CHAPTER FIVE

# Regulators of hepcidin expression

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## Contents



## Abstract

Iron, an essential nutrient, is required for many biological processes but is also toxic in excess. The lack of a mechanism to excrete excess iron makes it crucial for the body to regulate the amount of iron absorbed from the diet. This regulation is mediated by the hepatic hormone hepcidin. Hepcidin also controls iron release from macrophages that recycle iron and from hepatocytes that store iron. Hepcidin binds to the only known iron export protein, ferroportin, inducing its internalization and degradation and thus limiting the amount of iron released into the plasma. Important regulators of hepcidin, and

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therefore of systemic iron homeostasis, include plasma iron concentrations, body iron stores, infection and inflammation, hypoxia and erythropoiesis, and, to a lesser extent, testosterone. Dysregulation of hepcidin production contributes to the pathogenesis of many iron disorders: hepcidin deficiency causes iron overload in hereditary hemochromatosis and non-transfused β-thalassemia, whereas overproduction of hepcidin is associated with iron-restricted anemias seen in patients with chronic inflammatory diseases and inherited iron-refractory iron-deficiency anemia. The present review summarizes our current understanding of the molecular mechanisms and signaling pathways contributing to hepcidin regulation by these factors and highlights the issues that still need clarification.

## 1. Introduction

Iron is an essential trace metal involved in oxygen transport, cellular metabolism, DNA synthesis, innate immunity, growth, and development. By virtue of its ability to accept or donate electrons, it is crucial for many of the biologic reactions carried out by living systems. This same characteristic, however, allows free iron in solution to form highly reactive oxygen species that can oxidize lipids, DNA, and proteins, and lead to cell damage. Major sources of iron include recycling of iron from senescent erythrocytes by macrophages, intestinal iron absorption, and release of stored iron from hepatocytes. As there is no physiological means for excreting iron in mammals, systemic iron homeostasis must be maintained by tight regulation of intestinal iron absorption, as well as macrophage and hepatocyte iron release (Ganz, 2013).

Iron is exported from duodenal enterocytes, macrophages, and hepatocytes to the plasma compartment by the sole known cellular iron exporter, ferroportin (Donovan et al., 2000; McKie et al., 1999). The cellular concentrations of ferroportin are regulated by hepcidin, a 25 amino-acid peptide hormone produced predominantly by hepatocytes. Hepcidin blocks iron export into plasma by binding to ferroportin and inducing its endocytosis and proteolysis (Nemeth, Tuttle, et al., 2004), thereby causing iron sequestration in cells involved in dietary iron absorption and iron storage (Fig. 1). Hepcidin transcription in the liver is controlled by a complex interplay of signals. Most notably, it is increased by plasma and liver iron as a feedback mechanism to maintain stable body iron levels, increased by inflammation as a host defense mechanism to limit extracellular iron availability to microbes, and decreased by erythroid activity to ensure iron supply for erythropoiesis.

