

THE FATE OF IRON IN THE ORGANISM AND ITS REGULATORY PATHWAYS

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Summary: Iron is an essential element involved in many life-necessary processes. Interestingly, in mammals there is no active excretion mechanism for iron. Therefore iron kinetics has to be meticulously regulated. The most important step for regulation of iron kinetics is absorption. The absorption takes place in small intestine and it is implicated that it requires several proteins. Iron is then released from enterocytes into the circulation and delivered to the cells. Iron movement inside the cell is only partially elucidated and its traffic to mitochondria is not known. Surprisingly, the regulation of various proteins related to iron kinetics and energy metabolism at the molecular level is better described. On contrary, the complex control of iron absorption cannot be fully explicated with present knowledge.

Key words: Iron; Iron-regulatory proteins; Iron-binding proteins; Transferrin receptors; Ferritin

Introduction

Iron is an essential element for virtually all living cells. Many life-important processes, among others oxygen transport, ATP production and DNA-synthesis, could not exist without iron. Cellular iron deficiency stops the cell growth and ultimately leads to the cell death. The most important property of iron, for which iron is a necessary component of many enzymes, is its ability to donate and receive an electron, i.e. to convert between its ferrous (Fe^{2+}) and ferric form (Fe^{3+}). However this useful feature can be dangerous under some conditions, because iron is also known to generate free radicals.

The body of an adult man consists normally of 35 to 45 mg of iron per kilogram. The largest amount of iron is stored in circulating erythrocytes (1.8 g), parenchymatic cells of the liver (1 g), reticuloendothelial macrophages (0.6 g), bone marrow (0.3 g) and muscles (0.3 g) (6).

Absorption

Absorption takes place in absorptive villi of the small intestine, near of the gastro-duodenal junction (22). At this place, in the proximity of stomach the pH is still low and it assists in the decomposition of dietary iron. Iron is presented in food either locked in heme or as ferric ions bound to some molecule. The process of absorption of heme iron is little known while more information is available about non-heme iron. Firstly, ferric ions have to be converted to

ferrous ions. This transformation is realized on the apical (luminal) membrane (71) by means of a heme-based ferric reductase - duodenal cytochrome b (57). Expression of cytochrome b is stimulated by hypoxia, iron deficiency (71) and hypotransferrinaemia and depressed by iron overload (57).

Normally 1 to 2 mg of iron is absorbed daily, the absorption can rarely increase above 6 mg (22).

After the conversion, ferrous ions are transported through apical membrane by divalent metal transporter 1 (DMT-1 or previously named divalent cation transporter, DCT-1; natural resistance associated macrophage protein 2, Nramp2) localized on the same membrane. Except Fe^{2+} , DMT-1 has an unusually broad substrate range including also Zn^{2+} , Mn^{2+} , Co^{2+} , Cd^{2+} , Cu^{2+} , Ni^{2+} and Pb^{2+} , but interestingly it does not transport calcium and magnesium (34). Xu et al. discovered that a simple mutation can dramatically increase calcium permeability indicating similarity between DMT-1 and calcium channels (86). DMT-1 mediated transport is active, proton-coupled and depends on the cell membrane potential. This 561 amino acids protein with 12 transmembrane segments is ubiquitously expressed, most notably just in the proximal duodenum (34). DMT-1 acts also as intracellular transporter (*see the paragraph "Transport and endocytosis of iron into the cells" and Figure 2*). Importance of DMT-1 was manifested by Fleming et al. who found severe defects in intestinal iron absorption and erythroid iron utilization in microcytic, hypochromic anaemic homozygous mk/mk mice which carry

missense (glycine/arginine) mutation in DMT-1 (28). DMT1 is upregulated by dietary iron deficiency (34).

The enterocyte-entered iron can become a part of poorly defined intracellular labile iron pool, be incorporated into ferritin or be released into blood. The mechanism of release into the circulation has not been fully elucidated. Present knowledge indicates that a transmembrane protein named ferroportin1 (named also metal transport protein 1, MTP1) is responsible for this process (1, 50). Overexpression of ferroportin1 in tissue cultures caused intracellular iron depletion (1). Significance of ferroportin1 is documented in

