

# The liver: conductor of systemic iron balance

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**Iron is a micronutrient essential for almost all organisms: bacteria, plants, and animals. It is a metal that exists in multiple redox states, including the divalent ferrous ( $\text{Fe}^{2+}$ ) and the trivalent ferric ( $\text{Fe}^{3+}$ ) species. The multiple oxidation states of iron make it excellent for electron transfer, allowing iron to be selected during evolution as a cofactor for many proteins involved in central cellular processes including oxygen transport, mitochondrial**

**respiration, and DNA synthesis. However, the redox cycling of ferrous and ferric iron in the presence of  $\text{H}_2\text{O}_2$ , which is physiologically present in the cells, also leads to the production of free radicals (Fenton reaction) that can attack and damage lipids, proteins, DNA, and other cellular components. To meet the physiological needs of the body, but to prevent cellular damage by iron, the amount of iron in the body must be tightly regulated. Here we review**

**how the liver is the central conductor of systemic iron balance and show that this central role is related to the secretion of a peptide hormone hepcidin by hepatocytes. We then review how the liver receives and integrates the many signals that report the body's iron needs to orchestrate hepcidin production and maintain systemic iron homeostasis. (*Blood*. 2014;123(2):168-176)**

## Iron in the body

The total body iron content is approximately 3 to 5 g in the average adult human. The greatest amount of iron found in the body is complexed as  $\text{Fe}^{2+}$  in heme in the hemoglobin of red blood cells and myoglobin of muscles (2-3 g). Body iron balance is maintained by a daily intake of 1 to 2 mg from dietary iron to compensate for the losses of iron through the sloughing of skin, mucosal cells, and sometimes blood loss. Because 20 to 25 mg of iron are required for red blood cell production and cellular metabolism, the amount of iron absorbed from alimentation is insufficient to meet daily catabolic needs. Thus the bulk of iron for daily body needs is provided by the macrophages that recycle senescent red blood cells. Other organs, primarily the liver, serve as reservoirs of iron. There is no regulated pathway to excrete iron from the body, and thus iron balance is primarily preserved by the regulation of iron absorption from the duodenum and iron recycling from macrophages and other tissue stores.

Absorption of iron from food takes place at the brush border of the enterocytes in the duodenum. The mechanisms of absorption of inorganic iron (through the divalent metal transporter 1 (DMT1) and solute carrier family 11, member 2 (SLC11A2)) and iron complexes to heme have been described in detail in previously published reviews.<sup>1,2</sup>

Cytosolic iron that is not directly used by the cell is either stored in ferritin or exported into the plasma by the basolateral transmembrane iron exporter, ferroportin/SLC40A1<sup>3,4</sup> and loaded onto transferrin.

Aged or damaged erythrocytes are permanently degraded by macrophages primarily in the spleen, liver, and bone marrow through erythrophagocytosis and iron is exported from phagocytic vesicles via NRAMP1 (natural resistance-associated macrophages protein 1), a divalent metal transporter homologous to DMT1 expressed at the phagolysosomal membrane.<sup>5</sup> Iron is then stored

in ferritin and, when needed, can be released back into circulation through ferroportin.

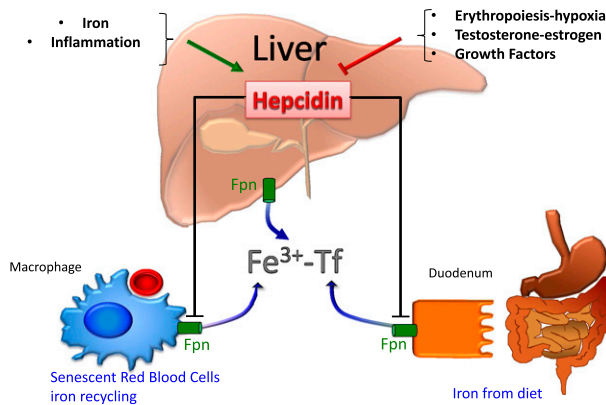
## Iron transport to cells and absorption by cells

After export from enterocytes and macrophages, iron circulates in the bloodstream bound to transferrin (Tf)<sup>6</sup> molecules for delivery to tissues for utilization or storage.

Cells acquire their iron from plasma iron-bound Tf by receptor-mediated endocytosis through the ubiquitous cell surface transferrin receptor 1 (TfR1).<sup>7</sup> TfR1 is a homodimeric membrane glycoprotein that binds 2 molecules of Tf to deliver iron into cells. The holo-Tf complex is then internalized and iron is exported to the cytosol through DMT1 or its homolog Nramp1 in macrophages. Finally, the apo-Tf/TfR1 complex is recycled to the cell surface, where Tf is dissociated from TfR1. Tf returns to the bloodstream and is available to rebind iron, whereas TfR1 remains at the plasma membrane.

