

* CHAPTER 19

Iron and erythropoiesis

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1. Introduction

Erythropoiesis is a very active process which leads to the daily production of 200 billion new mature erythrocytes to compensate for the destruction of senescent red cells by tissue macrophages. The control of erythropoiesis depends mainly on erythropoietin and on the availability of plasma iron. During erythroid differentiation, the expression pattern of erythroid-specific genes are governed mostly at the transcriptional levels and these different steps are reviewed in Chapter 2. However, the formation of haemoglobin requires that haem, α -globin and β -globin are synthesised at the appropriate 4:2:2 ratio, without excess production of any of these components. Several post-transcriptional mechanisms are also required for regulation of these processes as well as the systemic regulation of iron availability (reviewed in Chapter 20).

2. Iron acquisition by erythroid cells

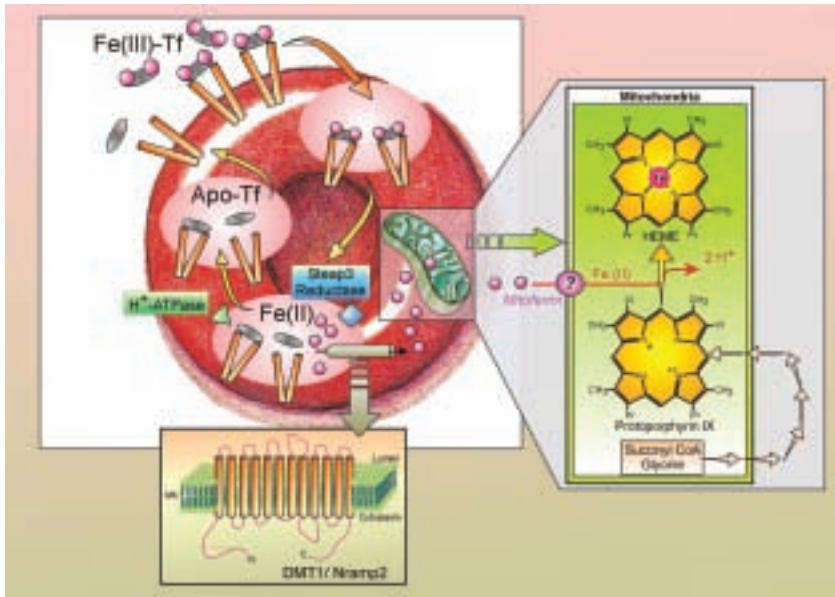
2.1 Plasma iron

Iron in plasma is bound to transferrin, a soluble protein synthesised and secreted by hepatocytes. Transferrin (Tf) has two high affinity iron binding sites, which will each bind one Fe(III) iron atom in the presence of a bicarbonate ion. Plasma transferrin can exist in four different molecular forms, either as apo-transferrin, mono-ferric transferrin with the iron atom bound to N-terminal lobe or to the C-terminal lobe, and as di-ferric transferrin. In normal conditions, only 30% of the iron binding sites are occupied by an iron atom. An increase in tissue iron stores is usually accompanied by an increase in transferrin saturation. Non transferrin-bound iron can also be present in plasma, when transferrin saturation is above 85-90%.

Erythroid cells rely entirely on transferrin-bound iron for iron acquisition (Figure 1). Low or undetectable levels of Tf result in defective haemoglobin synthesis and microcytic hypochromic anaemia, as seen in patients with atransferrinemia.

Iron bound to transferrin originates mostly from iron recycled by tissue macrophages following degradation of senescent erythrocytes. The daily amount of iron required by the developing erythroblasts in the bone marrow averages 25 mg and represents the amount of iron liberated by erythrophagocytosis. The systemic regulation of plasma iron levels is mediated by plasma hepcidin (see Chapter 20). Interestingly, the rapid reduction in plasma iron levels which follows a rise in hepcidin, induced by either direct hepcidin injection into mouse (1), transgenic hepcidin gene induction (2) or hepcidin stimulation by IL-6 infusion (3) illustrates the rapid turn over of plasma iron and the great iron demand of the erythron. Ferrokinetic studies performed in normal healthy individuals using ^{59}Fe -labelled transferrin injected into plasma have shown that within minutes, 80-90% of the radioactive iron is incorporated into haem in the bone marrow and most of this iron appears subsequently in circulating red cells (4).