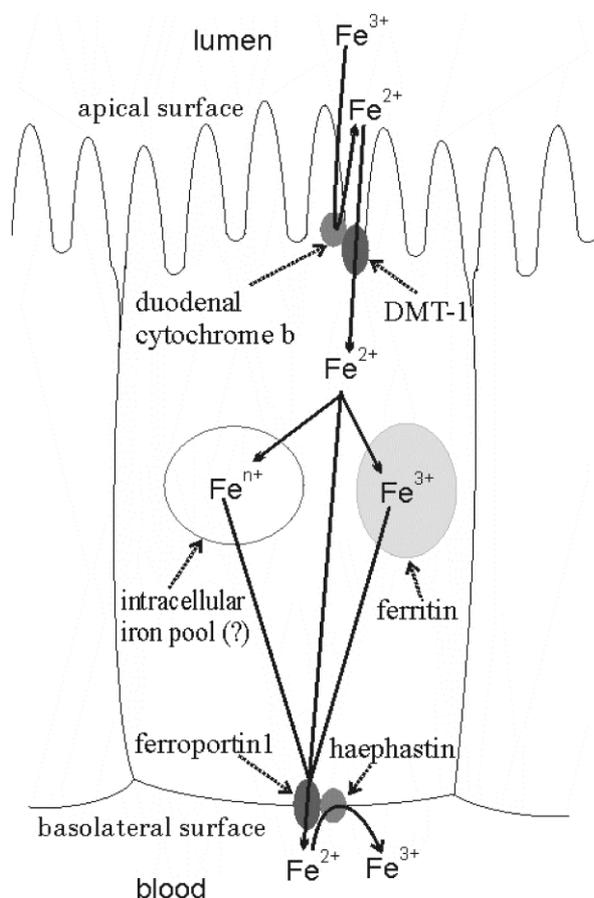


Fig. 1: Schematic illustration showing iron absorption from the lumen of small intestine and its transit through enterocyte - modified according to Andrews, 1999 (6). Iron is presented in food mainly in the form of ferric ions, which are converted by the use of duodenal cytochrome b to ferrous ions. Fe²⁺-ions are transported through the enterocyte membrane by divalent metal transporter-1 (DMT-1). The fate of iron in the enterocyte can be: 1) incorporation into ferritin, 2) becoming part of intracellular labile iron pool or 3) direct transit to the blood. Transport through the enterocyte/blood membrane occurs via ferroportin1. For loading of iron onto transferrin, its conversion to ferric ions, probably by haephastin, is needed.

zebrafish with mutations in ferroportin1 gene, such animals were unable to synthesize this transporter and developed hypochromic anemia (23). Ferroportin1 has 10 transmembrane domains and acts probably also as ferric reductase (a NADP/adenine specific site was identified). This may be due to assumption that ferric ions need to be firstly reduced to ferrous ions and then can be transported by ferroportin1 from the cell into the circulation. But a clear evidence that ferroportin1 transports ferrous or/and ferric ions has not yet been published (50). Ferroportin1 is expressed in many cells, but not in plasma membrane of macrophages (1), in jejunum and ileum (23). The function of ferroportin1 in iron metabolism could be different in miscellaneous cells (*see also Regulation of iron metabolism at the molecular level*). Ferroportin1 was also identified at the basal surface of placental syncytiotrophoblasts, which indicates its possible role in delivery of iron from the mother to the fetus (23).

Osaki et al. found that ferrous ions exported from the cell require before loading onto transferrin their conversion to ferric ions (66). For some decades it has been known that without copper, iron remains in enterocytes and its movement into the circulation is seriously impaired (52). These two facts together led finally to suggestion that ceruloplasmin, which has ferroxidase activity, can be involved in basolateral iron transport (66). Studying sex-linked anaemic (sla) mice, a ceruloplasmin analogue was discovered and it was named after a Greek god of metalworking, haephastin (81). Haephastin is a multicopper oxidase, which in contrast to soluble ceruloplasmin, has a transmembrane domain and therefore is a membrane protein (24). At the present, haephastin is considered to be involved in intestinal iron transport (20,24) while ceruloplasmin in iron transport in some other tissues, e.g. in the liver (24,36).

Transport and endocytosis of iron into the cells

Transferrin (Tf) is the main iron carrier in human, approximately 3 mg of iron is bound to this protein. Apo-Tf possesses two high affinity ferric binding sites, while it has very low affinity for ferrous ions. Normally only about 30% of Tf is saturated by iron (6). Plasma level of non-transferrin bound iron is extremely low and it normally does not exceed 1 μmol/l and is often under detection limits (4). Non-Tf bound iron represents iron bound to other proteins, like ferritin, and also non-protein iron, probably mainly attached to citrate or possibly the citrate-acetate complex (33). Importance of non-Tf bound iron is accentuated in iron-overload disorders.