TfR2 is a transferrin receptor homolog to TfR1 mainly expressed in hepatocytes. It has been primarily described to mediate iron-Tf uptake in nonhepatoma cell lines in vitro, possibly via receptor-mediated endocytosis, similar to TfR1 but with a lower affinity for Tf.<sup>8</sup> However, it is currently unclear whether TfR2 truly has a role in cellular iron uptake in hepatocytes.<sup>9</sup> Nevertheless, it is now well characterized that even if TfR2 does not participate directly in iron uptake, it is still required for iron homeostasis because mutations in the *TfR2* gene induce the iron overload disorder hereditary hemochromatosis, as discussed later.<sup>10</sup>

In conditions of iron excess, when Tf becomes largely saturated with iron, another form of iron can be found in plasma,



**Figure 1. The liver is the major source of hepcidin production.** Iron and inflammation stimulate, whereas erythropoiesis, hormones, and growth factors decrease, hepcidin production. Hepcidin downregulates ferroportin in macrophages, enterocytes, and hepatocytes, leading to decreased iron release into the serum that is subsequently bound to transferrin ( $\text{Fe}^{3+}$ -Tf).

non-transferrin-bound iron (NTBI). The topic of NTBI has been recently reviewed<sup>11</sup> but will not be discussed in detail here.

## The central role of the liver in systemic iron homeostasis

### The liver secretes hepcidin

Hepcidin is a small peptide hormone mainly secreted by hepatocytes in the liver (Figure 1). Hepcidin is the main regulator of systemic iron homeostasis that blocks the transfer of iron into the plasma from the 3 main sources: dietary absorption in the duodenum, recycling by macrophages, and efflux of stored iron from hepatocytes. Hepcidin was identified simultaneously for its role as an antimicrobial peptide<sup>12</sup> and its role in iron homeostasis.<sup>13</sup> Although hepatocytes are the main source of circulating hepcidin in the plasma, other cells can also produce hepcidin (such as macrophages<sup>14</sup> and adipocytes<sup>15</sup>) to a much lower extent, but the role of this extrahepatic secretion is still unclear. Because this review is focused on the role of the liver in iron homeostasis, we will only discuss the regulation of hepcidin expression in hepatocytes. The mature form of hepcidin is a 25-amino acid peptide generated from an 84-amino acid prepropeptide after peptidase and furin cleavage.<sup>16</sup> In plasma, the mature form of hepcidin circulates associated with  $\alpha 2$ -macroglobulin<sup>17</sup> and albumin,<sup>18</sup> although the proportion of this associated form compared with free hepcidin is still uncertain.<sup>18</sup>

The effect of hepcidin on iron body balance is related to its direct binding to the principal iron exporter ferroportin at the cell surface, leading to ferroportin internalization and subsequent lysosomal degradation.<sup>19</sup> Degradation of ferroportin results in the loss of the ability to export iron and leads to iron sequestration in enterocytes, macrophages, and hepatocytes.

The central role of hepcidin in iron metabolism has been supported by the pathological deregulation of hepcidin expression in humans and mice that results in iron overload or iron deficiency. For conditions in which there is inadequately low hepcidin expression because of mutations in the gene encoding for hepcidin (*HAMP*) itself,<sup>20,21</sup> or in the known positive regulators of hepcidin expression such as bone morphogenetic protein 6 (*BMP6*, mutations only identified in mouse studies),<sup>22,23</sup> hemojuvelin (*HFE2*, also known as *HJV* or *RGMc*),<sup>24</sup> mothers against decapentaplegic homolog

4 (*SMAD4*, confirmed only in mouse studies),<sup>25</sup> *TFR2*,<sup>10</sup> or the hemochromatosis protein (*HFE*),<sup>26</sup> the iron exporter ferroportin accumulates at the cell surface of enterocytes and macrophages, leading to unregulated absorption of dietary iron and supraphysiological release of iron from macrophages. This increase of iron export into the plasma rapidly saturates circulating Tf, resulting in an accumulation of NTBI that is easily absorbed by hepatocytes and other tissues, leading to the iron overload disorder known as hereditary hemochromatosis. Although hepatocytes also exhibit higher ferroportin expression under conditions of low hepcidin,<sup>27</sup> hepatocytes accumulate iron because the uptake of iron (thought to be a result of the high NTBI levels in plasma) supersedes the increased ferroportin iron export capacity. Conversely, in conditions of hepcidin overexpression, such as is seen in inflammatory conditions,<sup>28</sup> artificial hepcidin overexpression,<sup>29</sup> and mutations in negative regulator genes of hepcidin such as *TMPRSS6*,<sup>30-32</sup> the result is iron deficiency. Indeed, high hepcidin levels induce degradation of ferroportin that block (1) dietary iron uptake from enterocytes and (2) iron release from macrophages and hepatocytes. In chronic conditions, this iron deficiency can ultimately evolve into an anemia when iron is not sufficiently available for the demands of erythropoiesis (ie, production of red blood cells).

