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## Relationship Between Iron and Erythropoiesis

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### 1. Introduction

In recent years there has been important advancement in our knowledge of iron metabolism regulation that also has implications for understanding the physiopathology of some human disorders like beta-thalassemia and other iron overload diseases. In fact, progressive iron overload is the most salient and ultimately fatal complication of beta-thalassemia. Iron deposition occurs in visceral organs (mainly in the heart, liver and endocrine glands), causing tissue damage and ultimately organ dysfunction and failure. Both transfusional iron overload and excess gastrointestinal absorption are contributory. Paradoxically, excess gastrointestinal iron absorption persists despite massive increases in total body iron load. However, little is known about the relationship among ineffective erythropoiesis, the role of iron-regulatory genes, and tissue iron distribution in beta-thalassemia. The focus of this chapter is an update about iron homeostasis and erythroid differentiation with a particular attention to the molecular mechanisms of iron homeostasis deregulation in thalassemia and to the GDF15-BMP-Hepcidin-Ferroportin regulatory pathway in order to understand the contribution to iron overload. The chapter describes evidences for these relationships and discusses how recent discoveries on iron metabolism and erythropoiesis could lead to new therapeutic strategies and better clinical care of these diseases, thereby yielding a much better quality of life for the patients.

### 2. Overall view on erythroid cells differentiation and importance of iron homeostasis

Iron homeostasis depends on a coordinated regulation of molecules involved in the import of this element and those exporting it out of the cells. In some cell types, such as erythroid cells, iron import mechanisms are highly expressed, thus allowing massive iron uptake (Pietrangelo, 2002; Testa et al., 1995). Excessive iron, however, may be toxic for these cells, particularly in view of its capacity to generate superoxide radicals and  $H_2O_2$ , which may freely diffuse into the nucleus resulting in cell damage (Karthikeyan, 2002) and it seemed therefore of interest to investigate whether erythroid cells possess specific mechanisms for iron export. Within the hematopoietic differentiation, the maintenance of iron homeostasis is essential for erythroid cells and macrophages. Erythroid cells need to incorporate very high amounts of iron to support the continued synthesis of heme and hemoglobin, while the

macrophage cells play a key role in iron storage and recycling (Ponka, 1997; Testa et al., 1993; Testa, 2002). Human erythropoiesis is a dynamic complex multistep process that involves differentiation of pluripotent hematopoietic stem cells (HSCs) and early multipotent progenitors (MPP) to generate committed erythroid precursors, the erythroblasts, which then give birth to mature erythrocytes, i.e. the red blood cells (RBCs) (Orkin & Zon, 2008; Palis, 2008; Tsiftoglou et al., 2009; Weissman, 2000). Briefly, the early erythroid progenitors (BFU-E, burst-forming units-erythroid) differentiate into late colony-forming units erythroid (CFU-E) and proerythroblasts followed by a progressive wave of erythroblast maturation in polychromatic and orthochromatic erythroblasts coupled with a gradual increase of erythroid-specific markers (Fig.1). As the hematopoietic process progresses from the early stages into erythroid cell maturation, cells gradually lose their potential for cell proliferation and become mature enucleated cells (Fig.1). Mature erythrocytes are biconcave disks without mitochondria and other organelles but full of hemoglobin able to bind and deliver O<sub>2</sub> (Ingley et al., 2004; Koury et al., 2002; Stamatoyannopoulos, 2005; Tsiftoglou et al., 2009). The hematopoietic differentiation is a highly complex system in which, from a pool of totipotent stem cells, originate all the cells of peripheral blood (Golde, 1991; Metcalf, 1989; Orkin, 1996; Smith, 2003). The blood has a very important role in the functions of the organism from the earliest moments of its development, so that during embryonic life the various stages of the hematopoietic process alternate at different sites according to the different stages of development (Emerson et al., 1989). The embryonic→fetal hematopoiesis is characterized by three fundamental periods of activity progressively involving the yolk sac, liver and bone marrow. The first period, during which hematopoiesis is localized at the yolk sac, begins between the 14th and 19th days of embryonic life and continues until the completion of 3rd month (Emerson et al., 1989). Starting from the third month, the second phase of hematopoiesis takes place in the liver where it reaches its maximum during the 3rd-4th month and remains active until a few weeks before birth, when the definitive hematopoiesis (third phase) is concentrated only in the bone marrow and it will continue throughout adult life (Emerson et al., 1989). In this system of hematopoietic differentiation four compartments may be identified: stem cells and the progenitors (cellular compartments), precursors and mature elements of circulating blood (maturation compartments). Hematopoietic stem cells are characterized by the ability to self-renew (i.e. to generate other totipotent stem cells) and differentiate into hematopoietic progenitor cells. Stem cells also show the important property to remain for long time in a state of quiescence during adult life (Domen, 1999; Metcalf, 1989; Orkin, 1996; Smith, 2003). Primitive progenitors are able to generate blast colonies (CFU-B), the progenitors of high proliferative potential (HPP-CFC, colony-forming cells that power high-proliferative), and finally the multipotent progenitors that are still capable of generating mixed colonies, belonging to the different types of hematopoietic differentiation: erythroid, granulocyte, monocyte, and megakaryocytic (the CFU-GEMM) (Orkin, 1996; Ogawa, 1993; Grover, 1994). Mature progenitors are committed to the differentiation towards a singular hematopoietic lineage and are functionally defined as early burst forming units (BFUs) or more differentiated colony forming units (CFUs): erythroid progenitors are BFU-E and CFU-E; granulocytic-macrophagic progenitors are CFU-GM, CFU-G and CFU-M; finally megakaryocytic progenitors are BFU-Mk and CFU-Mk (Grover, 1994; Ogawa, 1993; Orkin, 1996; Smith, 2003). The survival, proliferation and differentiation of hematopoietic stem and progenitor cells are regulated by a complex network of hematopoietic growth factors collectively known as colony stimulating factors (CSFs), interleukins (ILs) or hemopoietins that are released from accessory cells such as fibroblasts, macrophages, lymphocytes and

endothelial cells. Depending on their mechanism of action during hematopoietic differentiation, these factors can be classified into three categories: the first category includes growth factors that exert their action at the earliest stages of hematopoiesis, e.g. the c-kit receptor ligand (KL) or stem cell factor (SCF) (Bernstein, 1991), FLT-3 ligand (FL) (Gabbianelli, 1995; Lyman, 1994;), the basic fibroblast growth factor (bFGF) (Berardi, 1995; Gabbianelli, 1990) and interleukin-6 (IL-6) [Leary, 1988]; in the second category are growth factors acting as multilineage, whose prototypes are the IL-3 and GM-CSF that are able to stimulate primitive progenitors to proliferate and differentiate into all hematopoietic lineage (Metcalf, 1993); and finally in the third category are included the growth factors acting as unilineage, i.e. those that stimulate the differentiation and proliferation of a single lineage and include erythropoietin (EPO) [Fried, 1995; Krantz, 1991], the granulocytic growth factor (G-CSF) [Demetri & Griffin, 1991], monocytic growth factor (M-CSF) [Sherr, 1990] and thrombopoietin (TPO) [Kaushanky et al., 1994]. These unilineage factors act on progenitors already moving towards hematopoietic lineage and promote the production of mature cells in the circulating blood, i.e. erythrocytes, neutrophils, eosinophils, monocytes/macrophages and megakaryocytes. During the hematopoietic differentiation, the maintenance of iron homeostasis is essential for erythrocytes and macrophages. Erythroid cells need to incorporate very high amounts of iron to support the continued synthesis of heme and hemoglobin, while the macrophage cells play a key role in the storage and recycling of iron (Testa et al., 1993; Testa, 2002; Tsiftoglou, 2009). During the differentiation of erythroid progenitors towards mature red cells the following morphologically recognizable stages can be distinguished: 1) the earlier stage of proerythroblast, that presents a nucleus relatively large in respect to the cytoplasm, and one or two nucleoli; 2) the more advanced basophilic erythroblast, characterized by a reduced cellular diameter, a nuclear volume reduced more rapidly than the cytoplasm, and a cytoplasm uniformly basophilic; 3) the polychromatophilic erythroblast, that shows the initial condensation of the nucleus, nucleoli no longer visible and the cytoplasm with acidophilic areas; 4) the orthochromatic erythroblast, with a nucleus:cytoplasm ratio of approximately 1:4, nucleus darker and subject to pyknotic degeneration, and cytoplasm slightly pink as a consequence of the progressive increase in hemoglobin concentration; 5) and finally the reticulocyte that has lost its nucleus and, through the complete degradation of ribosomes and mitochondria, proceeds to the transformation in mature erythrocyte (Grover, 1994; Loken et al., 1987; Okumura et al., 1992; Orkin, 1996) (Fig.1). In adult mammalian bone marrow erythroblasts are always associated to the erythroblastic islets, that represent the drive amplification stage anatomy of erythropoiesis and consists of 1 or 2 histiocytic crown cells surrounded by erythroblasts at all stages of maturation. The histiocytes have thin cytoplasmic extensions that insert between erythroblasts suggesting that factors of nutrition can be provided by the histiocytic cell, centrally located, to the peripheral maturing erythroblasts (Grover, 1994; Orkin, 1996). The circulating red cell mass is maintained constant by a homeostatic mechanism regulating erythropoiesis, based on an erythropoietic stimulus which ensures that, under physiological conditions, the production of red blood cells equals their destruction. Moreover, in response to hypoxia, hemorrhage or hemolysis, this stimulus causes increase in the production of red blood cells (Ponka, 1997). The most important factor involved in the control of erythropoiesis is erythropoietin, but other substances, particularly hormones, contribute to the regulation of this process [Fried, 1995; Ponka, 1997]. Transferrin comes out from the bone marrow sinusoids using ample fenestrature exits, and binds to surface receptors carried by erythroblasts. The iron transferred from transferrin and transported to the mitochondria is reduced from  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  and then inserted into protoporphyrin IX by