Iron-loaded transferrin (Tf-Fe₂) is cleared from the blood by specific binding to its cell membrane receptors, transferrin-receptor1 (TfR1) or cubilin receptor (48). Transferrin-receptor 2 (TfR2) does not seem to play a role in iron uptake; its role is discussed later. The binding of Tf to TfR1 has been described elsewhere (6,87). TfR1 exists as a membrane homodimer that binds one diferric Tf per monomer

(72). Attachment of Tf to TfR1 results in the endocytosis of the whole complex. The endosome is acidified by ATP-dependent proton pump, iron, apo-Tf and TfR1 are subsequently released from the complex. Apo-Tf and TfR1 return to the cell surface for re-utilization. Ferric ions are reduced to ferrous ions because only Fe^{2+} can be transported through the endocyte-membrane into cytoplasm by the use of DMT-1. There is, however, an important question, whether the reduction of iron occurs before or after being released from Tf. It was shown that Tf- Fe_2 loses ferric ions under acid conditions (30), but also NADH differic reductase activity on the cell membrane of hepatocytes was documented (77). Ferric ions could be reduced by that reductase to ferrous ions, which, as mentioned above, have low affinity to Tf and can be easily released from Tf. It was pointed out that TfR1 is needed for NADH differic reductase activity and it is well possible that this reductase enters into endosome together with Tf-TfR1 complex (77). Some authors disclaimed existence of a specific NADH differic reductase (10,78). Nowadays no clear evidence of such enzyme has been published, but there is also no evidence that differic transferrin reduction does not occur, it may be mediated by some non-specific NADH-reductase.

Recently, another transferrin membrane receptor was discovered – transferrin receptor 2 (TfR2) (44). TfR2 is composed of 2 protein transcripts: α and β . Transcript α manifests 45% identity and 66% similarity to TfR1 extracellular domain. TfR2 expression was found to be limited mainly to the liver, its elevated levels are presented in erythroid precursors while other tissues displayed low expression (44). Surprisingly no TfR2 was detected in mature red blood cells (17). It is suggested that the function of TfR2 is distinct from that of TfR1. There are some indices supporting this assumption: TfR2 affinity to Tf- Fe_2 is 30 times lower as compared to TfR1 and cell expression of TfR2 in cell culture corresponded with cell cycle, rather than with iron levels (43). Mice lacking TfR1 die before birth disclosing importance of TfR1 for erythropoiesis and neurological development (55) and insufficiency of TfR2 to substitute the function of TfR1. TfR2 acts probably as an iron regulator, its involvement in iron metabolism is described in detail in the paragraph „*Regulation of iron absorption.*“

Interestingly, mammals without Tf can live as it ensues from rare cases of atransferrinemia (12). They manifest hypochromic anemia and have increased iron absorption, but surprisingly their total plasma iron concentration is decreased rather than increased (4) and development of tissues except for the red blood cells is normal (22). Non-Tf iron must therefore be available for tissues apart from the red blood cells precursors that have absolute need for Tf bound iron. Wright and colleagues performed many experiments on rat liver and described a high efficient, carrier-mediated and saturable mechanism of non-Tf transport (85). But the responsible carrier has not been identified as yet.

Recently multifunctional membrane receptor cubilin was demonstrated to play quantitatively important role in

iron supply for the renal proximal tubules. Cubilin mediates the endocytosis of Tf- Fe_2 (48) and therefore can be classified as the third TfR.

At this place, it has to be mentioned a glycoprotein named lactoferrin. This protein reveals a high degree of homology at amino acid sequence level (60) and also the three dimensional conformation level with transferrin (2). Even if the structure of both proteins is very similar, they differ significantly in their localization and function. While transferrin does not allow the existence of free iron in the circulation, lactoferrin may perform the same function on mucosal surfaces. Lactoferrin was also documented to have antimicrobial, anti-inflammatory and antitumoral properties (15,53,79).