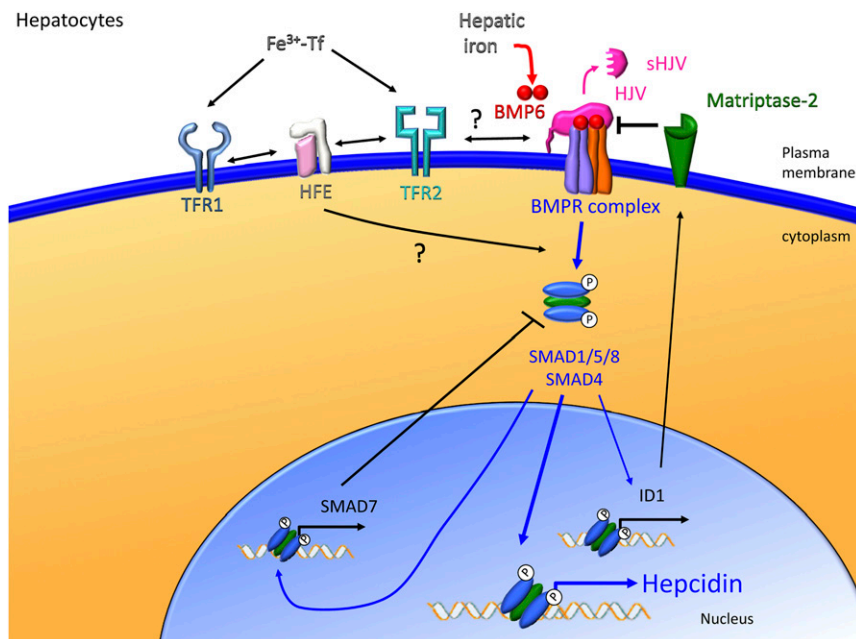
In addition, the central role of the liver in systemic iron homeostasis has also been recently highlighted in a study by Bardou-Jacquet et al.<sup>33</sup> They demonstrated that in patients with mutations in the *HFE* gene, a liver transplant could rescue the low hepcidin phenotype and prevent iron overload.

It is now well characterized that to provide the appropriate amount of iron available for erythropoiesis and cell metabolism, but to prevent toxic iron overload, hepcidin expression by the liver must be tightly regulated. Hepcidin expression is induced by hepatic iron, plasma iron, inflammation, and endoplasmic reticulum stress, whereas it is downregulated by iron deficiency, erythropoiesis, and hypoxia.

### The BMP signaling pathway is central for hepcidin regulation

It is now clearly established that the central signaling pathway involved in the regulation of hepcidin expression by iron is the BMP-SMAD pathway.<sup>34</sup> The hepcidin promoter contains key BMP-responsive elements, which regulate its transcription.<sup>35</sup> Bone morphogenetic proteins (BMPs) represent a large subfamily belonging to the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily of ligands. BMPs mediate many fundamental processes such as embryonic morphogenesis, bone development, and tissue repair.<sup>36</sup> Specificity of the BMP-SMAD pathway in the liver and its role in iron homeostasis seems to be dependent on the combination of two factors that are mainly expressed in the liver: an iron-regulated ligand BMP6 and a GPI-membrane-anchor co-receptor hemojuvelin (*HJV*) (Figure 2).

All TGF- $\beta$  superfamily members, including BMPs, share common structural features and a common model of signaling transduction. The active form of BMPs is a disulfide-linked dimeric protein, which is cleaved from a larger precursor protein and secreted. The essential role of BMP6 in hepcidin regulation is highlighted by the inappropriately low level of hepcidin and massive iron overload exhibited in mice lacking BMP6.<sup>22,23</sup> However, it is worth noting that the essential role of BMP6 in the regulation of hepcidin expression has not yet been described in humans. Other endogenous BMPs are not able to compensate for the loss of BMP6 in hepcidin regulation (at least in mice) despite the capacity of exogenous BMP2, 4, 5, 7, and 9 to stimulate hepcidin expression.<sup>37</sup> Once secreted, BMPs act by binding to 2 distinct receptor types: type I



**Figure 2. The liver responds to iron signals to regulate hepcidin production.** Hepatic iron accumulation leads to increased BMP6 production, which is an important ligand for the HJV/BMP receptor complex on the surface of hepatocytes. The activation of the BMP signaling pathway leads to nuclear translocation of SMAD1/5/8 with SMAD4 and subsequent activation of hepcidin transcription. Circulating iron-bound transferrin ( $\text{Fe}^{3+}$ -Tf) also stimulates hepcidin production by activating the BMP/HJV/SMAD signaling pathway. However, the exact details of the necessary and sufficient interactions of iron-bound transferrin with transferrin receptor 1 (TFR1), transferrin receptor 2 (TFR2), and HFE at the surface of hepatocytes to stimulate hepcidin expression are still unknown. Other modulators such as matriptase-2 are important for regulating iron-induced signals that affect hepcidin production.