tetrapyrroles heme synthetase (Heme synthetase HS) for the synthesis of heme (Ponka, 1997; Testa et al., 1993; Testa, 2002). The reticuloendothelial system is a functional unit that includes cells having heterogeneous histologically different identities and a widespread distribution throughout the body, which share the common property of phagocytic activity, e.g. endothelial cells of blood capillaries of liver, spleen, bone marrow and lymph nodes, tissue and circulating macrophages (Andrews, 2000; Ponka, 1997). Reticuloendothelial system is the most important source of iron that enters the blood compartment. The flow of iron from reticuloendothelial plasma is unidirectional, as the reticuloendothelial cells are not able to pick up the metal from transferrin, but receive only hemoglobin or ferritin iron (Ponka, 1997; Andrews, 2000). Senescent erythrocytes at the end of their life (approximately 120 days) are phagocytized by endothelial cells and represent the largest source of iron entering the reticuloendothelial system. About 85% of the iron that enters the reticuloendothelial cells is promptly transferred to plasma transferrin and the remaining 15% is stored as intracellular ferritin, and transferred to plasma much more slowly [(Andrews, 2000; Grover, 1994; Ponka, 1997).

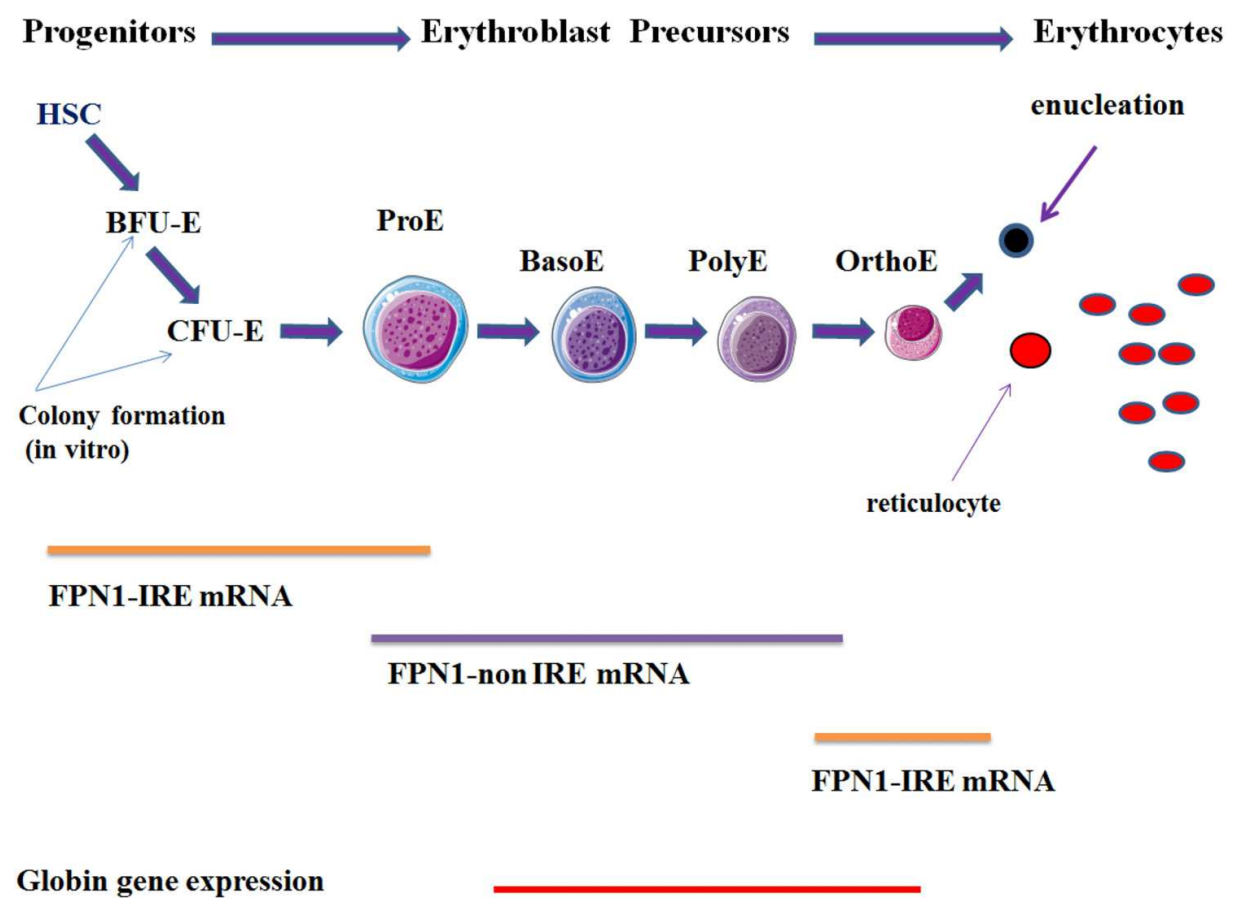


Fig. 1. **Pathway of the erythropoiesis from progenitors to mature cells.** Different stages are indicated: hematopoietic stem cell (HSC), burst-forming unit erythroid (BFU-E), colony-forming unit erythroid (CFU-E), proerythroblast (ProE), basophylic (BasoE), polychromatic (PolyE) and orthochromatic erythroblast (OrthoE). Coloured bars indicate timing of FPN1 alternative transcript expression (bottom) and hemoglobin synthesis referred to stages of erythropoiesis (bottom).



