MOLECULAR CONTROL OF IRON METABOLISM IN MAN

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Introduction
Iron participates in several biologic reactions and is essential to virtually all forms of living organisms. Iron-containing proteins of the respiratory chain are involved in electron transport to provide the energy for cellular functional activities. Iron is also required for cell growth and multiplication in view of its role in the activity of ribonucleotide reductase, a key enzyme in DNA synthesis, responsible for the reduction of ribonucleotides to deoxyribonucleotides. This enzyme turns over rapidly and needs a continuous supply of iron to maintain activity. On the other hand, iron is a potentially toxic element, being a catalyst of the transformation of the non-toxic superoxide radical (O_2^-) into poisonous free radicals such as OH^-.
Thus, the essentiality of this metal together with its potential toxicity suggests that cellular iron metabolism needs to be highly regulated, and that abnormalities in any of its different steps may affect both the survival and the proliferative activity of the cell.

For a long time, only three proteins of iron metabolism were known: transferrin, transferrin receptor (TfR), and ferritin (L and H subunit). Also, the only established genetic disorder of iron metabolism was HLA-related hereditary hemochromatosis, whose gene was unknown. In the last few years, our understanding of iron metabolism in health and disease has been explosive: novel genes and proteins have been identified, and several genetic disorders have been characterized.

Post-transcriptional regulation of human iron metabolism and its pathology
Cellular iron homeostasis in mammalian cells is maintained by the coordinated regulation of transferrin receptor and ferritin synthesis that occurs at the translational level and is mediated by cytoplasmic mRNA-binding proteins, known as iron regulatory proteins (IRPs). These proteins are capable of sensing cellular iron status and of interacting with mRNA stem-loop structures known as iron-responsive elements (IREs). IREs constitute the first well-characterized family of co-acting non-coding regulatory sequences in eukaryotic mRNA. A single functional IRE is found in the 3'-UTR of mRNAs for ferritin H and L subunits and in ALAS2. In contrast, multiple IREs are present in the 3'-UTR of the mRNA for transferrin receptor.

Two IRP family members, IRP1 and IRP2, have been identified in humans. Under conditions of intracellular iron depletion, both IRP1 and IRP2 function as RNA-binding proteins that bind IREs with high affinity. The binding of an IRP to the ferritin IRE prevents the association of the 43S translation pre-initiation complex with the mRNA by precluding the recruitment of the small ribosomal subunit. Thereby, translation of the ferritin protein is repressed. Conversely, binding of IRPs to the IREs in the 3'-UTR of transferrin receptor increases the stability of mRNA and improves the efficiency of translation. When intracellular iron concentrations rise, the IRPs dissociate from the IREs. IRP1 may acquire a [4Fe-4S] cluster and behave as a cytosolic aconitase. IRP1 exhibits approximately 30% sequence homology to mitochondrial aconitase. IRP2 lacks aconitase activity and functions solely as an RNA-binding protein. At elevated intracellular iron concentrations, IRP2 is targeted for degradation by the proteasome.

Thus, translational regulation by IRPs allows rapid and coordinated control of proteins that are crucial for cellular iron homeostasis. When cells have adequate iron, the expression of transferrin receptors decreases and the levels of ferritin rise to accommodate the excess iron. On the other hand, when cellular iron becomes scarce, the levels of ferritin fall while the expression of transferrin receptors increases to import more iron from the outside.

The above advances in the molecular regulation of iron metabolism have allowed a better understanding of genetic disorders of iron metabolism. Mutations that cause disease through increased efficiency of ferritin mRNA translation have been discovered, defining translational pathophysiology as a novel mechanism of human disease. Hereditary hyperferritinemia/cataract syndrome (HHCS, OMIM 600886) arises from various point mutations or deletions within a protein-binding sequence in the 5'-UTR of the L-ferritin mRNA. Each unique mutation confers a characteristic degree of hyperferritinemia and severity of cataract in affected individuals. Evaluation of in vitro affinity of the IRPs for the mRNA...
important IREs from HHCS patients has shown a close relationship between decreases in binding affinity and clinical severity. Having identified HHCS as a genetic disorder due to LI-ferritin IRE mutations, the next question is whether any human disorder of H-ferritin synthesis exists. So far, the only potential genetic disorder of H-ferritin synthesis has been reported by Kato and coworkers. They reported a single point mutation (A49U) in the IRE motif of H-ferritin mRNA in a family with hyperferritemia, claiming that this is a gain-of-function mutation responsible for decreased H ferritin synthesis and elevated L ferritin production.

**HFE and HLA-related genetic hemochromatosis**

A major step forward in our understanding of the molecular control of iron metabolism was the cloning of HFE on the short arm of chromosome 6. Seven years later the role of HFE in the process of normal iron metabolism is still uncertain, although a series of studies have documented that the HFE protein is able to bind TfR thereby inhibiting the interaction between TfR and dimeric transferrin. Townes& Drakesmith have proposed a molecular model in which HFE has two mutually exclusive activities in cells: inhibition of uptake or inhibition of release of iron. The balance between serum transferrin saturation and serum transferrin-receptor concentrations determines which of these functions predominates.

Following the identification of HFE as the gene of HLA-related genetic hemochromatosis (HFE, OMIM 235200), homozygosity for the HFE C282Y mutation has been found in more than 90% of North European origin clinically diagnosed with genetic hemochromatosis. However, it became later clear that both genetic and acquired factors play a role in the phenotypic expression of HFE-related hemochromatosis. Recently Beutler et al. reported that the penetrance of HFE-related hereditary hemochromatosis is much lower than generally thought, with less than 1% of homozygotes developing frank clinical hemochromatosis.