and type II. There are 4 type I receptors (ALK1, ALK2, ALK3, ALK6) and 3 type II receptors (ACTRIIA, ACTRIIB, BMPRII) for the BMP subfamily. For hepcidin regulation in response to iron, the BMP receptors involved are most likely ALK3, ALK2, and ACTRIIA<sup>38</sup> because liver-specific deletion of either *Alk3* or (to a lesser extent) *Alk2* causes iron overload in mice,<sup>39</sup> and because ACTRIIA is the predominant type II receptor expressed in human liver.<sup>38</sup> Upon BMP binding, the type II receptors phosphorylate the type I receptors, leading to the phosphorylation and activation of specific SMAD proteins. The receptor-regulated SMADs activated in response to BMPs binding to the signaling receptors are SMAD1, 5, and 8. These phosphorylated SMADs in turn bind SMAD4, and the SMAD complex translocates to the nucleus. In the nucleus, the SMAD complex binds to the specific promoter elements of the target genes, including hepcidin to regulate their transcription.<sup>36</sup> The importance of the BMP-SMAD pathway in hepcidin expression regulation has also been demonstrated in liver-specific *Smad4*<sup>-/-</sup> mice that also present with a massive iron overload similar to the phenotype of hepcidin knockout mice.<sup>25</sup>

To promote signal transduction under physiological conditions where BMP ligand levels are low, and to generate a specific signal in response to a subset of BMP ligands using a subset of BMP receptors, a BMP co-receptor is required. RGM proteins are the first known family of high-affinity co-receptors that are specific for BMPs. The RGM family is composed of 3 members in mammals; RGMc, also known as HJV, is the one expressed in the liver that is involved in the regulation of hepcidin expression in response to iron. This gene was identified as a hemochromatosis gene in 2004 by a positional cloning strategy for a locus associated with juvenile hemochromatosis in humans.<sup>24</sup> However, the link between the regulation of hepcidin by iron and the HJV/BMP-SMAD pathway was established 2 years later by Babitt et al,<sup>34</sup> when it was demonstrated that treatment of hepatoma cells with BMPs in combination with HJV overexpression results in the upregulation of hepcidin expression. Interestingly, by surface plasmon resonance, it has been demonstrated that among all RGMs, HJV has the highest affinity for BMP6.<sup>40</sup> Global *Hjv* knockout mice and human patients with *HJV* mutations do not present with any

other phenotype of iron-unrelated BMP functions,<sup>41,42</sup> suggesting that HJV has a role that is uniquely nonredundant for iron metabolism regulation. Although HJV is expressed in other tissues such as heart and muscle,<sup>24</sup> analysis of HJV tissue-specific knockout mice thus far suggests that HJV expression is predominantly important in hepatocytes.<sup>43,44</sup>

HJV can be released from cells as soluble HJV (sHJV) and detected in the serum of several species including humans.<sup>45,46</sup> In addition, it has been demonstrated that soluble recombinant HJV has the capacity to inhibit the BMP-SMAD signaling pathway and hepcidin expression,<sup>37</sup> but the source and function of endogenous sHJV are still poorly understood. In vitro, it has been shown that full-length sHJV can be released into the cell culture media by the action of endogenous phosphatidylinositol-specific phospholipase C (PI-PLC).<sup>47</sup> Furin, a pro-protein convertase, can also cleave HJV to generate a smaller fragment of sHJV.<sup>48,49</sup> Matriptase-2, encoded by the transmembrane serine protease *TMPRSS6*, has also been demonstrated to cleave sHJV in in vitro overexpression systems,<sup>50,51</sup> and this has been proposed as a mechanism by which mutations in *TMPRSS6* lead to hepcidin excess and iron-refractory iron deficiency anemia.

Neogenin, a protein of the deleted in colon cancer (DCC) family has been shown to be able to interact with HJV<sup>52</sup> and matriptase-2<sup>53</sup> and may play a role in iron metabolism because the hypomorphic neogenin mouse has iron accumulation in the liver.<sup>54</sup> Neogenin has been suggested in some studies,<sup>55</sup> but not others,<sup>38</sup> to affect HJV secretion and BMP-SMAD signaling. The exact role and function of neogenin in iron metabolism remains to be fully elucidated. Further support that the HJV/BMP pathway plays a role in hepcidin regulation comes from work showing that SMAD7, an inhibitory SMAD protein that mediates a negative feedback loop to both TGF- $\beta$  and BMP signaling, serves as an inhibitor of hepcidin expression.<sup>56</sup>