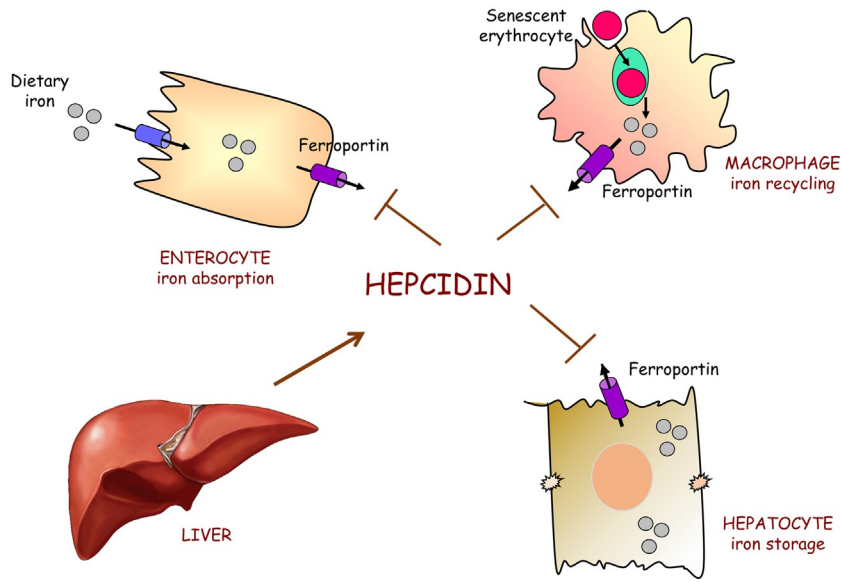


**Fig. 1** Hepcidin is the master regulator of systemic iron homeostasis. It is produced predominantly by the liver and blocks iron export from enterocytes, macrophages, and hepatocytes by the iron exporter ferroportin.

Although impressive advances in our understanding of how hepcidin is regulated by these various stimuli have been made since its discovery in 2001 (Park, Valore, Waring, & Ganz, 2001; Pigeon et al., 2001), a number of unresolved questions remain, and new discoveries keep challenging models thought to be established. This review focuses on the main issues that still require clarification.

## 2. Hepcidin regulation by plasma and liver iron

Hepcidin is indispensable to maintain iron homeostasis. Indeed, mice lacking hepcidin have massive iron overload (Lesbordes-Brion et al., 2006; Nicolas et al., 2001), whereas overexpression of hepcidin leads to severe iron deficiency (Nicolas, Bennoun, et al., 2002; Roy et al., 2007). In humans, mutations in the genes encoding HFE (Feder et al., 1996), an atypical major histocompatibility complex class I-like molecule that heterodimerizes with β2-microglobulin, transferrin receptor 2 (TFR2) (Camaschella et al., 2000), hemojuvelin (HJV) (Papanikolaou et al., 2004), as well as hepcidin itself (Roetto et al., 2003), lead to hereditary hemochromatosis, a variably severe iron overload disorder. Common to these subtypes of hemochromatosis is

an inappropriately low expression of hepcidin given the iron load, which suggested that HFE, TFR2, and HJV were upstream regulators of hepcidin. Conversely, mutations in the *TMPRSS6* gene encoding matriptase-2 lead to elevated hepcidin levels and iron-refractory iron-deficiency anemia (IRIDA) (Finberg et al., 2008), which implied that matriptase-2 was a negative regulator of hepcidin. The study of these iron-associated disorders and the corresponding mouse models has greatly increased our understanding of how iron homeostasis and hepcidin are regulated and the signaling pathways involved.

## 2.1 Hepcidin expression is modulated by body iron status via the BMP/SMAD signaling pathway

Bone morphogenetic proteins (BMPs) are a subset of the transforming growth factor beta (TGF-β) superfamily of ligands. These cell-signaling molecules form homodimers as well as heterodimers, with various combinations differing in their potency to activate the signal. These ligands bind two type I and two type II serine-threonine kinase receptors. The type II receptors possess constitutive serine-threonine kinase activity and, upon BMP binding, activate the type I receptor kinases by phosphorylation of a glycine-serine-rich domain. Phosphorylation of the type I receptors favors the recruitment and subsequent phosphorylation of its substrate, the intracellular SMADs 1, 5, and 8. The receptor-activated SMADs then form heteromeric complexes with the common mediator SMAD4, and translocate to the nucleus where they regulate the transcription of specific targets (Anderson & Frazer, 2006; Derynck & Zhang, 2003).

The link between the SMAD pathway and iron homeostasis was discovered when it was shown that mice with hepatocyte-specific deletion of Smad4 develop a severe iron overload phenotype in association with markedly decreased hepcidin expression (Wang et al., 2005). Additional evidence implicating the BMP/SMAD pathway in iron homeostasis came when HJV, the protein mutated in the majority of cases of juvenile hemochromatosis, was shown to act as a BMP coreceptor facilitating the activation of the BMP receptor complex (Babitt et al., 2005, 2006). Whereas stimulation of BMP signaling by HJV was paralleled by an increase in hepcidin expression, injection of a soluble HJV fusion construct (HJV.Fc) to mice decreased Smad1/5/8 phosphorylation and hepcidin mRNA levels, indicative of decreased BMP signaling (Babitt et al., 2007; Nili, Shinde, & Rotwein, 2010).

