (reviewed and discussed in Refs 3, 8), which differentially regulates the binding activity of the two IRPs, with a decrease of IRP1, accompanied by a rise in aconitase activity, and an increase of IRP2 at low oxygen concentrations. Why IRP1 and IRP2 respond in opposite ways to hypoxia remains to be established, but it should be kept in mind that at physiological oxygen tension (3–5%) IRP2 is the predominant RNA-binding protein, thus possibly explaining its major role in the regulation of iron metabolism (Fig. 2).

Clinical applications

Hyperferritinaemia with autosomal dominant congenital cataract – a disorder of iron metabolism characterised by early-onset, bilateral nuclear cataracts and elevated serum ferritin concentrations – is the best-characterised human disease involving the IRE–IRP regulatory system (reviewed by Refs 88, 89). In patients affected by this disorder, serum iron and transferrin saturation are normal or low, and body iron (as evaluated by phlebotomy), is not increased, thus excluding iron overload as the underlying cause of hyperferritinaemia. Molecular studies of tissues from these patients have identified multiple point mutations in the IRE of L-ferritin mRNA, which affects the highly conserved CAGUGU motif in the IRE loop responsible for the high-affinity interaction with IRPs (reviewed by Refs 88, 89). In cultured lymphoblastoid cells from affected patients, the mutation was found to abolish the binding of IRPs and results in constitutively high L-ferritin synthesis, which correlates with serum ferritin levels (Ref. 90). The affected subjects show no haematological or biochemical abnormalities and the phenotype of this mutation is characterised only by an accumulation of L-ferritin in the lens, resulting in cataract, although a direct relationship between the mutation and the lens deposit of aggregated and crystallised ferritin has not been formally demonstrated.

A point mutation in the IRE of the human H-ferritin gene (approved gene symbol FTH1) has been found in members of a Japanese family affected by iron overload (Ref. 91), but this finding has not been confirmed in other pedigrees.

Further support for the clinical implication of alterations in the IRE–IRP network is provided by the expression of ferroportin – the major or only iron exporter from cells – which can be altered upon loss of its 5’ IRE, as seen in polycythaemic mice (Ref. 92). Mutations in the ferroportin mRNA 5’ UTR were detected in a patient with iron overload because of ferroportin disease (Ref. 93), which may depend on altered IRE–IRP recognition. Similarly, the presence of alternative ferroportin transcripts without an IRE in erythroid cells (Ref. 94) leaves open the possibility that alterations in ferroportin mRNA splicing may be relevant in pathological conditions of altered erythroid differentiation.

The relevance of dysregulated iron metabolism in the neurological disorders mentioned above makes the finding that polymorphisms in the promoter region of the IRP2 gene are statistically associated with Alzheimer disease potentially interesting (Ref. 95). However, this preliminary report needs to be confirmed in a larger independent population, and the functional significance of the haplotype determined. In addition, the unexpectedly high IRP1 activity found in patients with Parkinson disease may account for the low ferritin levels present in neurons of the substantia nigra in spite of iron accumulation (Ref. 96).

Research in progress and outstanding research questions

[4Fe–4S] cluster assembly

In recent years, significant advances have been made to define the processes and the molecular participants underlying the assembly of the IRP1 [4Fe–4S] cluster, indicating that iron availability is not the only requirement. In fact, detailed analysis of the pathways of [4Fe–4S] cluster assembly in mammalian cells has indicated a key role of mitochondria (Ref. 97), although a cytosolic [4Fe–4S] cluster assembly machinery has also been identified (discussed in Ref. 8). Impairment of both pathways of [4Fe–4S] cluster assembly leads to induction of IRP1-binding activity and may also alter iron homeostasis. Indeed, it has been found that a mutation in mitochondrial glutaredoxin, a protein involved in [4Fe–4S] cluster biogenesis
(Ref. 53), shifts the IRP1/aconitase balance in favour of the apoform and induces anaemia in zebrafish by repressing eALAS translation and hence haem formation. Future work will define the role of the flux of iron through the mitochondrial and cytosolic [4Fe–4S] assembly pathways in iron homeostasis, possibly leading to treatments for disease, because mutations in the human counterparts of the proteins involved in these processes are likely to be found in human pathological settings (Ref. 98). Similarly, the impairment of [4Fe–4S] cluster formation that characterises Friedreich ataxia may result in uncontrolled IRP1 activation and pathological abnormalities of iron homeostasis (Refs 99, 100).