#### Tissue iron levels are sensed by the liver to regulate hepcidin

Liver BMP6-SMAD signaling is stimulated by iron administration<sup>57,58</sup> and, importantly, hepcidin regulation by iron is dependent on the BMP6-SMAD pathway because BMP6-SMAD pathway inhibitors

prevent hepcidin induction by iron.<sup>59</sup> There appear to be multiple mechanisms by which iron stimulates liver BMP6-SMAD signaling. First, there is a tight correlation between liver iron content and liver *Bmp6* mRNA expression in mice,<sup>57,60</sup> suggesting that hepatic iron loading regulates *Bmp6* mRNA expression. Although the mechanism for this regulation is still unclear, it does not appear to involve the hemochromatosis proteins HFE, Tfr2, or HJV because liver *Bmp6* mRNA is appropriately increased by iron loading in mice with mutations in these genes.<sup>61,62</sup> Interestingly, it was recently reported that although nonparenchymal cells and hepatocytes produce BMP6 mRNA under basal conditions, only nonparenchymal cells increase BMP6 mRNA expression in iron overload conditions, and this increase does not correlate with increased intracellular iron content of these nonparenchymal cells.<sup>63</sup> However, another study did show an increase in Bmp6 protein detected by immunohistochemistry in the hepatocytes of mice with iron overload owing to a high iron diet or *Hfe* inactivation.<sup>64</sup> Future studies will be needed to understand the exact mechanism by which hepatic iron loading leads to increased liver *Bmp6* mRNA expression.

However, as described by Ramos and al,<sup>65</sup> despite the absence of *Bmp6*, *Bmp6*<sup>-/-</sup> mice still have a partial increase in hepcidin expression in response to higher tissue iron loading, suggesting the involvement of others proteins beside Bmp6 in the regulation of hepcidin expression.

#### Circulating iron levels are sensed by the liver to regulate hepcidin

Hepcidin expression by hepatocytes is also stimulated by an increase in circulating iron via mechanisms that are distinct from hepcidin induction by tissue iron stores. Increases in serum iron, in the absence of hepatic iron loading, do not stimulate BMP6 expression,<sup>58</sup> but instead stimulate downstream SMAD1/5/8 phosphorylation by as yet unknown mechanisms. Although the signaling pathway is not fully characterized, it likely involves HFE, TFR2, and HJV.<sup>65</sup> The crucial role of HFE and TFR2 in hepcidin regulation by iron is illustrated by the hepcidin deficiency and iron overload that is induced by *HFE* and *TFR2* mutations in humans (hemochromatosis) and mice. In addition, *Hfe*<sup>-/-</sup> and *Tfr2*<sup>-/-</sup> mice fail to increase hepcidin expression in response to an increase of circulating Tf-iron but maintain the ability to increase hepcidin in response to an increase of hepatic iron, suggesting that the specific role of these 2 proteins to regulate hepcidin expression is mainly in response to circulating Tf-iron.<sup>65</sup> There is also evidence that the regulation of hepcidin expression by HFE and Tfr2 involves the SMAD pathway because humans and mice lacking *HFE*, *Tfr2*, or both, exhibit reduced SMAD1/5/8 phosphorylation relative to iron and BMP6 levels.<sup>58,61,66-68</sup> The exact link between Tf-iron, HFE, TFR2, and the SMAD pathway is still unclear. HFE has been shown to bind to TFR1,<sup>69</sup> and Tf-iron competes with HFE for binding to TFR1 because of overlapping binding sites on TFR1.<sup>70</sup> It has been suggested that in the presence of high Tf saturation, HFE is released from TFR1 and interacts with TFR2 (via domains that differ from those involved in HFE/TFR1 interaction<sup>71,72</sup>) to activate hepcidin transcription. An in vitro overexpression study has recently suggested that in response to Tf-iron, HFE and TFR2 could be part of the HJV-BMP receptor complex to regulate hepcidin.<sup>73</sup> Whether this interaction occurs in vivo in the liver has not yet been verified.

#### Iron deficiency and the regulation of hepcidin expression

Although iron stimulates hepcidin expression, iron deficiency inhibits hepcidin expression through a mechanism that may