## 2.2 BMP6 and BMP2 are the predominant ligands responsible for hepcidin regulation in vivo

Whereas many ligands of the BMP subfamily could positively regulate hepcidin *in vitro* (Babitt et al., 2006; Truksa, Peng, Lee, & Beutler, 2006), only Bmp6 and, to a lesser extent, Bmp2 were increased with tissue iron loading and seemed to have a role *in vivo* (Kautz et al., 2008). Although the significant correlation between *Bmp6* liver expression and body iron stores in mice strongly suggested a role for BMP6 in particular in the regulation of hepcidin by iron stores (Kautz et al., 2008), the most compelling evidence that BMP6 was the predominant BMP ligand signaling the SMAD-mediated upregulation of hepcidin in response to iron was provided by the knockout of *Bmp6* in mice. These mice indeed had very low hepcidin expression and severe iron overload (Andriopoulos et al., 2009; Meynard et al., 2009). Although hepcidin is expressed almost exclusively by hepatocytes, BMP6 is produced predominantly by the non-parenchymal liver sinusoidal endothelial cells (LSECs) (Enns et al., 2013; Rausa et al., 2015). Loss of Bmp6 in LSECs recapitulated the hemochromatosis phenotype of global *Bmp6* knockout mice, whereas hepatocyte and macrophage *Bmp6* conditional knockout mice exhibited no iron phenotype (Canali, Zumbrennen-Bullough, et al., 2017), supporting a model in which BMP6 has paracrine actions on hepatocyte hemojuvelin to regulate hepcidin transcription and maintain systemic iron homeostasis. Chronic, but not acute, iron administration is associated with increased *Bmp6* mRNA levels (Corradini et al., 2011) and iron overload leads to an increase in the expression of *Bmp6* in all the animal models of genetic hemochromatosis studied so far (Corradini et al., 2011; Kautz et al., 2008, 2009; Zhang, Gao, Koeberl, & Enns, 2010). Some evidence suggests that iron-containing ferritin might serve as a signaling molecule in the regulation of *Bmp6* expression in LSECs (Feng, Migas, Waheed, Britton, & Fleming, 2012).

The massive iron overload in *Bmp6* knockout mice indicates that Bmp6 is critical for iron homeostasis and that it is functionally non-redundant with other members of the Bmp subfamily. It was then surprising to discover that mice with a conditional ablation of Bmp2 in endothelial cells also exhibit hepcidin deficiency, serum iron overload, and tissue iron loading in liver, pancreas and heart (Canali, Wang, Zumbrennen-Bullough, Bayer, & Babitt, 2017; Koch et al., 2017). Obviously, BMP2 and BMP6 functions are non-redundant, as neither of the two ligands compensates for the lack of the other in knockout mice. Further work will be needed to determine

whether, in the liver, BMP2/6 heterodimeric ligands are more effective at activating hepcidin than BMP2 or BMP6 homodimers, which would provide an explanation for the present data. Notably, in the past, BMP2/6 heterodimers have already been shown to possess a higher affinity to both type I and II receptors and induce higher levels of SMAD1/5 phosphorylation than their homodimeric counterparts (Isaacs et al., 2010; Valera, Isaacs, Kawakami, Izpisua Belmonte, & Choe, 2010).

## 2.3 BMP/SMAD signaling requires specific BMP receptors and the coreceptor hemojuvelin, but is attenuated by matriptase-2

BMP type I receptors that are expressed on hepatocytes and regulate hepcidin expression include ALK2 (ACVR1) and ALK3 (BMPR1A). Hepatocyte-specific deletion of either receptor results in iron overload, although the iron loading phenotype in the *Alk3* knockout mice is more severe. Indeed, Alk3 deficiency is associated with a nearly complete ablation of basal BMP signaling and hepcidin expression (Steinbicker, Bartnikas, et al., 2011). The two BMP type II receptors expressed on hepatocytes are ACTR2A and BMPR2. Genetic mouse models and primary hepatocytes were used to determine that both receptors are involved in BMP6-induced hepcidin expression. However, the two receptors have redundant roles in regulating hepcidin, and only deficiency of both ActR2a and liver Bmpr2 prevented iron-induced hepcidin expression, which resulted in severe iron overload (Mayeur, Leyton, Kolodziej, Yu, & Bloch, 2014).