Figure 1: The iron acquisition pathway in developing erythroblasts



The Fe(III)-Tf complex binds to specific receptors present on the cell surface, inducing the formation of an endocytic vesicle. Acidification of the endosome will release iron from transferrin and Steap3, an erythroid-specific reductase, will reduce iron to its ferrous Fe(II) state. DMT1/Nramp2, a protons and Fe(II) co-transporter present in the endosomal membrane (see putative structure in lower insert) will export iron into the cytosol. Most of the iron will be targeted to mitochondria to participate in haem synthesis or in Fe/S cluster assembly. Mitoferrin is part of the mitochondrial iron transport machinery. Ferrochelatase, the last enzyme of the haem biosynthetic pathway, catalyzes the insertion of Fe(II) into PPIX to form haem (right insert). Illustration by Jean-Pierre Laigneau, reproduced with permission of Haematologica (© Ferrata Storti Foundation, Pavia, Italy).

2.2 The endosomal pathway

The iron-transferrin complex binds to cells through transferrin receptors, expressed in variable numbers on the surface of most cells. The transferrin receptor (TfR) is a dimer of two identical subunits of 95kDa molecular weight, bound by two disulfide bridges. There are two different molecular forms of the receptor, Tfr1, coded by a gene present on chromosome 3q39 and Tfr2, coded by a gene on 7q22. Tfr1 is highly expressed on developing erythroid progenitors in the bone marrow and on all dividing cells, whereas the expression of Tfr2 is restricted to the liver. The iron binding affinity of Tfr2 is much lower than that of Tfr1 (see (5) for review) and it is thought

to be a signalling molecule rather than an iron uptake molecule. Tfr2 mutations causes haemochromatosis with a very similar phenotype to HFE mutations and several studies suggest that HFE and Tfr2 are implicated in the signalling pathway that regulates hepcidin expression in response to changes in transferrin saturation.

After binding to its receptor on the plasma membrane, the diferric transferrin-receptor complex induces the formation of a clathrin-coated pit allowing its internalisation by endocytosis (6). An endosomal ATP-dependent proton pump lowers the pH to around 5-6. Protonation of the carbonate will open the iron-binding pocket in the transferrin molecule and induce release of the iron atoms, while apotransferrin remains bound to its receptor. Recently, Steap3 (6-transmembrane epithelial antigen of the prostate 3), an endosomal ferrireductase that facilitates Tf-mediated uptake of iron in erythroid precursors, has been identified (7). Steap3 is expressed highly in haematopoietic tissues, colocalises with the Tf cycling endosome and facilitates Tf-bound iron uptake. Erythroid cells from mice deficient in Steap3 (*nm1054* mice) are defective in Tf-dependent iron uptake, resulting in a hypochromic, microcytic anaemia typical of iron deficiency (8). Taken together, these findings indicate that Steap3 is an endosomal ferrireductase required for efficient Tf-dependent iron uptake in erythroid cells. The role of the other members of the family (Steap1, 2 and 4) in erythroid cells and in other tissues is not yet clear (9).

Ferrous iron is subsequently exported to the cytosol by Nramp2/DMT1, a co-transporter of Fe(II) and protons (10), with twelve transmembrane domains, expressed at the endosomal surface. Nramp2/DMT1 is a member of the Nramp (Natural Resistance-Associated-Macrophage Protein) family (11). Several isoforms of the *Nramp2/DMT1* mRNA are known, resulting from alternative splicing and/or the use of two alternative upstream promoter regions (12, 13). The isoform I of Nramp2/DMT1 is localised 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 (14). Therefore, Nramp2/DMT1 plays a role in intestinal iron uptake and in the transfer of iron from the endosome to the cytosol in developing erythroblasts. The endocytic vesicle containing apotransferrin, which remains bound to its receptor at the acidic pH of the endosomal lumen, is recycled back to the plasma membrane, where a pH of 7.4 will induce the release of the protein for another cycle of iron transport.