**Structural studies**

Insight into the structure–function relationship of IRPs has been recently provided by crystallography studies. The crystal structure of cytosolic aconitase has been solved (Ref. 101). Walden et al. (Ref. 26) also solved the crystal structure of IRP1 bound to ferritin IRE, detailing the molecular basis underlying the bifunctionality of this protein. Further investigation in this field, perhaps with a detailed crystal structure of IRP2 complexed with IRE, will hopefully determine the specificity of the IRE–IRP interaction, the preferential binding of individual IRPs to a subset of IREs and the effects of IRP modification on its binding activity and selectivity.

**Role of cytosolic aconitase**

Under normal conditions, most IRP1/aconitase is enriched with the [4Fe–4S] cluster and the ratio of aconitase to IRP1 may exceed 100:1 (Refs 27, 28); this finding hence leaves open the question of the function of cytoplasmic aconitase. A role in modulating the levels of NADPH and citrate has been proposed for cytosolic aconitase (Ref. 102).

**Mouse models**

The role of IRPs in mammalian iron metabolism has been thoroughly studied in cell culture, but relatively little in vivo information is available. Further information regarding their tissue-specific regulation could be gained by the characterisation of knockout mice bearing conditionally targeted IRP1 and IRP2 loci generated by tissue-specific promoters used to control the cre recombinase (Ref. 46). This approach could give indications of the particular role of individual IRPs in specific organs or tissues, as suggested by their different abundances (Ref. 2), and would also be helpful in understanding their physiological role. Further information could be obtained by subjecting animals with tissue-specific or general deficiency of IRPs to stressful stimuli that have been demonstrated to affect IRP activity.

**New targets**

One main area for future research is expected to be the search for new IRP targets. Integration of bioinformatic, biochemical and genomic approaches has recently led to the identification of new IRP-regulated genes involved in a number of pathways; implementation of these strategies will help in the identification of other IRE-regulated genes and lead to a wider understanding of iron-regulatory networks.

**Conclusion**

In recent years, the regulation of iron homeostasis has revealed its increasing complexity and its multifaceted and unforeseen interactions with a number of molecular pathways and processes. The work highlighted here has shown that significant advances have been made in elucidating the role of the IRE–IRP network in the post-transcriptional control of key mRNA molecules involved in iron metabolism, but also indicates that further studies are warranted. In particular, we need to gain further insight into the IRP-mediated metabolic remodelling that occurs in response to changes in iron availability and other iron-independent effectors. This information might shed light on the role of iron in the molecular pathophysiology of a wide range of disorders.

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**References**

1 Andrews, N.C. and Schmidt, P.J. (2007) Iron homeostasis. Annu Rev Physiol 69, 69-85
2 Hentze, M.W. and Kuhn, L.C. (1996) Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron,
nitric oxide, and oxidative stress. Proc Natl Acad Sci U S A 93, 8175-8182

3 Hanson, E.S. and Leibold, E.A. (1999) Regulation of the iron regulatory proteins by reactive nitrogen and oxygen species. Gene Expr 7, 367-376

4 Cairo, G. and Pietrangelo, A. (2000) Iron regulatory proteins in pathobiology. Biochem J 352, 241-250

5 Theil, E.C. and Eisenstein, R.S. (2000) Combinatorial mRNA regulation: iron regulatory proteins and iso-iron-responsive elements (Iso-IREs). J Biol Chem 275, 40659-40662

6 Hentze, M.W., Muckenthaler, M.U. and Andrews, N.C. (2004) Balancing acts: molecular control of mammalian iron metabolism. Cell 117, 285-297