### 3. Iron acquisition by erythroid cells

Most of the iron in the plasma is bound to transferrin, an 80-kDa glycoprotein with homologous N-terminal and C-terminal iron-binding domains that is synthesized in the liver (De Domenico et al., 2008). Plasma transferrin has two important roles in iron physiology: first, the high iron-binding affinity of transferrin and the presence of a high concentration of apotransferrin (the iron-free form of transferrin) ensure that when iron enters plasma it is chelated, so limiting the ability of iron to generate toxic radicals; second, transferrin also directs iron towards cells that express transferrin receptors (De Domenico et al., 2008). Erythroid precursors require efficient iron uptake from Tf so that hemoglobin can be produced. TfR1 mediates erythroid iron acquisition, and its expression parallels the maturation of erythroid progenitors (Hentze et al., 2004). The number of TfRs on erythroid cell, markedly higher than on other cell types (Sposi et al., 2000) is directly related to hemoglobin (Hb) production (Horton, 1983; Iacopetta et al., 1982; Nunez et al., 1977). In normal erythropoiesis, the hyperexpression of TfR1, starting from early erythroid HPC differentiation, is Epo-dependent and mediated via transcriptional and post-transcriptional mechanisms (Sposi et al., 2000). Both the number of TfRs present on the membrane and cellular ferritin concentration are regulated by intracellular iron level. Coordinate regulation of TfRs and ferritin is one of the most extensively studied mechanisms of post-transcriptional control of gene expression. In response to iron deprivation, the cytoplasmic stability of *TfR* mRNA is increased and ferritin mRNA translation inhibited (Klausner et al., 1993). As a consequence, enhanced iron uptake and diminished iron storage compensate for the lack of iron. The feedback regulation can be considered as a protective mechanism that prevents nutritional starvation and permits the biosynthesis of essential iron or heme-containing proteins. Under conditions of high iron supply, when cells need to store excess iron in order to prevent adverse effects of iron overload, the regulatory balance is inverted: *TfR* mRNA decays more rapidly and ferritin translation is no longer inhibited (Klausner et al., 1993). After binding to its receptor, the complex of Fe(III)-transferrin-TfR1 is rapidly internalized by receptor-mediated endocytosis through clathrin-coated pits (De Domenico et al., 2008; Ponka et al., 1998). Acidification of the endosome produces a conformational change in both transferrin-Fe(III) and TfR1 with the consequent release of iron (Bali et al., 1991; De Domenico et al., 2008; Sipe & Murphy, 1991). The endosomal Fe(III) is converted into Fe(II) by a STEAP3, an erythroid-specific reductase (Ohgami et al., 2005). DMT1/Nramp2, a protons and Fe(II) co-transporter present in the endosomal membrane, transports iron into the cytosol (De Domenico et al., 2008; Gunshin et al., 2001). DMT1/Nramp2 is a member of the natural resistance-associated macrophage protein (Nramp) family (Cellier et al., 1995). Several isoforms of the DMT1/Nramp2 mRNA are known, resulting from alternative splicing and/or the use of two alternative upstream promoter regions (Hubert & Hentze, 2002; Millot et al., 2009; Tabuchi et al., 2002). The isoform I is localized mainly at the apical site of the enterocytes and other epithelial cells whereas isoform II is found on the endosomal membrane of peripheral tissues and erythroid cells (Canonne-Hergaux et al., 2001; Millot et al., 2009). At acidic pH, apotransferrin remains bound to TfR1 and the complex is recycled to the cell surface (De Domenico et al., 2008). At the more neutral pH of plasma, apotransferrin dissociates from TfR1 and is free to bind iron and initiate further rounds of receptor-mediated endocytosis (De Domenico et al., 2008).

#### 4. Iron utilization by erythroid cells

Erythroblasts also handle large amounts of iron. In these cells, most of the iron leaving the endosome is then transported to the mitochondria for heme synthesis and iron-sulfur cluster assembly (Napier et al., 2005). Potentially, iron may be directly transported from endosome into mitochondria by a “kiss-and-run mechanism” through a direct contact between both organelles, effectively bypassing the cytosol (Sheftel et al., 2007). Mitoferrin (Mfrn1, SLC25a37), a protein belonging to the family of mitochondrial solute carrier proteins expressed in the inner mitochondrial membrane, is thought to be implicated in shuttling iron across mitochondrial membrane (Millot et al., 2009; Shaw et al., 2006). The zebra fish mutant *frascati* with a mutated Mfrn1 gene shows profound hypochromic anaemia due to defective iron uptake by mitochondria (Shaw et al., 2006). Mfrn1 has a paralogue in mammals, Mrfn2 that is ubiquitously expressed. Silencing of both Mrfn1 and Mrfn2 induces reduction in heme synthesis by 90% (Paradkar et al., 2009). Most of heme in the body is synthesized in erythroid cells, as a precursor to hemoglobin formation, although heme is also the prosthetic group of various types of proteins, such as cytosolic or mitochondrial cytochromes, catalase, peroxidase and NO synthase (Millot et al., 2009). The erythroid-specific first enzyme of protoporphyrin IX synthesis, 5-aminolaevulinate synthase (ALAS), is encoded by two different genes: ALAS1 which is ubiquitously expressed and ALAS2, which is expressed only in erythroid cells (Furuyama et al., 2007). Regulation mechanisms differ widely between the two isoforms, i.e. ALAS1 expression is negatively regulated by heme, whereas ALAS2 expression is only dependent on iron (Furuyama et al., 2007). Ferrochelatase, the last enzyme of the pathway, synthesizes heme from Fe(II) and protoporphyrin IX (PIX). Heme is then transported out of the mitochondria to be associated to globin chains and apocytochromes (Furuyama et al., 2007). Three molecules have been identified as possible mitochondrial heme exporters or transporters: the breast cancer resistance protein (ABCG2) (Jonker et al., 2002), the ABC-mitochondrial erythroid (ABC-me) transporter (Shirihai et al., 2000) and the feline leukemic virus subgroup C receptor (FLVCR) (Quigley et al., 2004). Heme export from mitochondria is thought to be mediated by ATP-Binding Cassette (ABC) transporters, i.e. ABCG2 and ABC-me (Shirihai et al., 2000), although the exact nature of the transporter has not been elucidated. FLVCR could be required for differentiation of erythroid precursors into colony forming units, potentially protecting cells against heme toxicity by exporting excess heme that can otherwise result in oxidative stress (Dunn et al., 2008; Quigley et al., 2004). However, the heme transporters responsible for heme release remain unclear. Another important function of iron in the mitochondria is to ensure the [Fe-S] cluster synthesis. It has been proposed that frataxin acts as a metabolic switch between [Fe-S] cluster and heme synthesis (Becker et al., 2002; Dunn et al., 2006). Frataxin expression is much decreased in the disease Friedreich’s ataxia, in which iron loading occurs in the mitochondria (Dunn et al., 2006; Puccio et al., 2001). The molecular form of this excess iron remains unknown, but it could be unbound iron or iron stored in mitochondrial ferritin or other proteins (Dunn et al., 2006). Ferritin mitochondrial (FtMt) mRNA does not contain IRE, contrary to the H and L ferritin mRNAs, and therefore the FtMt synthesis is not regulated by the IRE/IRP system (Drysdale et al., 2002; Levi et al., 2001). The role of this FtMt is not fully elucidated but is thought to be a protective molecule against iron-mediated oxidative damage rather than an iron-storage molecule (Millot et al.,

2009). Although erythroid cells consume large amounts of iron, they have to maintain safety mechanisms to avoid iron and/or heme excess. It can be stored in ferritin or exported by ferroportin. Additionally, erythroblasts also have the capacity to export excess heme by FLVCR (Keel et al., 2008).

## 5. Iron- and haem-dependent regulation in erythroid cells

Iron metabolism and cellular heme represent two of the most key regulators of erythropoiesis (Andrews, 2008; Nemeth et al., 2008; Tsiftoglou et al., 2009). The principal source of iron for erythrocyte precursors is plasma iron-transferrin (Fe-Tf), whereas heme derives from plasma as well as “de novo” biosynthesis inside the mitochondria as protoporphyrin IX first and then as iron-protoporphyrin IX (heme) after incorporation of iron with ferrochelatase (Tsiftoglou et al., 2009). Trafficking and storage of iron in the mitochondria is tightly regulated as excess free iron promotes the generation of harmful reactive oxygen species whereas an inadequate supply of iron prevents haemoglobin synthesis leading to microcytic hypochromic anaemia (Martin et al., 2006; Millot et al., 2009). Cellular iron homeostasis is coordinately regulated posttranscriptionally by IRE/IRP system during erythroid differentiation. The analysis of IRP expression in hemopoietic cells provided some potentially interesting findings. It is well established that two different IRPs, IRP-1 and IRP-2, exist in mammalian cells (Henderson et al., 1993; Sposi et al., 2000). Both these proteins interact with the IRE sequence, six nucleotide loops, present in the certain mRNA 5' or 3' untranslated regions. The binding of IRP to ferritin mRNA results in translational block, while the binding to TfR mRNA results in mRNA stabilization (Sposi et al., 2000; Thomson et al., 1999). In spite of these similarities, however, IRP-1 and IRP-2 exhibit some important differences. In fact IRP-1 is related to mitochondrial aconitase, an enzyme of the Krebs cycle. Under high iron conditions IRP-1 dissociates from the IRE and is converted to a cytoplasmic aconitase through insertion of a [4Fe-4S] cluster (Haile et al., 1992). IRP-2 shares 60% amino-acid homology with IRP-1, but differs having a 73-amino-acid insertion in its N-terminal region, which confers a sensitivity to degradation via the ubiquitin-proteasome pathway in iron loading conditions (Guo et al., 1995; Henderson et al., 1993). IRP-2 is unable to assemble a [4Fe-4S] cluster and thus lacks aconitase activity. IRP-1 and IRP-2 are differentially expressed in hemopoietic cells and their expression is modulated during differentiation/proliferation of these cells. Thus, during differentiation of Hemopoietic Progenitor Cells (HPCs) from progenitor cells to mature cells it was observed that, during the initial stages of hemopoietic cell differentiation both IRP-1 and IRP-2 mRNAs are induced in all hemopoietic lineages; however, at later stages of differentiation, IRP-2 is expressed in all hemopoietic lineages at different stages of differentiation/maturation, IRP-1 is selectively expressed only in erythroid cells, while its expression is lost in all the other hemopoietic lineages (Sposi et al., 2000). It is not clear whether both IRP1 and IRP2 contribute to stabilization of TfR1 mRNA but several lines of evidence suggest that IRP2 is the main iron-sensor in erythroid cells: i.e., heme deficiency stabilizes IRP2 whereas accumulation of free heme induces its ubiquitination and degradation by the proteasome (Ishikawa et al., 2005; Millot et al., 2009); IRP2 knock-out mice develop microcytic hypochromic anaemia with reduced TfR1 expression in bone marrow cells (Cooperman et al. 2005; Galy et al., 2005; Millot et al., 2009); primary erythroblasts deficient in Stat5 showed a reduction