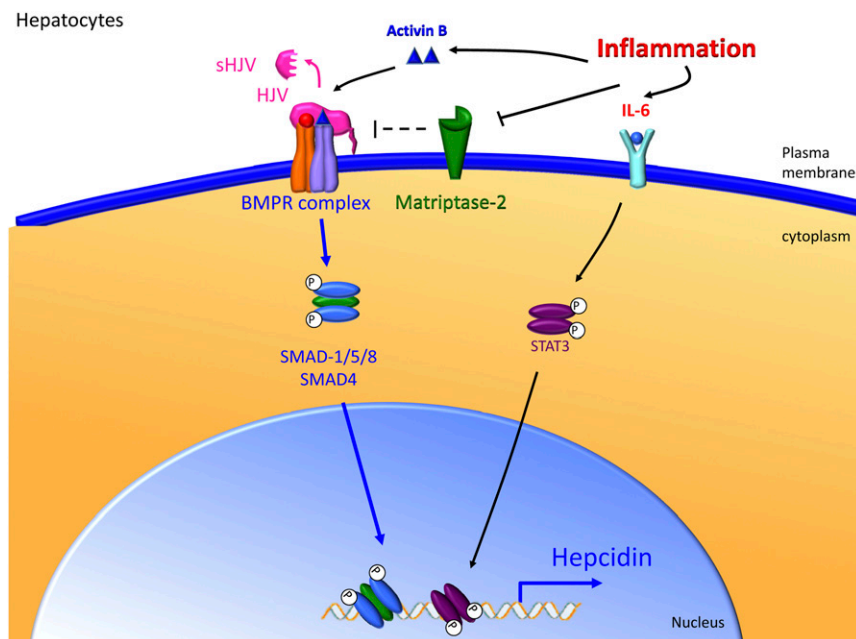
involve matriptase-2, as suggested by Zhang et al.<sup>74</sup> Encoded by the gene named *TMPRSS6*, matriptase-2 belongs to the family of type II transmembrane serine proteases. Matriptase-2 shares a number of structural features with the other members of the family: a short cytoplasmic domain, a type II transmembrane sequence, a central region with several modular structural domains including 2 CUB (complement factor C1s/C1r, urchin embryonic growth factor, bone morphogenetic protein) domains and 3 low-density lipoprotein-receptor tandem repeats, and, finally, a C-terminal catalytic domain with all the typical features of serine proteinases.<sup>75</sup>

Despite the identification of human matriptase-2 cDNA in 2002<sup>75</sup> and exploration of a suspected role as a tumor suppressor, its true physiological role remained unclear until 2008, when 3 laboratories demonstrated that matriptase-2 is essential for iron homeostasis.<sup>30-32</sup> Two studies in mice showed that the absence of matriptase-2 resulted in an inappropriately high level of hepcidin, leading to ferroportin degradation and the inhibition of iron absorption, and finally resulting in severe microcytic anemia.<sup>31,32</sup> A third study described that in humans, iron-refractory iron deficiency anemia is caused by mutations in the *TMPRSS6* gene, leading to a severe microcytic anemia with a high urinary hepcidin level.<sup>30</sup> Together these results indicate that matriptase-2 is a potent inhibitor of hepcidin expression in response to iron deficiency.

In parallel, genome-wide association studies identified common *TMPRSS6* single nucleotide polymorphisms (such as A736V) associated with iron status,<sup>76</sup> erythrocyte level,<sup>77</sup> and hemoglobin level,<sup>78</sup> suggesting that *TMPRSS6* is crucial in the control of iron homeostasis and normal erythropoiesis. The polymorphism A736V has also been shown to be a modifier of iron overload in hereditary hemochromatosis patients.<sup>79</sup> This result is also supported in mice models where *Tmprss6* is known to be a genetic modifier of the *Hfe*-hemochromatosis phenotype.<sup>80,81</sup>

It has been proposed by Silvestri et al that matriptase-2 could regulate hepcidin expression by cleaving HJV to decrease BMP-SMAD signaling. Indeed, the authors demonstrated that in vitro matriptase-2 does interact with HJV at the membrane cell surface and has the capacity to cleave HJV, preventing the induction of hepcidin expression during BMP2 treatment.<sup>50</sup> Moreover, interplay between matriptase-2 and the BMP-HJV-SMAD signaling pathway to regulate hepcidin expression is supported by double mutant mouse models *Hjv*<sup>-/-</sup> *Tmprss6*<sup>-/-</sup>,<sup>82,83</sup> and *Bmp6*<sup>-/-</sup> *Tmprss6*<sup>-/-</sup>,<sup>84</sup> which exhibit a low hepcidin mRNA level similar to that of the simple *Hjv*<sup>-/-</sup> or *Bmp6*<sup>-/-</sup> single knockout mice, suggesting at least a genetic interaction between *TMPRSS6* and the BMP6-HJV-SMAD pathway. However, 2 recent studies performed in vivo provide results that do not support a role for HJV in *TMPRSS6* action. Analysis of *Tmprss6*<sup>-/-</sup> mice reveals that compared with wild-type mice, HJV levels at the cell membrane surface are unexpectedly decreased<sup>85</sup> and soluble HJV in the serum is unchanged,<sup>46</sup> suggesting that HJV may not be the natural substrate of matriptase-2 in vivo. In addition, Gibert et al provided evidence in a zebrafish model suggesting that matriptase-2 could regulate hepcidin expression independently of HJV, because knockdown of matriptase-2 leads to high levels of the Hamp transcript in an *Hjv*-independent manner.<sup>86</sup> Thus, more work is needed to fully understand the mechanism of hepcidin regulation by matriptase-2.