7 Wallander, M.L., Leibold, E.A. and Eisenstein, R.S. (2006) Molecular control of vertebrate iron homeostasis by iron regulatory proteins. Biochim Biophys Acta 1763, 668-689

8 Rouault, T.A. (2006) The role of iron regulatory proteins in mammalian iron homeostasis and disease. Nat Chem Biol 2, 406-414

9 Aziz, N. and Munro, H.N. (1987) Iron regulates ferritin mRNA translation through a segment of its 5′ untranslated region. Proc Natl Acad Sci U S A 84, 8478-8482

10 Hentze, M.W. et al. (1987) Identification of the iron-responsive element for the translational regulation of human ferritin mRNA. Science 238, 1570-1573

11 Leibold, E.A. and Munro, H.N. (1988) Cytoplasmic protein binds in vitro to a highly conserved sequence in the 5′ untranslated region of ferritin heavy- and light-subunit mRNAs. Proc Natl Acad Sci U S A 85, 2171-2175

12 Rouault, T.A. et al. (1988) Binding of a cytosolic protein to the iron-responsive element of human ferritin messenger RNA. Science 241, 1207-1210

13 Mullner, E.W., Neupert, B. and Kuhn, L.C. (1989) A specific mRNA binding factor regulates the iron-dependent stability of cytoplasmic transferrin receptor mRNA. Cell 58, 373-382

14 Breuer, W., Shvartsman, M. and Cabantchik, Z.I. (2007) Intracellular labile iron. Int J Biochem Cell Biol, Mar 19 [Epub ahead of print]

15 Rouault, T.A. et al. (1990) Cloning of the cDNA encoding an RNA regulatory protein—the human iron-responsive element-binding protein. Proc Natl Acad Sci U S A 87, 7958-7962

16 Schalinske, K.L. et al. (1997) Iron regulatory protein 1 is not required for the modulation of ferritin and transferrin receptor expression by iron in a murine pro-B lymphocyte cell line. Proc Natl Acad Sci U S A 94, 10681-10686

17 Torti, F.M. and Torti, S.V. (2002) Regulation of ferritin genes and protein. Blood 99, 3505-3516

18 Bianchi, L., Tacchini, L. and Cairo, G. (1999) HIF-1-mediated activation of transferrin receptor gene transcription by iron chelation. Nucleic Acids Res 27, 4223-4227

19 Lok, C.N. and Ponka, P. (1999) Identification of a hypoxia response element in the transferrin receptor gene. J Biol Chem 274, 24147-24152

20 Patton, S.M. et al. (2005) Subcellular localization of iron regulatory proteins to Golgi and ER membranes. J Cell Sci 118, 4365-4373

21 Beinert, H. and Kennedy, M.C. (1993) Aconitase, a two-faced protein: enzyme and iron regulatory factor. FASEB J 7, 1442-1449

22 Hentze, M.W. et al. (1989) Oxidation-reduction and the molecular mechanism of a regulatory RNA-protein interaction. Science 244, 357-359

23 Hirling, H., Henderson, B.R. and Kuhn, L.C. (1994) Mutational analysis of the [4Fe–4S]-cluster converting iron regulatory factor from its RNA-binding form to cytoplasmic aconitase. EMBO J 13, 453-461

24 Wang, J. et al. (2007) Iron-dependent degradation of Apo-IRP1 by the ubiquitin-proteasome pathway. Mol Cell Biol 27, 2423-2430

25 Clarke, S.L. et al. (2006) Iron-responsive degradation of iron-regulatory protein 1 does not require the Fe-S cluster. Embo J 25, 544-553

26 Walden, W.E. et al. (2006) Structure of dual function iron regulatory protein 1 complexed with ferritin IRE-RNA. Science 314, 1903-1908

27 Kennedy, M.C. et al. (1992) Purification and characterization of cytosolic aconitase from beef liver and its relationship to the iron-responsive element binding protein. Proc Natl Acad Sci U S A 89, 11730-11734