in IRP2 expression, with a concomitant reduction in TfR1 mRNA and increasing of IRP1 (Kerenyi et al., 2008). The observation that in differentiating erythroblasts, TfR1 mRNA stability and IRP mRNA-binding affinity are no longer modulated by iron supply, has recently challenged the implication of the IRE/IRP system in iron homeostasis regulation in erythroid cells (Schranzhofer et al., 2006). This would be in agreement with the so called “kiss-and-run” hypothesis (Ponka et al., 1997; Richardson et al., 1996). It suggests that during terminal erythropoiesis endosomes come into close vicinity/physical contact with mitochondria to directly shuttle iron into this organelle for heme synthesis without modulating the mRNA-binding activity of the IRPs (Ponka et al., 2002; Zhang et al., 2005). This dual mechanism contributes to maintaining the high flux of incoming iron available for heme synthesis rather than being sequestered into ferritin (Millot et al., 2009). The level of cellular heme is very critical in the regulation of erythropoiesis. Heme biosynthesis is increased dramatically during Epo-moderated erythropoiesis to meet extra demand for red blood cell production under hypoxic conditions or stress erythropoiesis (Tsiftoglou et al., 2009). Heme is needed for the production of large number of hemoproteins involved in cell respiration, O<sub>2</sub> tension sensing and metabolism (Tsiftoglou et al., 2009). Heme is also needed to regulate the transcription of globin and nonglobin genes, because it has been found to regulate the action of transcription factors at nuclear level (Tsiftoglou et al., 2009). Heme itself functions as a transcriptional regulator. It can induce heme oxygenase 1, HO-1, a molecule which reciprocally induces heme degradation (Huihui and Ginzburg, 2010). Heme strongly stimulates HO-1 expression by inhibiting the transcriptional repressor Bach 1. Binding of a heterodimer of the small maf transcription factor and Bach 1 to the multiple MARE (maf recognition element) sites in HO-1 enhancer represses HO-1 gene expression (Millot et al., 2009). It has been shown that HO-1 mRNA decreases following erythroid differentiation of Friend erythroleukemia cells, while mRNAs coding for the enzymes of the heme biosynthetic pathway increase (Millot et al., 2009; Fujita and Sassa, 1989). Heme non participating in hemoglobin synthesis results in a downregulation of IRP2 which reduces TfR1 expression on the cell surface and thus the amount of iron entering cells so preventing excess heme from accumulating in erythroid precursors (Huihui and Ginzburg, 2010; Ishikawa et al., 2005). In order to prevent excess globin synthesis, heme deficiency represses globin synthesis by activating a stress protein kinase named heme regulated inhibitor (HRI) which phosphorylates eIF2 $\alpha$  (McEwen et al., 2005; Millot et al., 2009). Finally heme export has also recently been demonstrated in erythroid precursors. It has been shown that the feline leukemia virus, subgroup C, receptor (FLVCR) could function as a heme exporter (Quigly et al., 2004; Taylor et al., 1999). FLVCR is a member of the family of MFS (major facilitator superfamily) proteins which transport small solutes across membranes by using the energy of ion-proton gradient (Millot et al., 2009; Taylor et al., 1999). The absence of FLVCR results in arrest of proerythroblast differentiation and apoptosis, likely due to heme toxicity, whereas FLCVR overexpression in mice results in a mild microcytic hypochromic anemia suggesting that it is needed to maintain heme and globin balance and avoid accumulation of free heme or excess globin in the cytoplasm (Huihui and Ginzburg, 2010; Keel et al., 2008). In conclusion during erythroid differentiation the existence of an interplay of positive and negative feedback mechanisms maintains sufficient iron supply for heme synthesis and prevents formation or accumulation of heme in excess of globin chains.

## 6. Iron deficiency and anemia

Under physiological conditions, there is a balance between iron absorption, iron transport and iron storage in the human body. Iron deficiency anemia may result from the interplay of three distinct risk factors: increased iron requirements, limited external supply and increased blood loss (Munoz et al., 2010). Likewise, inappropriately high levels of hepcidin expression lower plasma iron levels and cause anemia. In this context, the common acquired anemia of chronic diseases (ACD) and the genetic iron-refractory iron deficiency anemia (IRIDA) are the most interesting examples. Several physiopathological features contribute to the anemia of chronic diseases, also known as the anemia of inflammation: impaired proliferation of erythroid progenitors and blunted response to erythropoietin, reduced erythropoietin synthesis as well as reduced life span of red blood cells (Millot et al., 2009). The basis of the disorder is that inflammatory stimuli, such as those caused by bacterial infections, cause acute hypoferremia presumably in an attempt to limit the growth of bacteria by limiting iron (De Domenico et al., 2008; Schaible and Kaufmann, 2004). During inflammation IL-6 seems to be the major pro-inflammatory cytokine implicated in hepcidin activation through a Stat3 dependent signaling pathway, so allowing the identification of a link between iron homeostasis and inflammation (Millot et al., 2009; Wrighting and Andrews, 2006). Hypoferremia develops rapidly as a result of decreased macrophage iron release leading to iron-limited erythropoiesis. Increased cellular iron retention is the result of decreased levels of cell-surface ferroportin, which, in turn, results from sustained secretion of hepcidin (De Domenico et al., 2008). Low or undetectable levels of hepcidin are normally observed in patients with iron deficiency. On the contrary, patients with IRIDA show very low iron stores and microcytic anemia refractory to iron treatment in consequence of inappropriately high hepcidin levels. IRIDA is caused by mutations in *TMPRSS6* (matriptase-2), a gene that encodes a protease that negatively regulates hepcidin expression (Du et al., 2008). Recently it has been observed that genetic variants in *TMPRSS6*, frequent in the general population, may modulate the ability to absorb iron and to synthesize hemoglobin for maturing erythroid cells (Andrews, 2009). Recent study suggest that *TMPRSS6* normally acts to down-regulate hepcidin expression by cleaving membrane-bound hemojuvelin, HJV, (Silvestri et al., 2008).