Exocyst proteins, a group of proteins that orchestrate transport of secreted material from the Golgi, and which are probably involved in vesicular fusion (17), are important for this recycling of apotransferrin, as shown by the phenotype of the haemoglobin-deficit mouse mutant (*hbd*) (15). These mutant mice are characterised by a hypochromic, microcytic anaemia and two independent groups have simultaneously identified the defect as being caused by the removal of an exon from

the *Sec15LI* gene (15, 16). The yeast homologue of the Sec15L1 protein is an integral member of the exocyst. In *hbd* reticulocytes, the Tf-Fe(III) complex appears to bind to the TfR and to enter the cells by the endosomal pathway (18), suggesting that the *Sec15L1* mutation only induces a reduction in the efficiency of vesicular trafficking, docking, fusing, and/or cargo delivery.

3. Iron utilisation by erythroid cells

3.1 From the endosome to the mitochondria

In erythroid cells, most of the iron leaving the endosome will be targeted to mitochondria where it participates in haem synthesis and iron-sulfur cluster assembly (19). There has been a huge controversy about the chemical form of iron in the cytosol and the possible existence of a pool of labile iron, loosely bound to low molecular weight compounds, able to generate the production of reactive oxygen species and readily chelatable. This iron pool is also the regulatory iron pool, inducing conformational changes in cytosolic iron sensors (see paragraph 4.1 below). The development of fluorescent metallosensors has allowed to confirm the existence of this labile iron pool in cultured cells and to estimate its concentration between 0.1 to 1 μM according to cell type or cell status (20). However, this proposal has been challenged in relation to erythroid cells, where a direct contact between endosome and mitochondria has been proposed (21), allowing iron to enter directly into mitochondria without transiting through the cytosol. This model can account for the specific features of the iron sensing pathway in erythroid cells (see paragraph 4.1).

Mitoferrin (Mfrn, 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 membranes. The zebra fish mutant *frascati* with a mutated Mfrn1 gene shows profound hypochromic anaemia due to defective iron uptake by mitochondria (22). Mouse erythroblasts derived from embryonic stem cells with a defective Mfrn1 gene show a complete inhibition of iron incorporation into haem as compared to wild type cells. Mfrn1 has a paralogue in mammals, Mfrn2 that is ubiquitously expressed. Silencing of both Mfrn1 and Mfrn2 induces reduction in haem synthesis by 90% (23). Interestingly, it also prevents iron-sulfur cluster assembly and maintains IRP1 in its apoform, with a high RNA binding affinity, thereby preventing ferritin synthesis. Deletion of the two yeast homologues Mrs3 and Mrs4 also affects incorporation of iron into protoporphyrin IX and the efficacy of iron sulfur cluster assembly, inducing poor growth on iron limited conditions (24).

3.2 Haem biosynthesis

The majority (80%) of haem in the body is synthesised in erythroid cells, as a precursor to haemoglobin formation, although haem is also the prosthetic group of various types of proteins, such as cytosolic or mitochondrial cytochromes, catalase, peroxidase and NO synthase. In mammals, eight enzymes are required for haem biosynthesis, located successively in the mitochondria, the cytosol and the mitochondria. The first enzyme of the pathway, 5-aminolaevulinate synthase (ALAS), which catalyzes the condensation of glycine and succinyl CoA to form 5-aminolaevulinic acid (ALA), is encoded by two different genes. ALAS1 is ubiquitously expressed while ALAS2, which is located on the X chromosome, is expressed only in erythroid cells. Regulation of these two genes is essential for the control of haem biosynthesis but the mechanisms differ widely between the two isoforms. Haem exerts a negative control over ALAS1 expression, especially in the liver, by inhibiting transcription of the gene, translation of the mRNA and mitochondrial targeting of the enzyme (see (25) for review), whereas regulation of ALAS2 expression in erythroid cells is only dependent on iron (see § 4.1).