28 Chen, O.S., Schalinske, K.L. and Eisenstein, R.S. (1997) Dietary iron intake modulates the activity of iron regulatory proteins and the abundance of ferritin and mitochondrial aconitase in rat liver. J Nutr 127, 238-248

29 Recalcati, S. et al. (2006) Iron regulatory proteins 1 and 2 in human monocytes, macrophages and duodenum: expression and regulation in hereditary hemochromatosis and iron deficiency. Haematologica 91, 303-310

30 Iwai, K. et al. (1998) Iron-dependent oxidation, ubiquitination, and degradation of iron regulatory protein 2: implications for degradation of oxidized proteins. Proc Natl Acad Sci U S A 95, 4924-4928

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31 Henderson, B.R., Menotti, E. and Kuhn, L.C. (1996) Iron regulatory proteins 1 and 2 bind distinct sets of RNA target sequences. J Biol Chem 271, 4900-4908
32 Butt, J. et al. (1996) Differences in the RNA binding sites of iron regulatory proteins and potential target diversity. Proc Natl Acad Sci U S A 93, 4345-4349
33 Hanson, E.S., Rawlins, M.L. and Leibold, E.A. (2003) Oxygen and iron regulation of iron regulatory protein 2. J Biol Chem 278, 40337-40342
34 Meyron-Holtz, E.G., Ghosh, M.C. and Rouault, T.A. (2004) Mammalian tissue oxygen levels modulate iron-regulatory protein activities in vivo. Science 306, 2087-2090
35 Cairo, G. et al. (1995) Induction of ferritin synthesis by oxidative stress. Transcriptional and post-transcriptional regulation by expansion of the “free” iron pool. J Biol Chem 270, 700-703
36 Minotti, G. et al. (2001) Doxorubicin irreversibly inactivates iron regulatory proteins 1 and 2 in cardiomyocytes: evidence for distinct metabolic pathways and implications for iron-mediated cardiotoxicity of antitumor therapy. Cancer Res 61, 8422-8428
37 Corna, G. et al. (2004) Doxorubicin paradoxically protects cardiomyocytes against iron-mediated toxicity: role of reactive oxygen species and ferritin. J Biol Chem 279, 13738-13745
38 Cairo, G. et al. (2002) The iron regulatory proteins: targets and modulators of free radical reactions and oxidative damage. Free Radic Biol Med 32, 1257-1243
39 Cairo, G. et al. (2002) Nitric oxide and peroxynitrite activate the iron regulatory protein-1 of J774A.1 macrophages by direct disassembly of the Fe-S cluster of cytoplasmic aconitase. Biochemistry 41, 7435-7442
40 Henderson, B.R., Seiser, C. and Kuhn, L.C. (1993) Characterization of a second RNA-binding protein in rodents with specificity for iron-responsive elements. J Biol Chem 268, 27327-27334
41 Recalcati, S., Conte, D. and Cairo, G. (1999) Preferential activation of iron regulatory protein-2 in cell lines as a result of higher sensitivity to iron. Eur J Biochem 259, 304-309
42 Recalcati, S. et al. (1998) Nitric oxide-mediated induction of ferritin synthesis in J774 macrophages by inflammatory cytokines: role of selective iron regulatory protein-2 downregulation. Blood 91, 1059-1066
43 Wang, W. et al. (2007) Excess capacity of the iron regulatory protein system. J Biol Chem 282, 24650-24659
44 Smith, S.R. et al. (2004) Severity of neurodegeneration correlates with compromise of iron metabolism in mice with iron regulatory protein deficiencies. Ann N Y Acad Sci 1012, 65-83
45 Galy, B. et al. (2004) Targeted mutagenesis of the murine IRP1 and IRP2 genes reveals context-dependent RNA processing differences in vivo. Rna 10, 1019-1025
46 Galy, B., Ferring, D. and Hentze, M.W. (2005) Generation of conditional alleles of the murine Iron Regulatory Protein (IRP)-1 and -2 genes. Genesis 43, 181-188
47 Galy, B. et al. (2005) Altered body iron distribution and microcytosis in mice deficient in iron regulatory protein 2 (IRP2). Blood 106, 2580-2589
48 Cooperman, S.S. et al. (2005) Microcytic anemia, erythropoietic protoporphyria, and neurodegeneration in mice with targeted deletion of iron-regulatory protein 2. Blood 106, 1084-1091
49 LaVaque, T. et al. (2001) Targeted deletion of the gene encoding iron regulatory protein-2 causes misregulation of iron metabolism and neurodegenerative disease in mice. Nat Genet 27, 209-214
50 Ghosh, M. et al. (2006) Reply to “Iron homeostasis in the brain: complete iron regulatory protein 2 deficiency without symptomatic neurodegeneration in the mouse”. Nat Genet 38, 969-970
51 Galy, B. et al. (2006) Iron homeostasis in the brain: complete iron regulatory protein 2 deficiency without symptomatic neurodegeneration in the mouse. Nat Genet 38, 967-969; discussion 969–970
52 Meyron-Holtz, E.G. et al. (2004) Genetic ablations of iron regulatory proteins 1 and 2 reveal why iron regulatory protein 2 dominates iron homeostasis. EMBO J 23, 386-395
53 Wingert, R.A. et al. (2005) Deficiency of glutaredoxin 5 reveals Fe-S clusters are required for vertebrate haem synthesis. Nature 436, 1035-1039
54 Gunshin, H. et al. (2001) Iron-dependent regulation of the divalent metal ion transporter. FEBS Lett 509, 309-316
55 Schalinske, K.L., Chen, O.S. and Eisenstein, R.S. (1998) Iron differentially stimulates translation of mitochondrial aconitase and ferritin mRNAs in mammalian cells. Implications for iron regulatory proteins as regulators of mitochondrial citrate utilization. J Biol Chem 273, 3740-3746
56 Cmejla, R., Petrak, J. and Cmejlova, J. (2006) A novel iron responsive element in the 3'UTR of human MRCKalpha. Biochem Biophys Res Commun 341, 158-166
Sanchez, M. et al. (2006) Iron regulation and the cell cycle: identification of an iron-responsive element in the 3′-untranslated region of human cell division cycle 14A mRNA by a refined microarray-based screening strategy. J Biol Chem 281, 22865-22874