The recently solved crystal structure of HJV in complex with BMP2 (Healey et al., 2015) has provided insights into how this coreceptor might modulate the BMP signaling cascade. The N-terminal domain of HJV mimics the structure of BMP type I receptors and HJV competes with BMP type I receptor for BMP2 binding (Healey et al., 2015). Therefore, BMPs are probably recruited to the membrane through their interaction with HJV. These complexes can then associate with BMP type II receptors and undergo endocytosis (Mueller, 2015). Owing to the acidification of endosomes and the pH dependence of the HJV–BMP interaction (Healey et al., 2015), HJV might dissociate from the complex and be replaced by BMP type I receptors, thus leading to activation and signaling from this cell compartment. Although very exciting, this model still needs confirmation by functional studies. It should be kept in mind that activation of the SMAD pathway is also possible through preformed BMP receptor complexes (Nohe et al., 2002). In that case, BMPs bind preformed but signaling-inactive

heteromeric BMP type I–type II receptor complexes. This results in their activation and triggers the SMAD pathway without requiring a coreceptor such as HJV. However, given the low hepcidin levels in patients and mice with mutated HJV, these complexes on their own are not likely to play a preponderant role in activating hepcidin transcription.

Matriptase-2, a liver-expressed transmembrane serine protease, is an additional regulator of iron homeostasis. In cultured cells, matriptase-2 cleaves HJV, physiologically attenuating the BMP-SMAD signaling and reducing hepcidin expression (Silvestri et al., 2008). Genetic inactivation of *Tmprss6* preserved the iron overload of *Hjv* knockout mice (Finberg, Whittlesey, Fleming, & Andrews, 2010), which is consistent with HJV being a substrate for matriptase-2. However, Hjv was not found to be increased in the liver of *Tmprss6* knockout mice as would be expected if it was cleaved by matriptase-2 (Frydlova et al., 2016; Krijt, Fujikura, Ramsay, Velasco, & Necas, 2011) and further studies are needed to clearly demonstrate that HJV is the target of matriptase-2 *in vivo* and possibly identify other substrates.

## 2.4 HFE and TfR2 are required for appropriate hepcidin regulation in response to an acute iron challenge

Regulation of BMP/SMAD pathway signaling by iron sensors is complex and not completely understood. Studies in transient transfection cell culture systems have indicated that HFE interacts not only with the iron uptake receptor TFR1 but also with TFR2, a homolog of TFR1 expressed predominantly in the liver (Trinder & Baker, 2003), and that an HFE–TFR2 complex is favored over the HFE–TFR1 interaction when high levels of diferric transferrin are present (Goswami & Andrews, 2006). On this basis, it was hypothesized that competition between TFR1 and TFR2 for HFE binding allows hepcidin to be regulated in response to the circulating amount of diferric transferrin (Gao et al., 2009; Goswami & Andrews, 2006; Schmidt, Toran, Giannetti, Bjorkman, & Andrews, 2008), but this issue continues to be discussed (Rishi, Crampton, Wallace, & Subramaniam, 2013; Schmidt & Fleming, 2012; Wallace et al., 2009).

Co-immunoprecipitation analyses have provided evidence that HFE, TFR2, and HJV form a multiprotein membrane complex on the surface of hepatocytes (D'Alessio, Hentze, & Muckenthaler, 2012), which would connect HFE and TFR2 to BMP/SMAD signaling. The observation that the phenotype of *Hjv* knockout females is not affected by the loss of Hfe or Tfr2 (Gutschow et al., 2015; Latour et al., 2016) is consistent with the

assumption that, *in vivo*, Hfe, Tfr2 and Hjv are involved in the same molecular pathway regulating hepcidin expression. Examination of the effects of acute iron loading on hepcidin expression in mice with mutations in *Hfe*, *Tfr2*, *Hjv*, or *Bmp6* (Ramos et al., 2011) showed that each of these proteins was required for appropriate hepcidin regulation in response to an acute iron challenge, suggesting that acute increases in holotransferrin concentrations transmitted through HFE, TfR2, and HJV augment BMP receptor sensitivity to BMPs. Interestingly, Hfe and Tfr2 are dispensable for an appropriate response to chronic iron loading which is mediated solely by Hjv and Bmp6 (Ramos et al., 2011). A model for hepcidin regulation by acute and chronic iron loading, which takes into account the latest developments, is shown in Fig. 2.