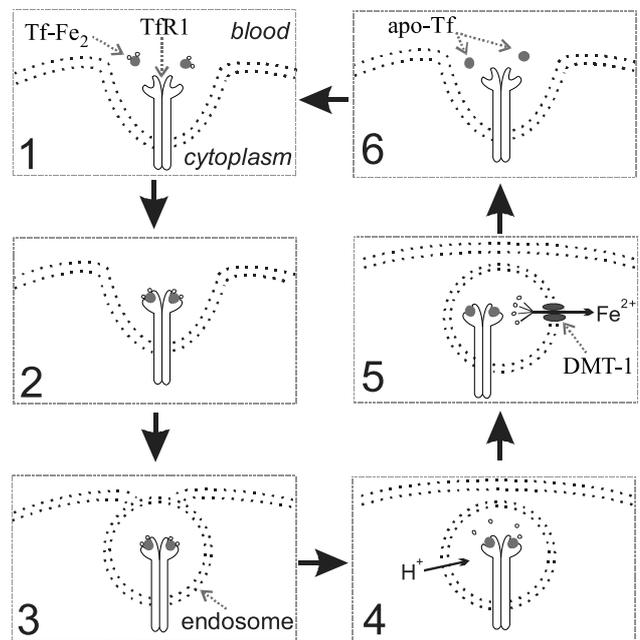


Fig. 2: The schematic view of iron uptake via TfR1-mediated endocytosis in erythroid precursors, as modified according to Andrews, 1999 (6). 1. Iron-loaded transferrin (Tf- Fe_2) in the blood and TfR1 receptor on the cell surface. 2. Two Tf- Fe_2 bind to one TfR1. 3. Internalization of complex Fe-Tf-TfR1. 4. The endosome is acidified by ATP-dependent proton pump and iron (ferric/ferrous?) is released from the complex. 5. Ferrous ions are transported through endosome membrane by use of divalent metal transporter 1 (DMT-1). 6. The recycling of TfR1 and release of apo-Tf into the blood.

Iron inside cells

After the DMT-1-mediated transport the intracellular fate of iron is somewhat unclear. It can be stored inside ferritin, moved to mitochondria, where it can be used for synthesis of heme or Fe-S clusters, or can become a part of mysterious chelatable (labile) iron pool.

Ferritin is a 24-subunits containing protein shell with an inner core where iron is stored as ferrihydrite. The cavity can store up to 4500 atoms of iron, but it usually accommodates about 2000 atoms. Apo-ferritin is heterogenous about 441 kDa large protein composed from variable numbers of 21 kDa heavy (H) or 19 kDa light (L) subunits. Ferritin composition is different in various tissues. Iron passes in form of its ferrous ion through one of the 6 pores of internal cavity of apo-ferritin being oxidized to ferric ion by ferroxidase activity of H-subunit. Inside the ferritin structure, iron becomes a part of a growing crystal of ferrihydrite (FeOOH)_x (40). If iron is needed, it can be easily released from ferritin, but the exact mechanism of iron “escape” and re-reduction to its ferrous form has not yet been published. Normally only isolated particles of ferritin can be seen inside the cells with the exception of the hemopoietic bone marrow and reticuloendothelial system cells, where more frequent particles can be found (40). Small amount of ferritin normally occurs in the blood and it is usually proportional to the quantity of total body iron store (41).

The second cellular iron store compound is hemosiderin. Hemosiderin is a heterogenous and rather insoluble particle, which contains except iron also proteins, carbohydrates and lipids. It is considered to be a degraded form of ferritin and its localization in siderosomes (40), iron containing lysosomes, suggests its possible danger for the cells. Heavily iron-loaded siderosomes were shown to be less stable probably due to the iron-induced lipid peroxidation of the lysosomal membrane (69).

While there is quite a good knowledge of iron storage compounds, there is little familiarity concerning iron transport into mitochondria. A protein named frataxin could play some role. Its mutation leads to neurodegenerative disorder described as Friedreich’s ataxia. Experiments on yeast with homologous gene revealed an accumulation of iron in the mitochondrion (70). Accumulation of iron inside mitochondria was observed also in patients suffering from Friedreich’s ataxia in some tissues (14). Therefore it has been thought that frataxin could play some role in the iron transport from and/or into mitochondria. Defects in Fe-S protein assembly have been recently observed in experiments on yeast implying possible involvement of frataxin in Fe-S cluster maturation (61).

Excretion and iron recirculation

Humans have no specific excretion mechanism for iron. Iron is eliminated only by exfoliation of enterocytes or by menstruation bleeding. The eliminated amount usually corresponds with the absorbed amount, which is normally 1 to 2 mg/day.

As mentioned above, most iron is localized in the red blood cells. At the end of their life, the red blood cells are phagocytosed by specialized population of reticuloendothelial macrophages. Degradation of hemoglobin liberates iron

which is then returned to the circulation by binding to transferrin. Transferrin then distributes the iron through the body and delivers it to iron-requiring cells, preferentially again for hemoglobin synthesis. When iron is needed for hemoglobin synthesis, most cells are capable to release it into the circulation.

Iron-containing proteins

Due to its ability to accept and donate electron iron forms the essential part of many enzymes. Iron is a common component of metalloproteins, where it can be directly ligated to the protein through Fe-S bound or be firmly closed in the heme structure. Heme proteins, including hemoglobin, myoglobin and many enzymes (cytochromes P-450, cytochromeoxidases, peroxidases) are known longer than the Fe-S proteins. Discovery of iron-sulfur clusters, where iron is bound to the protein through sulfur, opened in the 1960s new insight into iron metabolism and function (46). It was pointed out that Fe-S clusters form enzymatic sites of dehydratases, e.g. bacterial enzymes and human aconitases (8), and also the human enzyme succinate dehydrogenase (76).