Ferrochelatase, the last enzyme of the pathway, is located in the mitochondrial inner membrane and catalyses the insertion of Fe(II) iron into protoporphyrin IX (PPIX). In iron deficiency anaemia, PPIX will accumulate in erythroid cells in the form of Zn-PPIX, whereas free PPIX accumulates in erythropoietic protoporphyria, due to ferrochelatase deficiency (see Chapter 27). Following its synthesis, haem is exported out of the mitochondria to be associated to globin chains and apocytochromes. Haem export from the mitochondria is thought to be mediated by ATP-Binding Cassette (ABC) transporters, although the exact nature of the transporter has not been elucidated (26).

3.3 Iron sulfur cluster assembly

Another important function of iron in the mitochondria is to ensure the synthesis of the iron-sulfur clusters (ISC). These labile inorganic protein cofactors are associated with specific sites in the acceptor proteins through linkage to the sulfur of cysteine residues. Fe/S proteins play important roles in several cellular processes, including metabolism, electron transfer or regulation of gene expression. They are found in the mitochondria, the cytosol and the nucleus, but the ISC are primarily assembled in mitochondria. Eukaryotic mechanisms for the biogenesis of Fe/S proteins comprise the ISC assembly machinery in the mitochondria, the mitochondrial ISC export system and the cytosolic Fe/S protein assembly machinery (see (27) for review). It is not clear how these processes are regulated in erythroid cells or how the distribution of iron between haem synthesis and the ISC assembly machinery is controlled. However, there are several interrelationships between ISC and haem synthesis. For instance, IRP1 acts a cytosolic iron sensor through the

reversible acquisition of a 4Fe-4S cluster (see below) and the enzymatic activity of ferroxidase depends on the presence of an ISC. Furthermore, molecular defects in some of components of the ISC assembly or export can lead to microcytic anaemia (see Chapter 22).

3.4 Mitochondrial ferritin

Some years ago, Levi and Arosio reported the existence of a mitochondrial ferritin (FtMt), differing from the cytosolic ferritins (28). In humans, this FtMt is encoded by an intronless gene located on chromosome 5q23. The 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 (29). The sequence of the mature protein is about 80% identical to that of the cytosolic H ferritin but it differs from the H and L ferritin subunits by having a long amino acid N-terminal extension for mitochondrial import. FtMt has a ferroxidase activity and forms homopolymers. In human, FtMt mRNA is highly expressed in the testis (28) and FtMt protein was found to accumulate in iron loaded mitochondria of patients with sideroblastic anaemia (30). FtMt is not found in normal erythroid cells. The role of this mitochondrial ferritin is not fully elucidated but it is thought to be a protective molecule against iron-mediated oxidative damage rather than an iron-storage molecule.

4. Coordinated regulation of iron acquisition and haem synthesis in erythroid cells (HRI, IRPs)

Several mechanisms are operative in erythroid cells for coordinating iron acquisition and haem synthesis (31). Trafficking and storage of iron in the mitochondria must be tightly regulated as excess free iron promotes the generation of harmful reactive oxygen species (32) whereas an inadequate supply of iron prevents haemoglobin formation in developing erythroid precursors and lead to microcytic hypochromic anaemia.

4.1 Iron-dependent regulation

During erythroid differentiation, iron supply and PPIX production are tightly regulated via the IRE/IRP system. This post-transcriptional mechanism regulates the synthesis of ALAS2, the first enzyme of the haem biosynthetic pathway and also regulates the stability of TfR1 mRNA, to coordinate the iron uptake with the rate of PPIX synthesis.

There are two cellular iron sensors called Iron Regulatory Proteins 1 and 2 (IRP1 and IRP2) which regulate translation or stability of mRNAs encoding proteins of iron metabolism (see (33) for review). In their native conformation, both IRPs have a