Study of the regulation of matriptase-2 expression has recently demonstrated a link between matriptase-2 and hepcidin expression regulation in response to BMP6 and hepatic iron.<sup>87</sup> Injection of BMP6 or administration of an iron-enriched diet each induced an upregulation of *Tmprss6* expression in mice. In addition, the authors demonstrated that in vitro, this regulation is dependent on



**Figure 3. The liver responds to inflammatory signals to regulate hepcidin production.** Inflammatory signals such as IL-6 and Activin B stimulate the production of hepcidin by the liver. IL-6 increases phospho-STAT3 levels and subsequently stimulates transcription of hepcidin. Activin B may stimulate the BMP receptor complex directly. Matriptase-2 may also regulate inflammatory stimulation of hepcidin. Importantly, the proximal BMP-responsive element in the hepcidin promoter is necessary for the full effect of STAT-3 stimulation on hepcidin transcription.

expression of *Id1* mRNA, a transcript upregulated by the BMP-SMAD pathway and iron. Modulation of *TMPRSS6* expression could serve as a negative feedback inhibitor to avoid excessive hepcidin increases by iron to help maintain tight homeostatic balance of systemic iron levels.

Another mechanism to lower hepcidin expression in response to iron deprivation is to decrease the BMP6-SMAD pathway activity, as is suggested by the decrease of *Bmp6* mRNA observed in this condition.<sup>57</sup>

#### Inflammatory signals are sensed by the liver to regulate hepcidin

Inflammation caused by infection, autoimmune disease, or cancers stimulates the synthesis of many proinflammatory cytokines, such as interferon- $\gamma$ , interleukin-1, and interleukin-6 (IL-6), leading to a stimulation of hepcidin expression by the liver as well as white blood cells (Figure 3).<sup>88,89</sup> This induction of hepcidin production causes hypoferrremia and accumulation of iron in macrophages. This early host defense mechanism might be an advantage against specific infections such as malaria<sup>90</sup> by preventing the iron availability for pathogens. However, this innate immune response is also a disadvantage for the host in conditions of chronic inflammation. Indeed, the iron sequestration also limits the availability of iron for erythropoiesis and contributes to the anemia of chronic disease.<sup>91</sup>

The signaling pathways controlling hepcidin expression in hepatocytes under inflammatory condition are now well characterized. Inflammation stimulates IL-6 production that in turn activates the Janus kinase 2/signal transducer and activator of transcription 3 (STAT3) pathway, leading to the phosphorylation of STAT3. Translocation of phosphorylated STAT3 into the nucleus and binding to the canonical STAT3 binding site in the proximal hepcidin promoter results in upregulation of hepcidin mRNA expression.<sup>92,93</sup> Interestingly, interplay between the STAT3 pathway and the BMP-SMAD pathway is required to regulate hepcidin expression in response to inflammation. Thus mice lacking SMAD4 (common mediator of the BMP-SMAD pathway) lose the ability to induce hepcidin expression in response to IL-6 injection.<sup>25</sup> Moreover, in

cells, the presence of the proximal BMP response element, in addition to the STAT3 response element on the hepcidin promoter, is crucial to induce hepcidin expression during IL-6 treatment.<sup>35</sup> Indeed, increased liver SMAD1/5/8 phosphorylation has been observed in mice with anemia of inflammation in addition to increased STAT3 phosphorylation.<sup>94</sup> More recently, Besson-Fournier et al have suggested that the BMP/TGF- $\beta$  superfamily ligand participating in the SMAD1/5/8 pathway activation in response to LPS injection in mice is Activin B.<sup>95</sup>

Meynard et al have recently highlighted an additional layer of crosstalk between the BMP-SMAD pathway and the IL-6-STAT3 pathway. Those authors demonstrated that LPS or IL-6 injection in mice causes a downregulation of matriptase-2 expression, which may be part of the regulatory mechanism leading to the increase of hepcidin expression under inflammatory conditions.<sup>96</sup>

#### Erythropoiesis and hypoxia are sensed by the liver to regulate hepcidin

Because erythropoiesis depends on iron availability in the body, erythroid activity and iron absorption need to be coordinated. This coordination occurs through the downregulation of hepcidin expression in hepatocytes,<sup>97,98</sup> which results in an increase of iron absorption. The regulatory mechanism of hepcidin inhibition by erythropoiesis is still unclear, but it has recently been established that bone marrow cells are involved.<sup>99</sup> Growth differentiation factor 15 (GDF15) and twisted gastrulation-1 (TWSG1) are potential mediators of bone marrow signaling because their expression is stimulated under ineffective erythropoiesis conditions.<sup>100,101</sup> However, a nonredundant role for these proteins as erythroid regulators is excluded. Indeed, GDF15 is not required to balance iron homeostasis in response to blood loss,<sup>102</sup> and the involvement of TWSG1 has only been reported in thalassemia.<sup>101</sup> Further studies are still needed to identify the erythroid regulator of hepcidin expression and to characterize the molecular mechanism of its hepcidin regulation in hepatocytes.