## 7. Iron overload and hereditary hemochromatosis

Hereditary hemochromatosis is an iron overload disease characterized by excessive body iron that causes tissue damage in the liver, pancreas and heart (Pietrangelo A, 2004). Currently four types have been identified in Caucasian populations: type 1 is the common form and is an autosomal recessive disorder of low penetrance strongly associated with mutations in the *HFE* gene ; type 2 (juvenile hemochromatosis) is autosomal recessive, of high penetrance with causative mutations identified in the *HFE2* and *HAMP* genes; type 3 is also autosomal recessive with mutations in the *TfR2* gene; type 4, or *HFE4* (OMIM 606069), or ferroportin disease, is an autosomal dominant condition with heterozygous mutations in the ferroportin 1 (*FPN1*) gene (Worwood, 2005). *FPN1* (also known as *Ireg1* and *MTP1*), the product of the *Slc40a1* gene, was independently identified by three groups, using different approaches (Abboud & Haile, 2000; Donovan et al, 2000; McKie et al., 2000) and has been reported to be expressed and to play a critical role in several different tissue involved in mammalian iron homeostasis, including duodenal enterocytes (iron uptake and export into

the circulation; hepatocytes (storage); syncytiotrophoblasts (transfer to embryo) and reticuloendothelial macrophages (iron recycling from senescent red blood cells). FPN1 appears to act as an iron exporter (Donovan et al., 2000; McKie et al., 2000) and to be specifically regulated according to body iron requirements (Donovan et al., 2000; Martini et al., 2002; McKie & Barlow, 2004; Mok et al., 2004; Pietrangelo et al., 2004; Yang et al., 2002; Zoller et al., 2001) in these tissues. The FPN1 gene has been highly conserved during evolution and encodes for a protein composed of 571 aminoacids with a predict mass of 62 kDa (for review see Cianetti et al., 2010). The presence of a well-conserved IRE in the 5'-UTR of FPN1 mRNA indicated the possibility of post-transcriptional control through the IRP-IRE systems (Donovan et al., 2000; Liu et al, 2002; Lymboussaki et al., 2003; McKie & Barlow, 2004) but several recent observation have indicated a more complex regulation of FPN1 expression by iron (Cianetti et al., 2010). Hemochromatosis associated with mutations in FPN1 can result in two different types of iron loading: one type is phenotypically indistinguishable from classical HFE hemochromatosis (or hemochromatosis type 1) (Spelling), in that the patients have both an elevated transferrin saturation and serum ferritin, while the other type termed "ferroportin disease" is associated with microcytic anemia, a raised serum ferritin and iron deposition in macrophages rather than hepatocytes (Pietrangelo, 2004). FPN1 mutations have two effects, either causing misfolding of the protein and failure to reach the cell surface ("loss of function")(Schimanski, 2005), or the mutant protein is expressed at the cell surface but is not inhibited by hepcidin ("loss of regulation")(Drakesmith, 2005). Briefly it was shown that A77D, V162del, and G490D mutations, that are associated with typical pattern of disease in vivo, cause a loss of iron export function in vitro, but do not physically or functionally impede wild-type FPN1 (Drakesmith, 2005; Schimanski, 2005). These mutations may, therefore, lead to disease by haploinsufficiency. By contrast the Y64N, N144D, Q248H and C326Y mutations, which can be associated with greater transferrin saturation and more prominent iron deposition in liver parenchyma in vivo, retained iron export function in vitro (Drakesmith, 2005; Schimanski, 2005). Because the peptide hormone hepcidin inhibits ferroportin as part of a homeostatic negative feedback loop, it was postulated that this group of mutations may resist inhibition to hepcidin resulting in a permanently "turned on" iron exporter (Drakesmith, 2005; Schimanski, 2005). All these results with A77D, V162del and G490D mutations of FPN1 are consistent with the scheme proposed by Montosi et al (Montosi et al., 2001)) to explain the macrophage iron loading observed in patients with these mutations (Schimanski et al., 2005): lower serum iron resulting from iron sequestration in macrophages reduces availability to the bone marrow for erythropoiesis thus leading to anemia that was effectively observed in some patients with mild anemia in the early stages of disease and that respond poorly to phlebotomy (Pietrangelo, 2004; Schimanski et al, 2005). So iron overload may be a consequence of the erythron signalling to the gut enterocyte to increase iron uptake from the diet to compensate for the anemia. According to recent progress in this field it is likely that the erythron signalling is directly working through hepcidin-ferroportin interaction. By contrast the Y64N, N144D, Q248H and C326Y mutations, which can be associated with greater transferrin saturation and more prominent iron deposition in liver parenchyma in vivo, retain iron export function in vitro (Schimanski et al., 2005; Drakesmith et al., 2005). It was postulated that this group of mutations may resist inhibition by hepcidin, so interfering with its homeostatic negative feedback loop and resulting in a permanently "turned on" iron exporter (Schimanski et al., 2005; Drakesmith et al., 2005).

## 8. Ineffective erythropoiesis and thalassemia

In recent years there has been important advancement in our understanding of iron metabolism, mainly as a result of the discovery of hepcidin, a key regulator of whole-body iron homeostasis (for an exhaustive review see Ganz & Nemeth, 2006; Piperno et al., 2009; Lee & Beutler, 2009). Increasing experimental evidence suggested that a single molecule could be the “stores”, the “erythropoietic” and the “inflammation” regulator of iron absorption and recycling [Cianetti et al., 2010; Fleming & Sly, 2001; Nicolas et al., 2002], and that hepcidin acted principally or solely by binding to ferroportin, the only known cellular iron exporter, causing ferroportin to be phosphorylated, internalized, ubiquitinated, sorted (Nemeth et al., 2004) through the multivesicular body pathway and degraded in lysosomes (Ganz, 2005; Nemeth et al., 2004). Different stimuli can modulate hepcidin and act as positive or negative regulators. Four major regulatory pathways (erythroid, iron store, inflammatory and hypoxia-mediated regulation) that act through different signaling pathways to control the production of hepcidin are known (Cianetti et al., 2010). It is obvious that this complex network of interactions must be subjected to very close control in order to ensure that the iron erythropoietic demand is met and, in turn, adequate concentrations of iron in the circulation are always present (Cianetti et al., 2010; Piperno et al., 2009). Under normal conditions iron store and inflammatory regulation activate hepcidin transcription in the hepatocytes through the bone morphogenetic proteins (BMPs)/SMAD4 and signal transducer and activator of transcription-3 (STAT-3) pathways, respectively (Andrews, 2008; Piperno et al., 2009). The hemochromatosis protein HFE, transferrin receptor 2 (TfR2) and the membrane isoform of hemojuvelin (mHJV) are all positive modulators of hepcidin transcription and when defective, lead to hemochromatosis (HH) in humans (De Domenico et al., 2008; Piperno et al., 2009). Oppositely, hypoxia, anemia, increased erythropoiesis and reduced iron stores all negatively regulate hepcidin expression (Piperno et al., 2009). Emerging evidence suggests that erythropoiesis modulates hepcidin expression, with increased erythropoietic activity suppressing the action of hepcidin (Dallaglio et al., 2006; Dunn et al., 2007; Kattamis et al., 2006; Pak et al., 2006; Vokurka et al., 2006). This in turn facilitates export of iron from the reticuloendothelial system and enterocytes, increasing the availability of iron for erythropoiesis (Dunn et al., 2007; Pak et al., 2006). Anemia and hypoxia also suppress hepcidin expression, although recent experiments indicate that functional erythropoiesis is required (Dunn et al., 2007; Pak et al., 2006; Vokurka et al., 2006) for these conditions to regulate hepcidin expression. Finally it is evident that erythropoiesis and iron metabolism are extremely intertwined in that alteration of one of the two may have a major impact on the second (Gardenghi et al., 2007; El Rassi et al., 2008; Rivella, 2009; Rund & Rachmilewitz, 2005; Weatherall & Clegg, 2001; Weatherall, 2001). That's the reason why thalassemia intermedia and thalassemia major are the best studied human models of hepcidin modulation by ineffective erythropoiesis. Beta-thalassemias are caused by mutations in the beta-globin gene resulting in reduced or absent beta-chain synthesis (for exhaustive reviews see Weatherall, 1998; Olivieri, 1999; Cao & Galanello, 2010; Ginzburg & Rivella, 2011). A relative excess of  $\alpha$ -globin chain synthesis leads to increased erythroid precursor apoptosis, causing ineffective erythropoiesis which together with extramedullary expansion, splenomegalia and shortened red blood cells survival result in anemia (Huihui & Ginzburg, 2010). Patients either homozygous or compound heterozygous for mutation in the  $\beta$ -globin gene present with a broad range of clinical severity due to genotypically different mutations, combination inheritance with