high binding affinity for short hairpin structures called Iron Responsive Elements (IRE) present in the mRNAs of their target genes. Binding of IRPs to the IRE present in the 5' untranslated region of the H and L ferritin mRNAs, as well as in the ferroportin and in the ALAS2 mRNA, represses translation. Binding of IRPs to the multiple IREs present in the 3' untranslated region of Tfr1 mRNA stabilises the mRNA. A [4Fe-4S] cluster is required for IRP1 iron sensing and when ISC assembly is defective, as in the patient with GLRX5 mutation or in the *shiraz* zebra fish mutant, IRP1 remains in the apo-form with a high RNA binding affinity. It is not clear whether both IRP1 and IRP2 contribute to stabilisation of Tfr1 mRNA but several lines of evidence suggest that IRP2 is the main iron-sensor in erythroid cells. IRP2 is stabilised by haem deficiency whereas accumulation of free haem induces its ubiquitination and degradation by the proteasome (34). IRP2 knock-out mice develop microcytic hypochromic anaemia with reduced Tfr1 expression in bone marrow cells (35, 36), suggesting that Tfr1 mRNA are rapidly degraded in the absence of IRP2. Finally, IRP2 expression is decreased in primary erythroblasts deficient in Stat5, with a concomitant reduction in Tfr1 mRNA, whereas IRP1 is moderately increased (37). These observations highlight the importance of IRP2 in regulating Tfr1 expression in erythroid cells. However, the implication of the IRE/IRP system in the regulation of iron homeostasis in erythroid cells has been recently challenged by the observation that in differentiating erythroblasts, Tfr1 mRNA stability and IRP mRNA-binding affinity are no longer modulated by iron supply (38). This would be in agreement with the proposal that in erythroid cells, endosomes dock to mitochondria allowing the direct transfer of iron, without transiting through the cytosol and without modulating the mRNA-binding activity of the IRPs. This dual mechanism is not fully elucidated but in developing erythroblasts, it contributes to maintaining the high flux of incoming iron available for haem synthesis rather than being sequestered into ferritin.

4.2 Haem-dependent regulation

Besides its function as the prosthetic moiety in haem proteins, haem itself can influence gene expression at the level of transcription, miRNA processing, protein synthesis or post-translational modifications. Haem is involved in the transcriptional regulation of the haem oxygenase 1 (HO-1) gene, the enzyme which catalyses haem catabolism, and of globin genes. Haem strongly stimulates HO-1 expression by inhibiting the transcriptional repressor Bach1. Binding of a heterodimer of the small maf transcription factor and Bach1 to the multiple MARE (maf recognition element) sites in HO-1 enhancers represses HO-1 gene expression. "Uncommitted" haem can bind to the haem-binding motif on Bach1, causing a decrease in DNA binding activity and allowing the binding of maf-Nrf2 or maf-NFE2 heterodimers which act as activators of transcription (39). Although HO-1 expression has not been extensively studied in erythroid cells,

it has been shown that HO-1 mRNA decreases following erythroid differentiation of Friend erythroleukaemia cells, while mRNAs coding for the enzymes of the haem biosynthetic pathway increase (40). MAREs are also present in the enhancer of the L ferritin gene (41) and in the β globin Locus Control Region (42). Although Bach1 may not be a major regulator of globin gene expression during erythroid differentiation, it might allow a fine-tuning between haem and globin chain synthesis.

In contrast, haem deficiency will repress protein translation, and especially globin synthesis by activating a stress protein kinase named haem regulated inhibitor (HRI) which phosphorylates eIF2 α (43). When eIF2 is phosphorylated, it remains bound to its binding protein, thereby preventing the regeneration of GDP into GTP and shutting down mRNAs translation. This system will prevent the formation of globin chains in excess of haem during erythroid differentiation. In support of this view is the observation that HRI deficient mice present hyperchromic normocytic anaemia with erythroid hyperplasia and erythrocytes loaded with multiple globin inclusions (44). Finally, the amount of haem can also be controlled during erythroid differentiation by haem trafficking. It has recently been shown that the feline leukemia virus, subgroup C, receptor (FLVCR) could function as a haem exporter (45, 46). This protein was originally cloned as a cell surface protein which serves as a receptor for feline leukaemia virus, subgroup C. Cats viraemic with FeLV-C develop pure red cell aplasia, characterised by a block in erythroid differentiation at the CFU-E-proerythroblast stage, reticulocytopenia, and severe anaemia. FLVCR belongs to the family of MFS (major facilitator superfamily) proteins which transport small solutes across membranes by using the energy of ion-proton gradients. Overexpression of FLVCR induced significant reduction in cellular haem content, suggesting that FLVCR is involved in haem transport (45).