Nurtjahja-Tjendraputra, E. et al. (2007) Iron chelation regulates cyclin D1 expression via the proteasome: a link to iron deficiency-mediated growth suppression. Blood 109, 4045-4054

Sanchez, M. et al. (2007) Iron-regulatory proteins limit hypoxia-inducible factor-2alpha expression in iron deficiency. Nat Struct Mol Biol 14, 420-426

Zecca, L. et al. (2004) Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 5, 863-873

Friedlich, A.L., Tanzi, R.E. and Rogers, J.T. (2007) The 5′-untranslated region of Parkinson’s disease alpha-synuclein messengerRNA contains a predicted iron responsive element. Mol Psychiatry 12, 222-223

Rogers, J.T. et al. (2002) An iron-responsive element type II in the 5′-untranslated region of the Alzheimer’s amyloid precursor protein transcript. J Biol Chem 277, 45518-45528

Kohler, S.A., Menotti, E. and Kuhn, L.C. (1999) Molecular cloning of mouse glycylate oxidase. High evolutionary conservation and presence of an iron-responsive element-like sequence in the mRNA. J Biol Chem 274, 2401-2407

Recalcati, S. et al. (2003) Oxidative stress-mediated down-regulation of rat hydroxyacid oxidase 1, a liver-specific peroxisomal enzyme. Hepatology 38, 1159-1166

Tsuij, Y. et al. (2000) Coordinate transcriptional and translational regulation of ferritin in response to oxidative stress. Mol Cell Biol 20, 5818-5827