Under hypoxia, hepcidin is also reduced. In mice, stabilization of HIF1 and HIF2 (hypoxia-inducible factors) both inhibit hepcidin

expression.<sup>103,104</sup> A direct regulation of hepcidin expression by the binding of HIF on hepcidin promoter was suggested by a study published by Peyssonau et al,<sup>103</sup> but has not been confirmed by subsequent studies.<sup>105</sup> In vivo, hypoxia stimulates erythropoietin production that in turn could decrease hepcidin expression. This hypothesis is supported by recent findings showing that hepatic HIF2 is not directly involved in hepcidin repression but contributes to the repression of hepcidin through erythropoietin-mediated increased erythropoiesis.<sup>104</sup>

### Signaling pathways that downregulate hepcidin in the liver

Several factors have been shown to downregulate hepcidin expression, in particular epidermal growth factor (EGF), hepatocyte growth factor,<sup>106</sup> tumor necrosis factor- $\alpha$ ,<sup>107</sup> and estrogen.<sup>108</sup> The role, if any, of these pathways in iron homeostasis remains unknown. In addition, recently, 2 laboratories showed independently that testosterone downregulates hepcidin,<sup>109,110</sup> explaining the difference seen in tissue iron loading between male and female animals. Latour et al also demonstrated that the hepcidin regulation by testosterone was mediated through EGFR. Alcohol is also an inhibitor of hepcidin expression. This inhibition is dependent on both HIF-1 $\alpha$  and HIF-2 $\alpha$ , which leads to a decrease of C/EBP $\alpha$ ,<sup>111</sup> a transcription factor critical in maintaining basal hepcidin expression.<sup>112</sup> In the same manner, it is now well established that hepatitis C infection results in a decrease of hepcidin expression and that this regulation occurs through a destabilization of C/EBP $\alpha$  and STAT3 on the hepcidin promoter.<sup>113</sup>

## Conclusion

To provide the appropriate amount of iron available for erythropoiesis and cell metabolism, but to prevent toxic iron overload, hepcidin expression by the liver must be tightly regulated. The liver is the central organ that senses a variety of signals related to iron, oxygenation, and erythropoiesis for the purpose of regulating hepcidin expression. HJV/BMP/SMAD and IL6/STAT3 seem to be the main pathways involved in the regulation of hepcidin expression in response to multiple stimuli. However, the important pathway modulating hepcidin in response to erythropoiesis and hypoxia in hepatocytes is still unknown and needs to be elucidated. The liver interprets and integrates these disparate signals to regulate the production of hepcidin mRNA and subsequently systemic iron homeostasis.

The identification of the liver as a central regulator of iron homeostasis and especially the discovery of hepcidin and its regulation were important steps for the development of new promising therapeutic strategies for the management of iron disorders. All the strategies for the treatment of anemia of chronic disease or anemia of inflammation have been recently well summarized in the review by Sun et al,<sup>91</sup> such as antihepcidin antibodies, short interference

RNA and antisense oligonucleotides against hepcidin, BMP6-HJV-SMAD pathway inhibitors, and IL-6 pathway inhibitors.

For iron disorders associated with an iron overload induced by a low hepcidin state, such as hemochromatosis or  $\beta$ -thalassemia, new therapeutic strategies are also being studied.<sup>114,115</sup> Minihepcidins, small druglike hepcidin agonists, are a new promising approach for the prevention and treatment of iron overload that have been tested in hepcidin-deficient mice. In this mouse model, administration of large doses of minihepcidins could prevent iron overload.

With lower doses, there is a more moderate effect and partial redistribution of iron from the liver to the spleen.<sup>116</sup> Another strategy currently under study is the activation of the hepcidin pathway by the inhibition of Tmprss6 expression to inhibit hepcidin expression. Indeed, 2 groups have recently demonstrated that inhibition of *Tmprss6* expression by injection of silencing RNA formulated in lipid nanoparticles<sup>117</sup> or antisense oligonucleotides<sup>118</sup> into *Hfe*<sup>-/-</sup> mice and *HBB(th3/+)* mice achieved knockdown of *Tmprss6* expression, leading to an elevation in hepcidin level. These therapies were both efficient in reducing liver iron concentrations in both mouse models.

Because increasing hepcidin does not cause the removal or excretion of iron, these potential new treatments are not likely to be sufficient to replace current treatments (ie, iron chelation and phlebotomy) in patients. They may rather be considered as an additional treatment to be used in combination with current strategies to decrease the frequency of iron chelation or phlebotomy, which have undesirable side effects.

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## Authorship

Contribution: D.M. wrote the initial draft of the review and generated the figures; J.L.B. edited and helped write the review; and H.Y.L. edited and wrote key sections of the review.

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