Mice with neonatal inactivation of FLVCR develop a severe macrocytic anaemia with maturation arrest at the proerythroblast stage, which suggests that erythroid precursors export excess haem to ensure survival (47).

Therefore, during maturation of erythroid precursors, there is an interplay of positive and negative feedback mechanisms which maintain sufficient iron supply for haem synthesis and prevent formation or accumulation of haem in excess of globin chains.

5. Congenital microcytic anaemia related to genetic defects in iron pathways

5.1 Sideroblastic anaemia

Congenital or acquired sideroblastic anaemias (SA) are a heterogeneous group of disorders characterised by the presence of ring sideroblasts (erythroblasts with

abnormal mitochondrial iron deposits) in the bone marrow (see Chapter 22 for details and (48) for review). X-linked SA (XLSA) is due to mutations in the *ALAS2* gene encoding the erythroid-specific isoform of the first enzyme of the haem biosynthetic pathway. Some rare forms of SA associated with ataxia in early childhood result from a mutation in *ABCB7*, a mitochondrial membrane protein thought to export components of ISC out of the mitochondria to participate in the maturation of cytosolic Fe-S proteins. The identification of the molecular defect in these rare patients was the first hint that the ISC assembly is an important player in the control of haem formation in erythroid cells. Further demonstration was brought about by the discovery of a large deletion in the *glutaredoxin 5 (glrx5)* gene in the *shiraz* zebra fish mutant (49), displaying hypochromic microcytic anaemia at birth, and by the identification of a patient with *GLRX5* deficiency, microcytic sideroblastic anaemia and iron overload (50). In this patient, *IRP1* failed to acquire an ISC and translation of the IRE-containing mRNA coding for *ALAS2* was repressed, preventing *PPIX* and thereby haem formation. Molecular defects in the *ALAS2* gene as found in patients with XLSA also block *PPIX* formation. In both cases, there is an activation of *TfR1* expression through *IRPs*-mediated stabilisation of *TfR1* mRNA, increased influx of iron into the mitochondria despite the lack of haem precursor and formation of abnormal deposits of iron bound to mitochondrial ferritin, contributing to the formation of ring sideroblasts

5.2 Non-sideroblastic microcytic hypochromic anaemia

Recent progress in our understanding of the iron acquisition pathway in erythroid cells and in the systemic regulation of iron availability has come from the elucidation of the molecular defects in animal models of microcytic anaemia in the mouse or Zebra fish. In some cases (*DMT1*, *TMPRSS6*), human patients have been found carrying mutations in these newly identified genes, but several candidate genes remain which have not so far been found implicated in human diseases (see (51) for review).

5.2.1 *DMT1* mutants

The first *DMT1* mutation (G185R in TM4) was found both in the *mk/mk* mouse (52) and in the Belgrade rat (53), causing hypochromic microcytic anaemia due to a defect in intestinal absorption of iron and in iron utilisation for erythropoiesis. In the mouse, selective inactivation of *DMT1* confirmed the role of *DMT1* in gut and bone marrow (54). Up to now, three human patients have been reported with *DMT1* mutations. The first mutation in human *DMT1* was found in the homozygous state in a Czech patient with congenital severe hypochromic microcytic anaemia and normal to slightly increased serum ferritinem. Liver iron overload was diagnosed at the age of 19 (55). The mutation, G1285C, affected the last nucleotide of exon