hemoglobinopathies, and additional modifying factors (Ginzburg & Rivella, 2011; Huihui & Ginzburg, 2010). Individuals with thalassemia major require regular red blood cell (RBC) transfusions to ameliorate anemia and suppress extramedullary erythropoiesis. Patients with beta-thalassemia intermedia show a milder clinical picture with more beta-globin chains synthesis and require only intermittent transfusions (Huihui & Ginzburg, 2010). Patients with beta-thalassemia have increased intestinal iron absorption which, in addition to transfusion dependence, contributes to iron overload (Huihui & Ginzburg, 2010). Progressive iron overload is the most salient and ultimately fatal complication of beta-thalassemia. Iron deposition occurs in visceral organs (mainly in the heart, liver and endocrine glands), causing tissue damage and ultimately organ dysfunction and failure (Fig.2). Both transfusional iron overload and excess gastrointestinal absorption are contributory. Paradoxically, excess gastrointestinal iron absorption persists despite massive increases in total body iron load (Fleming & Sly, 2001; Gardenghi et al., 2007; Rivella, 2009)(Fig.3). However, little is known about the relationship among ineffective erythropoiesis, the role of iron-regulatory genes, and tissue iron distribution in beta-thalassemia. If iron were a dominant regulator, patients with beta-thalassemia should express very high levels of hepcidin in serum; in contrast, the levels are very low, suggesting that the ineffective erythropoiesis alone is able to suppress the synthesis of hepcidin in spite of the presence of a severe iron overload (Cianetti et al, 2010; Piperno et al., 2009). Furthermore, serum from patients with thalassemia inhibited hepcidin mRNA expression in the HepG2 cell line, which suggested the presence of a humoral factor that down-regulates hepcidin (Weizer-Stern et al., 2006). The nature of the erythropoietic regulator of hepcidin is still uncharacterized, but may include one or more proteins during active erythropoiesis. Recent observations in thalassemia patients has suggested that one of these regulators could be the cytokine growth differentiation factor-15 (GDF15) (Piperno et al., 2009; Tanno et al., 2007). GDF15 is a divergent member of the transforming growth factor-beta superfamily that is secreted by erythroid precursors and other tissues. It has been identified as an oxygen-regulated transcript responding to hypoxia and as a molecule involved in hepcidin regulation (Cianetti et al., 2010; Bottner et al., 1999; De Caestecker, 2004; Tanno et al., 2007). Serum from thalassemia patients suppressed hepcidin mRNA expression in primary human hepatocytes and depletion of GDF15 reversed the hepcidin suppression (Piperno et al., 2009; Tanno et al., 2007). It was suggested that GDF15 overexpression arising from an expanded erythroid compartment contributed to iron overload in thalassemia syndromes by inhibiting hepcidin expression, possibly by antagonizing the BMP pathway. Without going into a detailed analysis of the GDF15 regulation mechanisms, we would like to recall the results obtained recently, that are in our view important to start reflecting on the existence of alternative ways that regulate hepcidin production (Cianetti et al., 2010). Recently a very interesting study demonstrated that expression of both GDF15 mRNA and protein was strongly and specifically responsive to intracellular iron depletion in a number of human cell lines and in vivo in humans (Lakhal et al., 2009; Cianetti et al., 2010). This up-regulation is independent of IRP1, IRP2 and the HIF pathway suggesting the involvement of a novel iron-regulatory pathway (Lakhal et al., 2009). This study showed that GDF15 was induced by over-expression of wild type ferroportin (Lakhal et al., 2009). This observation is very intriguing because it connects the iron-mediated regulation of GDF15 concentration to patho-physiological levels of iron: despite systemic iron overload, ineffective erythropoiesis and associated iron-fluxes in beta-thalassemia might generate an iron deficiency signal in a relevant molecular or cellular context and consequent stimulation of GDF15 expression in a



particular erythroid compartment (Cianetti et al., 2010; Lakhal et al., 2009). Recent literatures provided at least two more molecules potentially involved in the regulation of hepcidin by erythropoiesis, i.e. the human twisted gastrulation factor (TWSG1) (Tanno et al., 2009) and the Oncostatin M (OsM) (Chung et al., 2010; Kanda et al., 2009). In contrast to GDF15, the highest-level expression of TWSG1 was detected at early stages of erythroblast differentiation before hemoglobinization of the cells (Tanno et al.,2009). In human cells, TWSG1 suppressed hepcidin through a BMP-dependent mechanism (Tanno et al., 2009). In vivo studies on thalassemic mice showed that TWSG1 expression was significantly increased in the spleen, bone marrow and liver. So it was proposed that TWSG1 might act with GDF15 to dysregulate iron homeostasis in beta-thalassemia (Tanno et al., 2009). In contrast to GDF15 and TWSG1, recent observations have showed that OsM could induce hepcidin expression in human hepatoma cell lines mainly through the JAK/STAT pathways (Kanda et al., 2009). Finally, results obtained by HuH7 hepatoma cells cocultured with primary human erythroblasts or erythroleukemic UT7 cells presented a 20- to 35-fold increase of hepcidin expression and identified OsM as responsible for increased levels of hepcidin (Chung et al., 2010). Furthermore, this study described the biological involvement of OsM in iron metabolism “in vivo” through direct transcriptional regulation of hepcidin gene expression and suggested a new OsM-hepcidin axis that might be critical in the development of hypoferremia in inflammation (Chung et al., 2010).

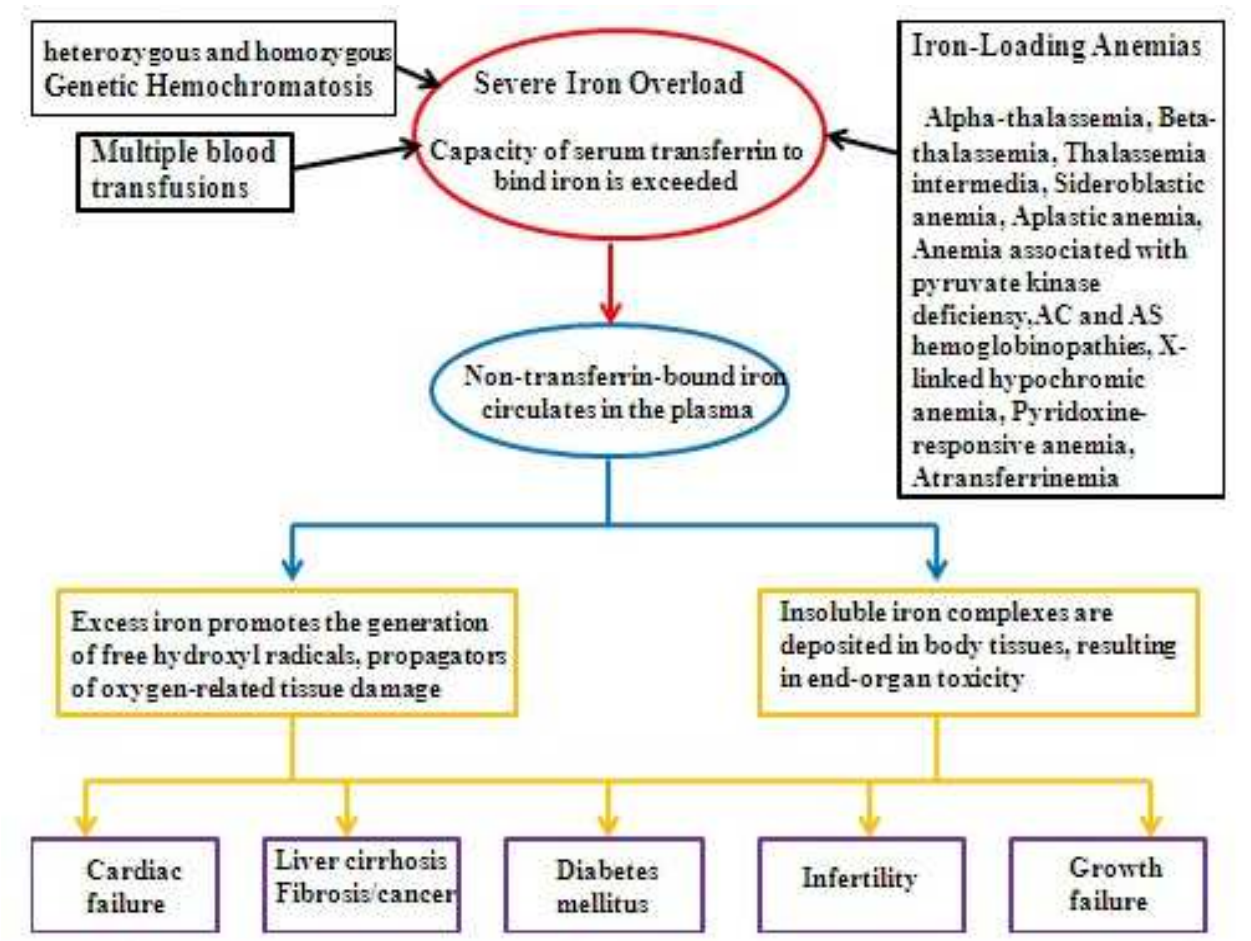


Fig. 2. **A summary of the causes of iron overload.** A schematic representation of the main causes of severe iron overload and its most important clinical manifestations.