Caltagirone, A., Weiss, G. and Pantopoulos, K. (2001) Modulation of cellular iron metabolism by hydrogen peroxide. Effects of H2O2 on the expression and function of iron-responsive element-containing mRNAs in B6 fibroblasts. J Biol Chem 276, 19738-19745

Cairo, G. et al. (1996) Superoxide and hydrogen peroxide-dependent inhibition of iron regulatory protein activity: a protective stratagem against oxidative injury. FASEB J 10, 1326-1335

Tacchini, L. et al. (1997) Induction of ferritin synthesis in ischemic-reperfused rat liver: analysis of the molecular mechanisms. Gastroenterology 113, 946-953

Smith, A.G. et al. (1998) Interaction between iron metabolism and 2,3,7,8-tetrachlorodibenzo-p-dioxin in mice with variants of the Ahr gene: a hepatic oxidative mechanism. Mol Pharmacol 53, 52-61

Gehring, N.H., Hentze, M.W. and Pantopoulos, K. (1999) Inactivation of both RNA binding and aconitase activities of iron regulatory protein-1 by quinone-induced oxidative stress. J Biol Chem 274, 6219-6225

Epsztejn, S. et al. (1999) H-ferritin subunit overexpression in erythroid cells reduces the oxidative stress response and induces multidrug resistance properties. Blood 94, 3593-3603

Cozzi, A. et al. (2000) Overexpression of wild type and mutated human ferritin H-chain in HeLa cells: in vivo role of ferritin ferroxidase activity. J Biol Chem 275, 25122-25129

Orino, K. et al. (2001) Ferritin and the response to oxidative stress. Biochem J 357, 241-247

Drapier, J.C. (1997) Interplay between NO and [Fe-S] clusters: relevance to biological systems. Methods 11, 319-329

Kim, S. and Ponka, P. (2002) Nitric oxide-mediated modulation of iron regulatory proteins: implication for cellular iron homeostasis. Blood Cells Mol Dis 29, 400-410

Kim, S. and Ponka, P. (2000) Effects of interferon-gamma and lipopolysaccharide on macrophage iron metabolism are mediated by nitric oxide-induced degradation of iron regulatory protein 2. J Biol Chem 275, 6220-6226

Oliveira, L. and Drapier, J.C. (2000) Down-regulation of iron regulatory protein 1 gene expression by nitric oxide. Proc Natl Acad Sci U S A 97, 6550-6555

Recalcati, S. et al. (1998) Response of monocyte iron regulatory protein activity to inflammation: abnormal behavior in genetic hemochromatosis. Blood 91, 2565-2572

Minotti, G. et al. (1996) Paradoxical inhibition of cardiac lipid peroxidation in cancer patients treated with doxorubicin. Pharmacologic and molecular reappraisal of anthracycline cardiotoxicity. J Clin Invest 98, 650-661

Minotti, G., Cairo, G. and Monti, E. (1999) Role of iron in anthracycline cardiotoxicity: new tunes for an old song? FASEB J 13, 199-212

Minotti, G. et al. (1998) The secondary alcohol metabolite of doxorubicin irreversibly inactivates aconitase/iron regulatory protein-1 in cytosolic fractions from human myocardium. FASEB J 12, 541-552