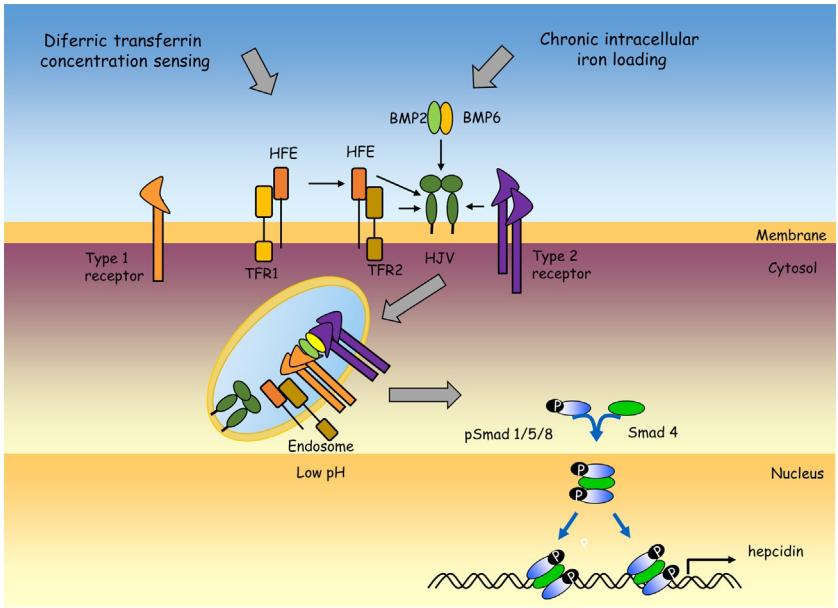


**Fig. 2** Proposed model for the regulation of hepcidin by chronic and acute iron loading. The ligands BMP2 and BMP6, whose production by liver sinusoidal endothelial cells is induced by chronic iron loading, are recruited to the hepatocyte membrane by hemojuvelin (HJV), most likely in the form of heterodimers. After association with the BMP type II receptors, the complexes undergo endocytosis into endosomal compartments where HJV is replaced by BMP type I receptors, leading to Smad1/5/8 activation. Increases in holotransferrin concentrations are signaled by HFE and TFR2 which, by associating with hemojuvelin, augment BMP receptor sensitivity to BMPs.

## 2.5 Lessons from double knockout animals

Over the past few years, almost all pairs of knockout mice have been intercrossed (Rishi & Subramaniam, 2017), allowing comparison of the different models between them and better delineation of the role of each partner in the BMP/SMAD pathway signaling to hepcidin. The observation that the loss of Hfe or Tfr2 did not affect the phenotype of *Hjv* knockout females but considerably worsened that of *Bmp6* knockout females (Latour et al., 2016) led us to speculate that BMP2, HJV, HFE and TFR2 could be involved in a pathway of hepcidin regulation sensing extracellular diferric transferrin concentration, and BMP6 in a pathway sensing intracellular iron (Latour et al., 2016). Further repression of hepcidin and aggravation of the phenotype in animals where both Bmp6 and Hjv were deleted, compared with single knockout animals (Latour et al., 2017), would fit with such a regulatory mechanism. However, taken individually, each of these potential pathways is not very effective at maintaining appropriate levels of hepcidin, which is not exactly in favor of two independent ways of signaling as outlined above.