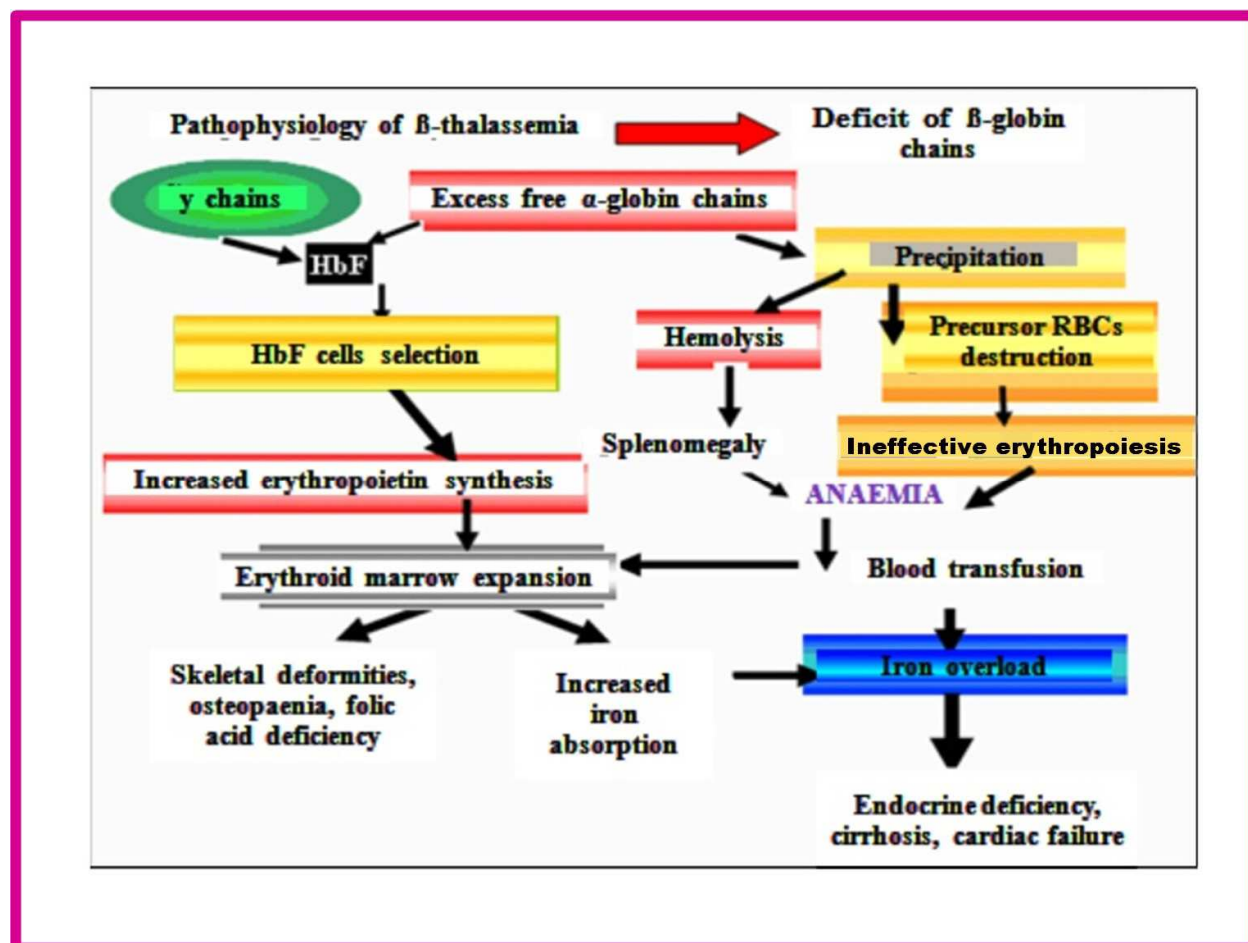


Fig. 3. Pathophysiology of beta-thalassemia and corresponding clinical manifestations.

A summary of the effects of excess production of free alpha-globin chains. Excess unbound alpha-globin chains and their degradation products precipitate in red-cell precursors, causing defective maturation and ineffective erythropoiesis.

## 9. Ferroportin and erythroid cells

We reported for the first time the expression of FPN1 mRNA and protein in normal human erythroid cells at all stages of differentiation (Cianetti et al., 2005). The presence of an iron exporter was very surprising because the erythroid cells need to incorporate very high amounts of iron to support the continued synthesis of heme and hemoglobin (Cianetti et al., 2010). The IRE element in the 5'-UTR of FPN1 mRNA was demonstrated to be functional in erythroid cells and able to mediate translational modulation by cellular iron levels (Cianetti et al., 2005). Nonetheless, FPN1 protein expression appeared to maintain a constant level during different steps of erythroid differentiation and after iron treatments (Cianetti et al., 2005). A solution to this problem could be to use an upstream alternative promoters to produce mRNA species in which the 5'-UTR IRE could be spliced out or made non-functional (Cianetti et al., 2010). We described for the first time the existence of two alternative FPN1 transcripts (*variant II* and *III*), other than the IRE-containing canonical one (*variant I*), that did not contain the IRE element in their 5'-UT region, did not respond to iron treatments and together accounted for more than half of total FPN1 mRNA present in erythroid cells (Cianetti et al., 2005; Cianetti et al., 2010). These transcripts arise from the

usage of alternative upstream promoters and differential splicing of 5'-UTR sequences. Interestingly, these transcripts were expressed mainly during the middle steps (4-11 days) of in vitro erythroid differentiation, corresponding to the maturation from late erythroid progenitors to polychromatophilic erythroblasts (Cianetti et al., 2005) (Fig.1). At these stages of erythroid differentiation TfR1, the receptor responsible for iron import in erythroid cells, is strongly and increasingly expressed (Sposi et al., 2000). Therefore, the non-IRE (*variant II* and *III*) FPN1 transcripts were expressed when erythroid progenitor/precursor cells need to accumulate iron into the cells (Cianetti et al., 2005). It was speculated that expression of the non-IRE FPN1 transcripts could produce a constant level of the transporter, unresponsive to the very high iron levels present in maturing erythroid cell. In contrast, IRE-containing FPN1 transcripts were mainly expressed in undifferentiated erythroid progenitors and in mature terminal erythroblasts, suggesting a possible role at these particular stages of erythroid differentiation (Cianetti et al., 2005; Cianetti et al., 2010). The existence of multiple FPN1 alternative transcripts indicated a complex regulation of the FPN1 gene in erythroid cells and the possibility that the control of FPN1 expression by iron conditions in different cell types might be complex. So in erythroid cells the regulation of FPN1 mRNA translation through the 5'-UTR IRE mechanism might be silenced because in this cell type a high level of iron uptake is needed to accumulate high amounts of iron required for optimal heme synthesis (Cianetti et al., 2005). A solution for this problem might be the utilization of an upstream alternative promoter to produce mRNA species in which the 5'-UTR IRE might be spliced out or made non functional (Cianetti et al., 2005; Cianetti et al., 2010). The alternative FPN1 transcripts are differentially expressed during erythroid differentiation, in particular indicating a sequential and specific activation pathway, with an apparently mutual exclusion between *variant I* IRE and *variant II/III* not containing the IRE transcripts (Cianetti et al., 2005) (Fig.1). These observations suggest that erythroid precursor cells need FPN1 transcript without a IRE to evade translational control by IRP-IRE system in order to export iron during the critical period when cells are committed to proliferate and differentiate (Cianetti et al., 2010). Once the precursor erythroid cells begin to produce hemoglobin, FPN1 without a IRE diminishes and FPN1 with a IRE predominates allowing erythroid cells to limit iron export through the IRP-IRE system and synthesize heme without developing microcytic anemia (Cianetti et al., 2010) (Fig.1).