**DMT1**

Another major advance was represented by the recognition of the divalent metal transporter in mammals, DMT1 (also known as DCT1 and Nramp2), active in the gut and expressed on the luminal membrane of the enterocyte (Fleming et al., 1997; Gunshin et al., 1997). DMT1 transfers iron across the apical surface of intestinal cells and out of transferrin cycle endosomes, and is not iron-specific, but serves also other metals. DMT1 mRNA in the gut has a 3' UTR IRE and is iron-regulated, as shown in mouse models in conditions of iron deficiency or iron overload. Both mk/mk mice and Belgrade rats, which have a microcytic, hypochromic anemia, carry a missense mutation (G185R) in DMT1. A homolog of the transferrin receptor has been identified as transferrin receptor 2 (TfR2). TfR2 is a member of the “transferrin-like receptors” family with an unknown function, characterized by a restricted pattern of expression in the liver. TfR2 lacks an IRE and allows continued uptake of transferrin-bound iron by hepatocytes even after TfR has been down regulated by iron overload. Camaschella and coworkers have conducted studies whose findings support the hypothesis that TfR2 contributes to body iron sensing.

Hemochromatosis type 3 (HFE3, OMIM 604250) is a familial iron overload syndrome. The two groups found different missense mutations in the gene coding TfR2, suggesting that this may represent the molecular basis for point mutations in the gene coding TfR2, suggesting that this may represent the molecular basis for this familial iron overload syndrome. The TfR2 mutations responsible for hemochromatosis type 3 appear to be private mutations.

**Ferroportin and hemochromatosis type 4 (HFE4, OMIM 606069)**

Ferroportin (Ferroportin) is a newly discovered molecule that plays a crucial role in iron export. The gene (SLC11A3) has at least 10 transmembrane domains, a reductase site and a basalolateral localization signal. Ferroportin is expressed on the basolateral surfaces of mature enterocytes within the duodenum and in macrophages of the spleen and liver. Hemochromatosis type 4 (HFE4, OMIM 606069) is a dominantly inherited. The two groups found different missense mutations in the gene encoding the iron export protein ferroportin. This dominant type of this genetic reticuloendothelial iron overload is currently defined as hemochromatosis type 4, or HFE4 (OMIM 606069), or ferroportin disease. A peculiar type of this condition, typically presenting as selective reticuloendothelial iron overload, is the genetic disorder associated with a three base pair deletion in the coding region of the ferroportin gene (SLC11A3) involving the deletion of valine 162 of the protein.

**Hepcidin, hemojuevelin, anemia of inflammation and juvenile genetic hemochromatosis**

Hepcidin is an antimicrobial peptide that plays a major role in metabolism and is a mediator of ane-
mia of inflammation. This peptide, encoded by the HAMP gene, is produced by the hepatocyte and is expressed as a precursor of 84 residues that is processed to mature forms of 25, 21 and 20 amino acids. It is a secretory protein and has the structure of a beta-hairpin connected by 4 disulfide bridges resulting in a molecule with hydrophilic and basic surfaces. Hepcidin expression is modulated by the body iron status in the mice, and the inactivation of its gene in KO mice leads to a severe iron loading. Overexpression of this antimicrobial peptide in transgenic mice causes severe, and often lethal, iron deficiency anemia. Hepcidin expression is positively regulated by inflammation, specifically by interleukin 627, and by iron overload, while it is negatively regulated by the gene, and very little is known on processing, maturation and secretion pathways of the protein. This is due to the lack of adequate antibodies and cellular models.

Recently a new protein called “hemojuvelin” was identified, encoded by the HVJ gene on chromosome 1q31. Camaschella and coworkers later found that the juvenile locus was mapped to chromosome 1q29. This protein has a leader and a C-terminal trans-membrane sequence, with properties typical of a GPI anchor protein. However, alternative splicing products have been reported, which are possibly expressed in skeletal muscle and other organs, and are predicted to encode proteins without the leader sequence. The observations that HJV is expressed in the same tissues that express HAMP suggests that hemojuvelin is an hepcidin chaperon rather than the hepcidin receptor.

Juvenile genetic hemochromatosis, or hemosiderosis type 2 (HFE2, OMIM 602390). These patients present with hypochromic hypogonadism in the second decade of life and, unless proper treatment is started, die early because of cardiac dysfunction. In an Italian cooperative study, the juvenile locus was mapped to chromosome 1q11. Camaschella and coworkers later found that the juvenile condition which is not associated with the 1q locus (HFE2B, OMIM 602390) is caused by mutations in the HAMP gene encoding hepcidin. Finally, we and others have shown that the most common type of juvenile genetic hemosiderosis (HFE2A, OMIM 602390) is caused by mutations in the HJV gene encoding hemojuvelin.

Thus, hepcidin is a key regulator of iron metabolism and mediator of anemia of inflammation.

Conclusions

This outline documents the recent remarkable advances in our understanding of molecular regulation of iron metabolism, and shows how these achievements have provided us with opportunities to identify novel genetic disorders and their molecular basis, and to develop new diagnostic and therapeutic strategies for these conditions.

References

4. Rouault TA. The iron regulatory protein-1 \\