Rather, there could be only one mega-complex linking up all the different players and capable of regulating hepcidin in response to both diferric transferrin and intracellular iron. In this system, the ligands BMP2 and BMP6 would most likely be recruited to the membrane in the form of heterodimers through their interaction with HJV. When one actor of this signaling mega-complex is lacking as observed in the different mouse models examined, alternative pathways, although less effective to activate Smad1/5/8, succeed in maintaining hepcidin to a level avoiding extrahepatic iron accumulation in females. These can be initiated by interaction of HJV with BMP2 homodimers when BMP6 is missing, or by direct binding of BMP2/6 heterodimers to preformed BMP type I/type II receptor complexes when HJV is missing. The existence of these alternative pathways could be justified by the critical importance of hepcidin in the maintenance of stable body iron levels. Their suppression, as in Bmp6/Hjv double knockout animals, leads to a greater repression of hepcidin in mice of both sexes and substantial exacerbation of the extrahepatic iron overload phenotype in females. Interestingly, whereas genetic inactivation of *Tmprss6* preserved the iron overload of *Hjv* knockout mice (Finberg et al., 2010), it almost completely normalized the phenotype of *Bmp6* knockout mice (Nai et al., 2016), which could be explained by the boosting of the alternative pathway initiated by interaction of HJV with BMP2 in the absence of matriptase-2.

The deletion of both *Hfe* and *Tfr2* in mice also results in a more severe iron phenotype than does deletion of either gene alone (Schmidt & Fleming, 2012; Wallace et al., 2009), and this has long been considered an argument against the transmission of acute increases in holotransferrin concentrations by a complex formed of HFE and TFR2. However, it is well possible that, as suggested above, whereas optimal signaling requires such a complex, each partner separately can still convey some kind of signal to avoid complete loss of sensing of the circulating amount of iron.

## 3. Hepcidin regulation by inflammation

Iron is an essential nutrient for nearly all infectious microorganisms, and host defense mechanisms target this dependence to deprive invading extracellular microbes of iron (Ganz, 2018). Within hours after infection or other inflammatory stimuli, plasma iron concentrations decrease. This response, referred to as hypoferremia of inflammation, may impair erythropoiesis and develop into anemia if the inflammatory condition persists. Administration of lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, or of turpentine, a product of resin distillation, leads to systemic or local inflammation, respectively. These two models have been widely used to decipher the mechanisms of hypoferremia of inflammation. Interestingly, an increase in hepcidin was observed in mice treated with LPS (Pigeon et al., 2001) or turpentine (Nicolas, Chauvet, et al., 2002), and in humans injected with LPS (Kemna, Pickkers, Nemeth, van der Hoeven, & Swinkels, 2005). Increased hepcidin downregulates ferroportin (Nemeth, Tuttle, et al., 2004) and thereby decreases iron influx from enterocytes, macrophages, and hepatocytes into the plasma compartment. Iron recycled from senescent erythrocytes is then retained in macrophages and iron absorption is decreased. The increase in hepcidin was thus thought to be a key mediator of hypoferremia of inflammation, especially since hypoferremia is absent in hepcidin knockout mice (Gardenghi et al., 2014; Kim et al., 2014; Nicolas, Chauvet, et al., 2002).

### 3.1 IL-6 is an important mediator of hepcidin induction by inflammation

Induction of hepcidin by inflammation can be attributed, at least in part, to the inflammatory cytokine IL-6 which is the major regulator of the acute phase response in hepatocytes. IL-6 treatment stimulates hepcidin expression in isolated hepatocytes and in hepatoma cell lines (Nemeth, Rivera, et al., 2004).

Administration of IL-6 to mice (Mayeur, Lohmeyer, et al., 2014) and human volunteers (Nemeth, Rivera, et al., 2004) stimulates hepcidin production and results in hypoferremia. Furthermore, although mice lacking Il-6 still respond to LPS (Besson-Fournier et al., 2012; Lee, Peng, Gelbart, & Beutler, 2004), they fail to induce hepcidin and do not become anemic in response to turpentine (Nemeth, Rivera, et al., 2004).