12, leading to an E399D replacement. This DMT1 mutant seems to have retained full iron transport activity (56), the clinical phenotype of the patient resulting from preferential exon 12 skipping. The second patient, reported by Iolascon et al., presented severe neonatal microcytic hypochromic anaemia and an early onset of liver iron overload (57). He was a compound heterozygote, including a 3-bp deletion (delCTT) in intron 4 that partially impaired normal splicing and an amino acid substitution (R416C) at a conserved residue in TM9 of the protein. The anaemia was present from birth. Functional studies of the mutant proteins suggested some phenotype- genotype correlation (58). The third patient also presented a neonatal anaemia, although with a less severe haemoglobin defect. No functional studies were performed on the mutated proteins from this French patient but it can be speculated that the milder phenotype reflects residual transport activity of the rather conservative G212V mutation in TM5, whereas the delVal114 is likely to disrupt the TM structure (59). Liver iron overload also developed rapidly and it is conceivable that the high serum transferrin saturation leads to the presence of non-transferrin bound iron and that this contributes to hepatocyte iron loading. Normal to low urinary hepcidin levels in relation to the levels of iron stores have been found in these patients, probably accounting for the increased intestinal iron absorption (60) and onset of liver iron overload. All three patients appeared to respond to erythropoietin (Epo) administration (60). Because mean corpuscular volume and mean cell haemoglobin did not change during Epo treatment, it was concluded that Epo did not improve iron utilisation by the erythroblasts, but probably reduced the degree of apoptosis known to be associated with iron deficient erythroblasts (61).

5.2.2 *TMPRSS6* mutants

The involvement of *TMPRSS6*, a membrane-bound serine protease, in the control of hepcidin expression and of iron availability for erythropoiesis came as a surprise. Beutler et al. recently described the mask mice which result from chemical mutagenesis and which present a progressive loss of body (but not facial) hair and microcytic anaemia (62). They were able to show that the mask phenotype results from reduced absorption of dietary iron caused by high levels of hepcidin, and is due to a splicing defect in the transmembrane serine protease 6 gene *Tmprss6*. Shortly after the initial description, several groups (63-65) including ours (66) found mutations in the human *TMPRSS6* gene in patients with very low iron stores and microcytic anaemia refractory to iron treatment. This condition was called iron refractory iron deficiency anaemia (IRIDA). The role of *TMPRSS6* in regulating hepcidin synthesis is discussed in more detail in Chapter 20.

5.2.3 Hereditary atransferrinemia

Atransferrinemia/hypotransferrinemia is a rare genetic disorder characterised by microcytic anaemia and iron overload. It was first described in 1961 (67) and since then, 11 patients have been reported. The molecular defect in the transferrin gene has so far been identified in only four of them (68) but all these patients had in common low to undetectable levels of plasma transferrin.

Because transferrin functions to deliver iron to the developing erythron, as well as to other tissues, atransferrinemia results in reduced delivery of iron to the bone marrow erythroid precursors and reduced haemoglobin synthesis. Some iron is present in the plasma in a non-transferrin bound form and results in severe iron overload in the liver, pancreas, and heart. This is exacerbated by increased intestinal iron absorption due to decreased hepcidin production (69). A similar phenotype is observed in hypotransferrinemic mice (hpx/hpx) which carry a spontaneous mutation if the Tf gene. Newborn hpx/hpx mice are viable but severely anaemic and they can only survive for up to 2 weeks after birth without blood transfusions or Tf infusions. However, they will only reach adulthood when receiving weekly injections of whole mouse serum or apo Tf (70). In human patients, correction of the anaemia and partial reduction of the iron overload have been obtained with monthly infusions of fresh-frozen human plasma (71). Tf infusions have also been used successfully; they induced a rapid rise in haemoglobin concentration within one week and the effect was maintained for 4-5 months (71, 72).

5.2.4 Hereditary aceruloplasminaemia

This is a rare autosomal recessive disorder of iron homeostasis caused by mutations in the caeruloplasmin gene. More than 25 disease-causing mutations have been identified. Affected individuals may present in adulthood with evidence of hepatic iron overload, diabetes, peripheral retinal degeneration, dystonia, dementia, or dysarthria. Laboratory studies demonstrate microcytic anaemia, elevated serum ferritin, and a complete absence of serum caeruloplasmin ferroxidase activity.