Enzyme aconitase (aconitase hydratase) converts citrate to isocitrate. There are two different aconitases in mammals, 83 kDa large mitochondrial (m-acon) and 98 kDa cytosolic (c-acon). Both aconitases are encoded by nuclear DNA but their genes are located on different chromosomes. Both are very similar 4-domain proteins with 30% sequence identity and containing [4Fe-4S] cluster necessary for their enzymatic activity (73). Interestingly, acon is an unusual case of Fe-S proteins, because only three irons are ligated directly to cysteines of protein while the fourth (marked as Fe_a, see also Fig. 4) is attached to an inorganic sulfur of cluster and a hydroxyl group (8). Indispensability of Fe_a for enzymatic activity of acon ensues from the fact that it represents the binding site for carboxyl and hydroxyl groups of citrate (9). After translation m-acon is directed to mitochondria, where it executes an important step of Krebs cycle. Conversely, the enzymatic role of c-acon is obscure, it may regulate the non-protein bound iron (65), the non-enzymatic role of c-acon is discussed in the next paragraph.

Succinate dehydrogenase is another Krebs cycle enzyme. It comprises 3 different types of Fe-S clusters, [4Fe-4S], [3Fe-3S] and [2Fe-2S] (76), representing the only known enzyme containing all known types of Fe-S clusters.

Regulation of iron metabolism at the molecular level

There is quite a good knowledge concerning the post-transcriptional regulation of iron metabolism. Iron regulatory elements (IREs) were identified in the untranslated regions of mRNA encoding miscellaneous proteins involved in iron kinetics and energy metabolism. IREs are about 30-nucleotides long stem-loop, or more precisely stem-bul-

ge-stem-loop (see Fig. 3), structures that present specific binding sites for cytoplasmic iron regulatory proteins (IRPs) IRP1 and IRP2.

Under conditions of iron excess, IRPs do not possess affinity to bind IREs, on the contrary, when iron is scarce, IRPs bind to IREs. It is generally accepted that if IRE lies in the 5' end of mRNA, IRE-IRP binding inhibits mRNA translation. If it lies in the 3' end, the IRE-IRP binding protects mRNA against degradation and synthesis of protein is enhanced (47). This rule can be applied in many proteins associated with iron and energy metabolism containing IREs. IREs were found in 5' end of mRNA of H- and L- ferritin chains (7), m-acon (75) or erythroid 5-aminolevulinic acid synthase (e-alas) (13,21,59), a key protein in erythroid heme synthesis. Binding of IRPs to IREs of these mRNAs stops protein synthesis by inhibiting the stable association of mRNA with the small ribosomal subunit (31). Increased levels of iron therefore raise synthesis of ferritin as well as heme/hemoglobin and ATP production (42,75). At the 3' end of TfR1 mRNA five types of IREs, named A-E, were found (19). Between the IRE C and IRE D lies an unstable site, which can be easily recognized by nucleases and the mRNA degradation consequently starts. If IRP binds to IRE, this unstable site is not accessible for endonucleotic clip, mRNA is stabilized and TfR1 synthesis enabled (39). In TfR2 mRNAs, no known IRP was detected, suggesting another type of regulation (44).

Discovery of ferroportin1 regulation represents only one known exception at this time. Ferroportin1 mRNA contains IRE as well in its 5' untranslated region (1,58). But its regulation is somewhat mysterious. In duodenal epithelial cells of iron-deprived mice an augmented expression of ferroportin1 was documented while in iron-replete it diminished. The reverse situation prevails in Kupffer cells of the liver: iron-deprived showed less expression while iron-repletion augmented expression (1).