Upon an inflammatory stimulus, IL-6 is released by many types of cells, including Kupffer cells, and activates a hexameric cell-surface signaling assembly composed of IL-6, the non-signaling IL-6 receptor, and a gp130 subunit capable of signal transduction. IL-6 binding activates the receptor-associated Janus kinases (JAKs), and specific tyrosine residues in the intracellular domains of gp130 become phosphorylated. These phospho-tyrosine residues provide docking sites for signaling molecules, including the transcription factor STAT3. STAT3 itself is phosphorylated on tyrosine residues that facilitate nuclear translocation of activated STATs and the binding of STAT3 dimers to specific DNA residues on target promoters. Studies on hepatoma cell lines have shown that STAT3 binds to a conserved sequence on the hepcidin promoter and that this sequence is necessary and sufficient for IL-6 responsiveness (Verga Falzacappa et al., 2007; Wrighting & Andrews, 2006), giving rise to the so far consensual mechanism of hepcidin regulation by inflammation through the JAK/STAT3 signaling pathway. In addition to IL-6, a wide variety of other cytokines can activate STAT3 in hepatocytes, most notably oncostatin M, the leukemia inhibitory factor LIF, and IL-22 (Wang, Lafdil, Kong, & Gao, 2011). Interestingly, these cytokines have all been shown to induce hepcidin *via* the JAK/STAT3 pathway (Armitage et al., 2011; Chung et al., 2010; Kanda et al., 2009; Smith et al., 2013; Wallace & Subramaniam, 2015) and probably explain why, although delayed in time, Stat3 can still be activated by LPS in the liver of *Il-6* knockout mice (Besson-Fournier et al., 2012).

## 3.2 In vivo, the kinetics of hepcidin induction does not coincide with that of Stat3 activation

Surprisingly, kinetic curves of Stat3 activation *versus* hepcidin induction in the mouse liver have never been compared. At the beginning of 2000s, immunologists have shown that the IL-6-signaling pathway is rapidly turned off by the suppressor of cytokine signaling SOCS-3, an immediate early gene whose transcripts are present at very low levels but are quickly induced by activated STAT3 upon stimulation by IL-6 (Croker et al., 2003; Kubo, Hanada, & Yoshimura, 2003). In the regenerating liver following partial hepatectomy, SOCS-3 mRNA is also promptly induced by IL-6 and the

SOCS-3 protein participates in a rapid feedback loop to inhibit STAT3 activity (Campbell et al., 2001). Our personal data show that *Socs-3* mRNA, a direct target of activated Stat3, is induced in a robust manner in the mouse liver already 1 h after LPS treatment, at a time-point where hepcidin is not induced (Fig. 3). This correlates with a massive translocation of activated Stat3 in the nuclei of the hepatocytes. As expected, Socs3 rapidly inhibits Stat3 phosphorylation which is strongly reduced in the nuclei of hepatocytes 4–6 h after LPS administration, when the peak of hepcidin induction is observed. *Socs3* mRNA is then stabilized by activation of $p38^{MAPK}$ (Ehlting et al., 2007) and sustained up to 8 h after injection, preventing further activation of Stat3. These opposing trends of Stat3 activation and hepcidin induction challenge the dogma that, *in vivo*, hepcidin stimulation by IL-6 is due to the binding of activated Stat3 to the hepcidin promoter. Interestingly, translocation of activated Stat3 to the hepatocyte nuclei and induction of *Socs3* mRNA are delayed and reduced in LPS-challenged *Il6*