Minotti, G. et al. (2001) Impairment of myocardial contractility by anticancer anthracyclines: role of secondary alcohol metabolites and evidence of...
reduced toxicity by a novel disaccharide analogue.
Br J Pharmacol 134, 1271-1278
83 Brazzolotto, X. et al. (2003) Interactions between
doxorubicin and the human iron regulatory
system. Biochim Biophys Acta 1593, 209-218
84 Kwok, J.C. and Richardson, D.R. (2002)
Unexpected anthracycline-mediated alterations in
iron-regulatory protein-RNA-binding activity: the
iron and copper complexes of anthracyclines
decrease RNA-binding activity. Mol Pharmacol 62,
888-900
85 Corna, G. et al. (2006) IRP1-independent
alterations of cardiac iron metabolism in
doxorubicin-treated mice. J Mol Med 84, 551-560
86 Harrison, P.M. and Arosio, P. (1996) The ferritins:
molecular properties, iron storage function and
cellular regulation. Biochim Biophys Acta 1275,
161-203
87 Minotti, G. et al. (2000) Anthracycline metabolism
and toxicity in human myocardium: comparisons
between doxorubicin, epirubicin, and a novel
disaccharide analogue with a reduced level of
formation and [4Fe–4S] reactivity of its secondary
alcohol metabolite. Chem Res Toxicol 13, 1336-1341
88 Sheth, S. and Brittenham, G.M. (2000) Genetic
disorders affecting proteins of iron metabolism:
clinical implications. Annu Rev Med 51, 443-464
89 Aguilar-Martinez, P., Schved, J.F. and Brissot, P.
(2005) The evaluation of hyperferritinemia:
an updated strategy based on advances in
detecting genetic abnormalities. Am J
Gastroenterol 100, 1185-1194
90 Cazzola, M. et al. (1997) Hereditary
hyperferritinemia-cataract syndrome: relationship
between phenotypes and specific mutations in the
iron-responsive element of ferritin light-chain
mRNA. Blood 90, 814-821
91 Kato, J. et al. (2001) A mutation, in the iron-
responsive element of H ferritin mRNA, causing
autosomal dominant iron overload. Am J Hum
Genet 69, 191-197
92 Mok, H. et al. (2004) Disruption of ferroportin 1
regulation causes dynamic alterations in iron
homeostasis and erythropoiesis in polycythaemia
mice. Development 131, 1859-1868
93 Liu, W. et al. (2005) Hemochromatosis with
mutation of the ferroportin 1 (IREG1) gene. Intern
Med 44, 285-289
94 Cianetti, L. et al. (2005) Expression of alternative
transcripts of ferroportin-1 during human
erthroid differentiation. Haematologica 90,
1595-1606
95 Coon, K.D. et al. (2006) Preliminary demonstration
of an allelic association of the IREB2 gene
with Alzheimer’s disease. J Alzheimers Dis 9,
225-233
96 Faucheux, B.A. et al. (2002) Lack of up-regulation
of ferritin is associated with sustained iron
regulatory protein-1 binding activity in the
substantia nigra of patients with Parkinson’s
disease. J Neurochem 83, 320-330
97 Lill, R. et al. (2006) Mechanisms of iron-sulfur
protein maturation in mitochondria, cytosol and
nucleus of eukaryotes. Biochim Biophys Acta 1763,
652-667
98 Camaschella, C. et al. (2007) The human
counterpart of zebrafish shiraz shows
sideroblastic-like microcytic anemia and iron
overload. Blood 110, 1353-1358
99 Seznec, H. et al. (2005) Friedreich ataxia: the
oxidative stress paradox. Hum Mol Genet 14,
463-474
100 Stehling, O. et al. (2004) Iron-sulfur protein
maturation in human cells: evidence for a function
of frataxin. Hum Mol Genet 13, 3007-3015
101 Dupuy, J. et al. (2006) Crystal structure of human
iron regulatory protein 1 as cytosolic aconitase.
Structure 14, 129-139
102 Tong, W.H. and Rouault, T.A. (2007) Metabolic
regulation of citrate and iron by aconitases: role of
iron-sulfur cluster biogenesis. Biometals 20,
549-564
Further reading, resources and contacts

Rouault, T.A. (2006) The role of iron-regulatory proteins in mammalian iron homeostasis and disease. Nat Chem Biol 2, 406-414

Wallander, M.L., Leibold, E.A. and Eisenstein, R.S. (2006) Molecular control of vertebrate iron homeostasis by iron-regulatory proteins. Biochim Biophys Acta 1763, 668-689

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http://www.bioiron.org/

Nomenclature of iron genes and proteins:
http://www.bioiron.org/pdf/nomenclature%202.pdf

Features associated with this article

Figures
Figure 1. Regulation of cellular iron homeostasis by the iron-regulatory proteins.
Figure 2. Effect of iron and other signals on the IRE–IRP regulatory system.

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