On the basis of phylogenetic comparison, IREs are considered as strong conserved mRNA structures (80). Henderson et al. examined an optimal sequence and structure of IRE. They confirmed that loop and bulge seem to be the most necessary part of IRE. The loop was formerly considered to have 6 unpaired bases - CAGUGX (X - any nucleotide). Henderson et al. outlined pairing between pyrimidine and purine bases at positions 1 and 5, respectively. In known IREs cytosine (pos. 1) and guanidine (pos. 5) are localized at these positions. Also combination of uracil (pos. 1) and adenine (pos. 5) is sufficient to maintain the function of IRP1, but such combination has not yet been discovered in natural mRNAs (37). In harmony with this finding, positions 1 and 5 are much less accessible to chemical and nuclease attack than other loop positions (11). NMR spectroscopy confirmed the existence of hydrogen bond between positions 1 and 5 (49). It should be emphasized, that IRP2 requires conserved cytosine-guanidine pair (45). Three "free" nucleotides (AGU) at loop positions 2, 3 and 4 and unpaired cytosine bulge seems to be specific

binding sites for IRP contact. Any substitution at these positions largely decreases the IRP-IRE binding in most cases (37). On the contrary, nucleotides in stems can vary if the base-pairing remains retained. Disrupting base-pairing of the upper stem prevents or broadly decreases IRE-IRP binding (11,54).

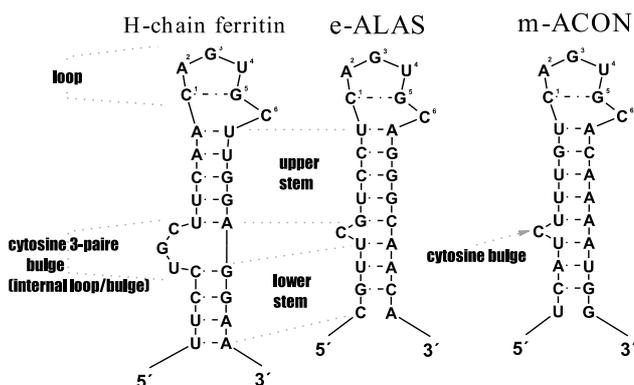


Fig. 3: Structures of some IREs. The primary structures of H-chain ferritin according to Henderson et al., 1994 (37), of e-alas and m-acon according to Ke et al., 1998 (45).

Iron regulatory proteins IRP1 and IRP2 have high sequence identity (57% identical and 79% similar) except for a 73 amino acid insertion in the case of IRP2 (73). IRP1 (formerly termed as iron regulatory factor IRF or iron responsive element binding protein IRE-BP) is 98 kDa bifunctional protein of 889 amino acids containing iron-sulfur cluster. When intracellular iron is abundant, protein possess [4Fe-4S] cluster and act as acon, while the condition of low intracellular iron level occurs, the cluster somewhat decomposes and acts as IRP1. The disintegration of cluster involves more profound changes than a simple loss of Fe_a atom (see previous section and Fig. 4) (16). It should be mentioned, that m-acon lacks the IRE-binding activity (46). IRP2 (formerly assigned as IRP_B) is a 105 kDa protein (38) containing 963 amino acids (73). It is not composed of iron-sulfur cluster and it lacks aconitase activity (35). Like IRP1 also IRP2 binds to IREs in the state of iron deficiency. When iron is in excess, IRP2 is enzymatically degraded (45). Though IRP1 and IRP2 are very similar, they bind various IREs with different affinities: IRP1 affinity to all known IREs was detected to be analogous, but IRP2 binds 10-times more IREs with 3-nucleotide bulge than IREs with single cytosine bulge (45).

The regulation of various proteins involved in iron kinetics and energy metabolism depends on the strength of IRP-IRE binding ensuing from IRE sequence. Suppression of ferritin synthesis in iron-deplete condition is more efficient than those of m-acon (75), e-alas (59) or TfR1 (45). Ferritin mRNA therefore seems to be the most sensitive target for IRPs. The easiest explanation can ensue from the C-bulge structure. Cytosine 3-base bulge (sometimes described

as internal loop/bulge) opposing one nucleotide presented in ferritin mRNA appeared to be 3-fold more effective than as single unpaired cytosine (37) presented in TfR, e-als and m-acon mRNAs. Discovery of IRP2 may play the crucial role because of its higher affinity to ferritin IRE containing 3-base bulge than to other IREs with single cytosine, as described above. The distribution of IRPs in the organism is different. IRP2 prevails in the brain while IRP1 is more profoundly expressed in other tissues (73). High concentration of IRP2 was found out also in the intestine in experiments with rodents (37). These findings, however, cannot sufficiently explain IRP2 contribution to iron protein regulation.