Tissue iron overload probably results from both increased intestinal iron overload and defective mobilisation of tissue iron stores. Caeruloplasmin (Cp) is a plasma multi-copper ferroxidase which catalyses the oxidation of ferrous to ferric iron, a change required for iron efflux from macrophages by ferroportin (73) and for the binding of iron to plasma transferrin. It is synthesised and secreted by hepatocytes but it is also expressed in the central nervous system where it is mostly found on the surface of astrocytes in a glycosylphosphatidylinositol (GPI)-anchored form, resulting from alternative RNA splicing (74). GPI-linked Cp has also been described in bone marrow macrophages. Cp knock-out mice display increased iron content in

liver and spleen macrophages as well as in hepatocytes (75). Interestingly, another multi-copper ferroxidase called hephaestin is expressed in duodenal enterocytes and present on the plasma membrane as a GPI-anchored protein. Hephaestin mutation in *sla* (sex-linked anaemia) mice results in reduced iron efflux from duodenal enterocytes and development of moderate microcytic anaemia (76). Therefore, Cp seems important for mobilisation of iron stores and hephaestin for intestinal iron absorption. No hephaestin mutations have been found in human patients. Acaeruloplasminaemia has been described mainly in Japanese patients and rarely in whites. In Japan, the incidence was estimated to be approximately one per 2,000,000 in the case of non-consanguineous marriages (77).

6. Anaemia of chronic disorders (ACD)

Several physiopathological features may contribute, although to a varying degree, to the anaemia of chronic disorders, also known as the anaemia of inflammation (reviewed in (78)). These consist in impaired proliferation of erythroid progenitors and blunted response to erythropoietin, reduced life span of red blood cells as well as reduced erythropoietin synthesis and finally, disturbances of iron homeostasis. The hallmark of ACD is increased uptake and retention of iron within macrophages, leading to a defect in iron availability for erythropoiesis. Macrophages acquire iron mostly through erythrophagocytosis of senescent red blood cells (79). Iron is liberated from haem by haem oxygenase and normally stored in the ferritin molecules, or exported back to the plasma by ferroportin, the sole iron exporter in mammals, which is expressed on macrophages and duodenal enterocytes. In chronic inflammation, the pro-inflammatory cytokines will activate the macrophages and consequently increase their erythrophagocytic activity, which is the main cause of the reduced red cell life span. Interferon- γ , LPS and TNF- α have been shown to reduce ferroportin expression (80) and to stimulate ferritin synthesis, thereby leading to iron retention within macrophages. However, the recent discovery of hepcidin has allowed the identification of another major link between iron homeostasis and inflammation. During inflammation, IL-6 seems to be the major pro-inflammatory cytokine implicated in hepcidin activation, through a Stat3 dependent signalling pathway (3). The symptoms characteristic of the anaemia of inflammation (reduction in serum iron, retention of iron in macrophages and blocking of intestinal iron absorption) are all compatible with the consequences of an increase in the production of hepcidin. Interestingly, stimulation of erythropoiesis by erythropoietin injections or repeated blood sampling can repress hepcidin, even in the presence of inflammation(81). Therefore, the signalling pathway which repress hepcidin expression in response to stimulation of erythropoiesis (see Chapter 20) dominates over the pro-inflammatory response.

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Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/iron-handbook2009answers.htm>

1. Bone marrow erythroblasts acquire iron by which one of the following mechanisms?

- a) Uptake of circulating ferritin
- b) Uptake of the Fe²⁺-Tf complex by specific receptors
- c) Uptake of non-transferrin bound iron
- d) Haem uptake

2. What is the phenotype associated with TMPRSS6 mutations?

- a) Haemochromatosis with parenchymal iron overload
- b) Haemochromatosis with macrophage iron overload
- c) Iron deficiency anaemia
- d) Anaemia and iron overload

3. What is the main route of utilisation of iron in erythroid cells?

- a) Storage into ferritin
- b) Iron-sulfur cluster assembly
- c) Haem synthesis
- d) Iron export by ferroportin

4. All the mechanisms below have been shown to contribute to the anaemia of chronic disorders except one:

- a) Reduced urinary hepcidin excretion
- b) Increased hepcidin expression
- c) Blunted erythropoietin response
- d) Reduced red blood cell lifespan

5. What is the principal mechanism regulating transferrin receptor expression in erythroid cells?

- a) Transcriptional regulation
- b) Changes in mRNA stability
- c) miRNA
- d) Degradation by the proteasome

NOTES