## 10. New potential therapeutic opportunities

It is increasingly evident that the iron metabolism, heme and cellular erythropoiesis are inextricably linked, because iron metabolism (Andrews, 2005; Nemeth, 2008) and cellular heme (for exhaustive review see Tsiftoglou et al., 2006) are two of the most relevant key regulators of erythropoiesis (Cianetti et al., 2010). The complex regulation of erythropoiesis suggests the existence of several molecular targets that could be exploited therapeutically for treatment of RBC disorders like thalassemias and anemias (Tsiftoglou et al., 2009). We must differentiate between primary iron overload, and iron overload that accompanies ineffective erythropoiesis: in the latter case the administration of hepcidin might be considered as a new potential therapeutic approach to reduce iron overload in thalassemias and other forms of anemia associated with ineffective erythropoiesis (Cianetti et al., 2010; Tsiftoglou et al., 2009). The reduced number or the absence of mature erythroid cells in beta-thalassemia patients is still very difficult to understand, and it has become one of the paradoxes among the most difficult to resolve : when the body has greater need for red blood cells instead it responds by decreasing their production (Rivella, 2009). The most



probable hypothesis to explain this phenomenon might rely on the existence of intrinsic and extrinsic mechanisms that would affect the process of differentiation: for example in cells where the synthesis of beta-globin gene is defective to the point that they ensure a stoichiometric between alpha and beta globin chains, a security mechanism can block the intrinsic maturation or, alternatively, an amount of heme in excess can be an extrinsic signal to prevent the differentiation that would lead to clusters of alpha globin chains production of reactive oxygen species (ROS) too toxic to survive (Cianetti et al, 2010; Rivella, 2009). There is much experimental evidence that oxidative stress may limit the process of differentiation. All this of course worsens the anemic outline (Rivella, 2009). So the contribution of these mechanisms to ineffective erythropoiesis might be different in each patient according to level of beta-globin synthesis and other extrinsic factors such as iron overload (Rivella, 2009). At this point the question arises: is there a meeting point between different signaling pathways, although activated by different signals? Recent discoveries indicate that there is a potential for therapeutic intervention in beta-thalassemia by means of manipulating iron metabolism (Mabaera et al., 2008; Rivella, 2009; Rund & Rachmilewitz, 2005). A recent study suggested a link between EpoR/Jak/Stat signaling and iron metabolism, showing that in mice that completely lack Stat5 activity the cell surface levels of TfR1 on erythroid cells were decreased more than 2-fold (Kerenyi et al., 2008). Another study suggested a direct involvement of Epo in hepcidin regulation through the transcriptional factor C/EBP alpha (Pinto et al., 2008). In addition a link has been shown between Jak 2 and FPN1: Jak2 phosphorylates FPN1 following binding of this protein to hepcidin (De Domenico et al., 2009). Phosphorylation of FPN1 then triggers its internalization and degradation (De domenico et al., 2009). Therefore Jak2 might represent one of the major links at the interface between erythropoiesis and iron metabolism suggesting that use of Jak2 inhibitors, antioxidant, and analog of the hepcidin might be used to reduce ineffective erythropoiesis and abnormal iron absorption (Cianetti et al., 2010; Rivella, 2009). Administration of synthetic hepcidin or of agents that increase its expression, may be beneficial in controlling absorption of this metal (Piperno et al., 2009). Hepcidin agonists or stimulators of hepcidin production are being developed for the treatment or prevention of iron overload in hepcidin deficiency states, including hereditary hemochromatosis and beta-thalassemia (Ganz, 2011). In the mouse model of beta-thalassemia, transgenic hepcidin therapy improved iron overload as well as erythropoiesis suggesting that hepcidin deficiency or iron overload may adversely impact erythropoiesis in this disease (Ganz, 2011). Hepcidin antagonists and inhibitors of hepcidin production may find utility in the treatment of iron-restricted anemias, alone or in combination with erythropoiesis-stimulating agents (Ganz, 2011). Also GDF15 could be another potential therapeutic target for beta-thalassemia syndromes (Tanno et al., 2007). A major goal of hemoglobinopathy research is to develop treatments that correct the underlying molecular defects responsible for sickle cell disease and beta-thalassemia (Mabaera et al., 2008). One approach to achieving this goal is the pharmacologic induction of fetal hemoglobin (HbF). Although many of the events controlling the activity of the beta-globin locus are known, the details of those regulating normal human hemoglobin switching and reactivation of HbF in adult hematopoietic cells remain to be elucidated (Cianetti et al., 2010). If the molecular events in hemoglobin switching or gamma-globin gene reactivation were better understood and HbF could be fully reactivated in adult cells, the insights obtained might lead to a cure for these disorders (Cianetti et al., 2010). Agents that increase human HbF in patients may work at one or more levels: for example, hydroxyurea and 5-azacytidine kill dividing cells

preferentially and may increase gamma-globin expression indirectly through this effect [for complete reviews see Mabaera et al., 2008; Bank, 2006). Butyrate may work both by histone deacetylase (HDAC) inhibition and by increasing gamma-globin translation on ribosomes (Bank, 2006; Mabaera et al., 2008). The Stem Cell Factor (SCF) induced an “in vitro” expansion of effective erythropoiesis and a reactivation of gamma-globin synthesis up to fetal levels, paving the way to its potential use in the therapeutic treatment of this disease (Gabbianelli et al., 2008). Recently it was reported the ability of thalidomide to increase gamma-globin gene expression and the proportion of HbF-containing cells in a human in vitro erythroid differentiation system (Aerbajinai et al., 2007) showing that thalidomide induced production of ROS that in turn caused p38 MAPK phosphorylation and globally increased histone H4 acetylation (Aerbajinai et al., 2007; Mabaera et al., 2008). All these experiments present a body of evidence that suggests an important role for intracellular signaling in HbF induction (Cianetti et al., 2010). However, the mechanisms of action of these agents are not yet defined and their role in beta-thalassemia therapy is still being explored in light of its acceptable toxicity profiles adding to their promise as therapeutic agents (Ginzburg & Rivella, 2011). Key genes controlling fetal/adult globin switching have been identified (e.g. BCL11 and cMYB) and may ultimately serve as direct targets for small molecules that would increase HbF levels in this patients (Bauer & Orkin, 2011; Ginzburg & Rivella, 2011; Wilber et al., 2011). Finally recent publications have demonstrated the importance of what has been termed the “integrated stress response” pathway in erythroid cells that is also activated from a variety of stress stimuli, including viral infection, NO, heat shock, ROS, endoplasmic reticulum stress, ultraviolet irradiation, proteasome inhibition, inadequate nutrients and, in erythroid cells, limiting amounts of heme (Chen, 2007; Cianetti et al., 2010; Mabaera et al., 2008; Wek et al., 2006).

## 11. Conclusion

Our understanding of the pathogenesis of iron-restricted anemias and iron-overload has been revolutionized by discovery of hepcidin and its role in iron homeostasis stimulating the development of new diagnostic and therapeutic modalities for these disorders. Further work is required to understand the mechanisms of hepcidin regulation by iron and erythroid activity and to understand the structure, the transport function and the complex regulation of the hepcidin receptor ferroportin. In conclusion we are increasingly convinced of the importance to study the molecular mechanisms of iron homeostasis dysregulation in thalassemia and in particular the GDF15-BMP-Hepcidin-Ferroportin regulatory way in order to understanding its contribute to iron overload.

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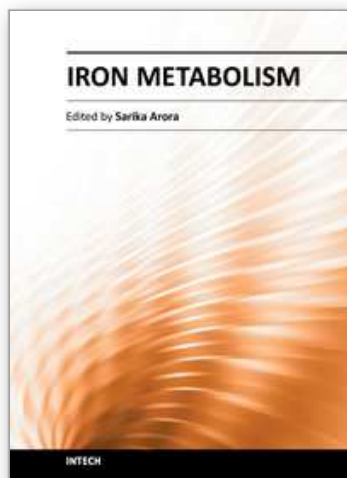
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## **Iron Metabolism**

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Iron has various functions in the body, including the metabolism of oxygen in a variety of biochemical processes. Iron, as either heme or in its "nonheme" form, plays an important role in key reactions of DNA synthesis and energy production. However, low solubility of iron in body fluids and the ability to form toxic hydroxyl radicals in presence of oxygen make iron uptake, use and storage a serious challenge. The discovery of new metal transporters, receptors and peptides and as well as the discovery of new cross-interactions between known proteins are now leading to a breakthrough in the understanding of systemic iron metabolism. The objective of this book is to review and summarize recent developments in our understanding of iron transport and storage in living systems.

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