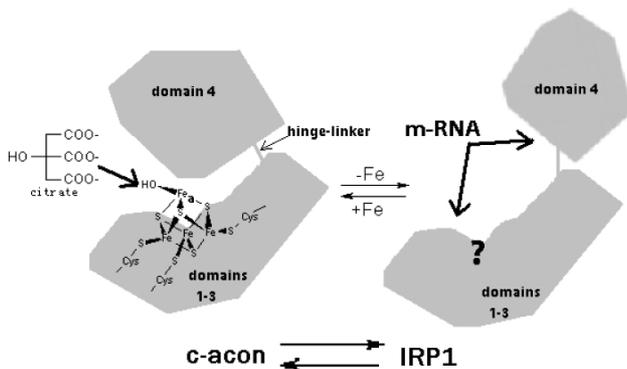


Fig. 4: The double function of cytosolic aconitase / iron regulatory protein 1. The structure of the cluster and protein according to Beinert and Kennedy, 1989 (9) and Klausner and Rouault, 1993 (46), respectively. In iron-replete status, the protein acts as c-acon, in iron deplete status, iron is released from the cluster, the cluster somewhat decomposes and the whole protein acts as IRP1. The double-function protein IRP1/c-acon is 4-domains protein with a cleft between domains 1-3 and 4 that is connected by a flexible hinge linker.

Phosphorylation of IRPs may explain some questions. Eisenstein et al. found two specific sites (around Ser 138 and Ser 711) of IRPs which can be phosphorylated. It is of interest that IRP1 contains both specific sites, but IRP2 only one with the important change of serine to alanine (25). Both sites were proven to be highly conserved in vertebrates (25,27). It was documented that phosphorylation of Ser-138 site of IRP1 causes loss of c-acon activity by an impairment of Fe-S cluster (16,27). Previously, the enhanced IRE-IRP binding of phosphorylated IRPs was found (25). Interestingly, on Ser-138 phosphorylated IRP1 appears to be stabilized by iron deprivation but in situations of iron abundance it undergoes degradation similar to dissociation of IRP2 (27). Therefore phosphorylation can represent a regulatory mechanism with involvement of either iron or some other signals.

At least, it can be stated that the affinities of IRPs to IREs and distribution of IRPs are not accidental and reflect

the accuracy of regulation. For the cell it is important to have some level of m-acon, because of its exigency in ATP synthesis. On the other hand, huge ferritin synthesis could create a deficiency in accessibility of iron for iron-protein synthesis (75). In the future, discovery of new proteins regulated by IRE sequences is awaited. At present their research is difficult due to variation of IRE-sequence not enabling fast analysis.

Iron was found to be the responsible element for regulation of some other proteins but not via the IRP-IRE system. In *Drosophila* IRE was found in the mRNA of succinate dehydrogenase (47), in human mRNA of the homologous enzyme such IRE is not comprised, but succinate dehydrogenase is still regulated by iron status. Iron supplementation was found to increase activity of two others enzymes of Krebs cycle: succinate dehydrogenase and isocitrate dehydrogenase (64). This finding in the concordance with the described regulation of m-acon accentuates enhanced NADH and ATP production in the case of iron abundance (64).

Regulation of iron absorption

As generally accepted, the excess of iron is toxic for the cells. Because mammals lack a regulatory pathway for an active iron excretion, iron levels have to be tightly regulated by iron absorption.

For many years the process of regulation of iron absorption was so foggy, that terms like "stores regulator", "erythropoetic regulator" and "mucosal block" (absorption regulator) were used to express three proposed ways for that regulation. The present knowledge is still not sufficient, but it is known that at least two of these vague regulators may represent protein called hepcidin.

Hepcidin is a 25-amino acid peptide hormone, which contains 8 cysteine-residues, all connected by 4 intramolecular disulfide bounds. It is produced by the liver as a 84-amino acid long precursor. The name reflects both its site of origin and its significant antimicrobial properties (68). The group of Nicolas demonstrated dependence of iron status on the hepcidin levels: Transgenic animals overexpressing hepcidin have decreased levels of body iron, on the contrary mice lacking hepcidin manifested progressive iron accumulation though surprisingly dramatic decrease of iron stores in reticuloendothelial cells. Experimentally induced anemia decreases hepcidin mRNA levels in mice (62,63).

Patients with large hepatic adenomas have severe iron refractory anemia. Adenoma produces inappropriately high levels of hepcidin, when adenoma was resected or the liver was transplanted, anemia resolved spontaneously (84).

Decreased iron content in the diet evoked augmented formation of duodenal cytochrome b, DMT-1 and ferroportin1 (hephaestin did not changed) and depression in hepcidin synthesis in the liver (5). In conclusion, hepcidin has been proposed as a negative regulator of iron absorption and reticuloendothelial macrophage iron release.

Important role in the regulation of iron absorption plays also HFE-protein. It is a 343 amino acids integral membrane protein which reveals a tight homology to a major histocompatibility complex class I-like protein. Its significance in iron metabolism has been acknowledged for many years because of its defect in hereditary hemochromatosis, the most common autosomal recessive disorder known in human. Patients suffering from this disease show increased iron absorption and develop iron overload. HFE-protein is localized in association with TfR1 and β_2 -microglobulin in duodenum, predominantly in the crypt enterocytes and in the placenta (83). Lebron et al. showed that if HFE-protein is bound to TfR1, it reduces affinity of receptor for iron-loaded transferrin (51). However this presumption is questioned by others as the reported nanomolar changes in affinity do not seem to influence receptor binding properties (83). It was found out that one type of mutation in HFE (C282Y) inhibited HFE-protein interaction with β_2 -microglobulin (26), leading to accelerated degradation of mutated HFE-protein (82). On the one hand, mice lacking β_2 -microglobulin were shown to have the same manifestations like in hereditary hemochromatosis, i.e. impaired iron absorption regulation associated with increased iron absorption and iron overload (74), seemingly proposing important role of β_2 -microglobulin in iron absorption control. But on the other hand, the second most common mutation of HFE gene (H63D), which was demonstrated to aggravate iron overload status (56), did not inhibit the interaction of β_2 -microglobulin with HFE-protein (26). Mice with decreased levels of TfR1 seemed to have reduced iron absorption (55).

knowledge proposes that the level of serum transferrin-bound iron informs crypt enterocytes via HFE-protein and TfR1 about the total body iron status. If the body iron stores are low, crypt cells are targeted to differentiate in enterocytes with programmed elevated absorption of iron (3).

This seems to be likely, because the response of iron demand lasts 2 or 3 days and the same period is necessary for crypt cells to migrate and differentiate in villus enterocytes. The programming of enterocytes involves the raised synthesis of DMT-1. This is well documented in hereditary hemochromatosis, where the augmented expression of DMT-1 was discovered (34, 88). Precise mechanism how HFE is involved in iron metabolism is not known nowadays.

From the recent studies it ensues that TfR2 plays an important role in the iron regulation. Mice with mutation of TfR2 manifested hemochromatosis (29). Humans with mutated TfR2 suffer also from hemochromatosis, a disorder very similar to the hereditary hemochromatosis caused by HFE mutation (18). The link between the HFE and TfR2 is supported by finding that TfR2 and HFE-protein co-localize in the crypt duodenal cells (32).

Recently, a new mutation in HFE2 gene responsible for protein named hemojuvelin was discovered. The mutation in HFE2 causes juvenile hemochromatosis, a disorder indistinguishable from hepcidin-deficiency. Hemojuvelin localization is restricted to similar tissues as that of hepcidin. Analysis of hemojuvelin reveals its possible function as membrane-bound receptor or secreted polypeptide hormone. Deleterious mutation of hemojuvelin reduces hepcidin levels despite iron overload (in such condition hepcidin expression is normally induced). It is thought that hemojuvelin acts as modulator of hepcidin expression (67) but further studies are needed for verification.

Conclusion

This review attempted to shed light on the fate of iron in the organism. Because the topic is large, it was necessary to omit willfully some important tasks. Anemias and diseases with iron overload were mentioned only for expli-

Tab. 1: The overview of proteins related to iron kinetics and its regulation.

Protein	The role in iron kinetics and its regulation
ceruloplasmin	oxidation of cell-released iron before binding to transferrin
cubilin	transport of iron in the renal proximal tubular cells
DMT-1	iron transport from the small intestine lumen into enterocytes and from endosome into cytoplasm
ferritin	iron storage
ferroportin1	iron transport from cell into the blood
haephastin	oxidation of cell-released iron before loading on transferrin
hemojuvelin	regulation of iron kinetics, modulator of hepcidin expression?
hemosiderin	iron storage (detoxication)
hepcidin	negative regulator of iron absorption and reticuloendothelial macrophage iron release?
HFE-protein	regulation of iron kinetics?
IRP1	regulation of iron kinetics
IRP2	regulation of iron kinetics
lactoferrin	iron chelation on mucosal surfaces and the involvement in the body defence against pathogens
TfR1	iron transport from the blood into cells
TfR2	regulation of iron kinetics?
transferrin	transport of iron in the circulation

cation of physiological ways of iron metabolism. The fate of iron in the brain was reviewed successfully by Zecca et al. (87).

Abbreviations

DMT-1, divalent metal transporter; IRP1, iron regulatory protein 1; IRP2, iron regulatory protein 2; Tf, transferrin; Tf-Fe₂, iron-loaded transferrin; TfR1, transferrin receptor 1; TfR2, transferrin receptor 2; c-acon, cytosolic aconitase; m-acon; mitochondrial aconitase; Ser, serin.

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