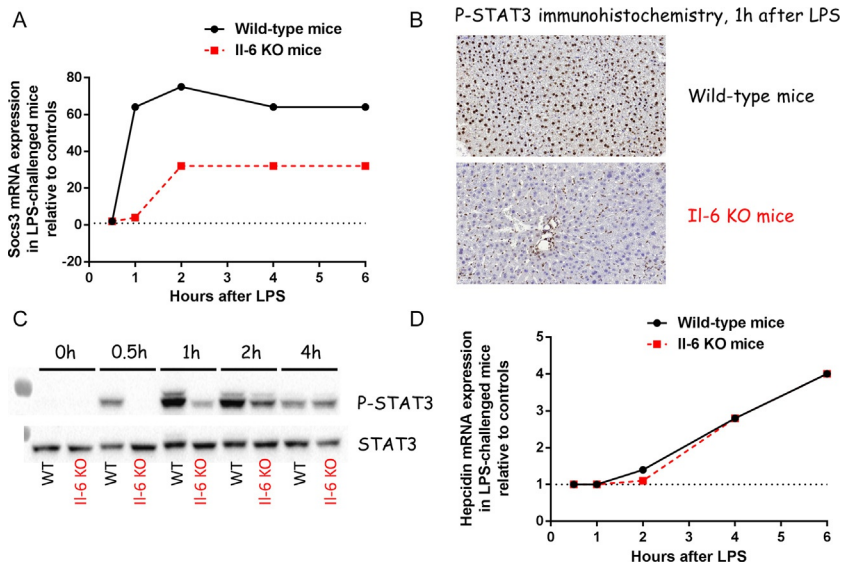


**Fig. 3** In vivo, the kinetics of hepcidin induction does not coincide with that of Stat3. In wild-type mice, *Socs3* mRNA, a direct target of activated Stat3, is strongly induced already 1 h after LPS administration (A). This correlates with massive translocation of activated Stat3 in the nuclei of hepatocytes at that time point (B). Stat3 activation is then rapidly inhibited by Socs3 (C) and is much lower when the peak of hepcidin induction is observed (D). In *Il-6* knockout mice, although translocation of activated Stat3 is delayed (B) and phosphorylation of Stat3 reduced (C) compared with wild-type mice, hepcidin is induced to levels similar to those in wild-type mice (D).

knockout mice, but not completely abolished, uncovering the actions of other cytokines of the IL-6 family or IL-22. However, despite these differences, the level of hepcidin induction is similar to that in wild-type mice, further challenging the consensual mechanism of hepcidin regulation by inflammation.

### 3.3 In mouse primary hepatocytes, hepcidin is cooperatively regulated by STAT3 and NF-KB

The systemic inflammatory response to pathogen infection or local tissue injury is also mediated by interleukin 1. IL-1 potently activates the nuclear factor κB (NF-κB) family of transcription factors. The nuclear import and activation of NF-κB is achieved by cytokine-dependent phosphorylation and subsequent degradation of the inhibitory protein IκB. Interestingly, IL-1 was shown to stimulate hepcidin in primary hepatocytes from both wild-type and *Il-6* knockout mice (Lee, Peng, Gelbart, Wang, & Beutler, 2005), suggesting that IL-1 may play an IL-6-independent role in the upregulation of hepcidin by inflammation. STAT3 and NF-κB are engaged in extensive crosstalks in the liver (He & Karin, 2011) and it has been reported that, whereas neither IL-6 nor IL-1 alone is a potent inducer of the hepcidin gene in primary hepatocytes, hepcidin expression is substantially enhanced upon combined stimulation with IL-6 and IL-1 (Bode, Albrecht, Haussinger, Heinrich, & Schaper, 2012; Goldstein, Paakinaho, Baek, Sung, & Hager, 2017). Analysis of available chromatin immunoprecipitation sequencing (ChIP-seq) data (Goldstein et al., 2017) shows that the two cytokines alter the hepatocyte chromatin at the hepcidin locus (Fig. 4). H3K27ac, a modification of histone H3 (acetylation of the lysine residue in position 27), is a well-established marker of active enhancer. Interestingly, the H3K27ac signal is much higher when mouse primary hepatocytes are treated with both IL-1 and IL-6 rather than with single treatments, showing that IL-1 and IL-6 synergistically induce hepcidin gene transcription. Furthermore, enhancer activity induced by IL-1 is negated in the presence of a dominant negative peptide inhibiting NF-κB activity, indicating that NF-κB is responsible for IL-1-mediated expression of hepcidin. Synergistic induction of hepcidin by dual treatment also requires NF-κB signaling. Altogether, these data show a clear synergy between IL-6/STAT3 and IL-1/NF-κB signaling pathways in the induction of hepcidin in mouse primary hepatocytes. Whether a role for NF-κB signaling in hepcidin induction is also important *in vivo* remains to be established but could explain why the level of hepcidin is significantly higher in LPS than in IL-6-treated mice.

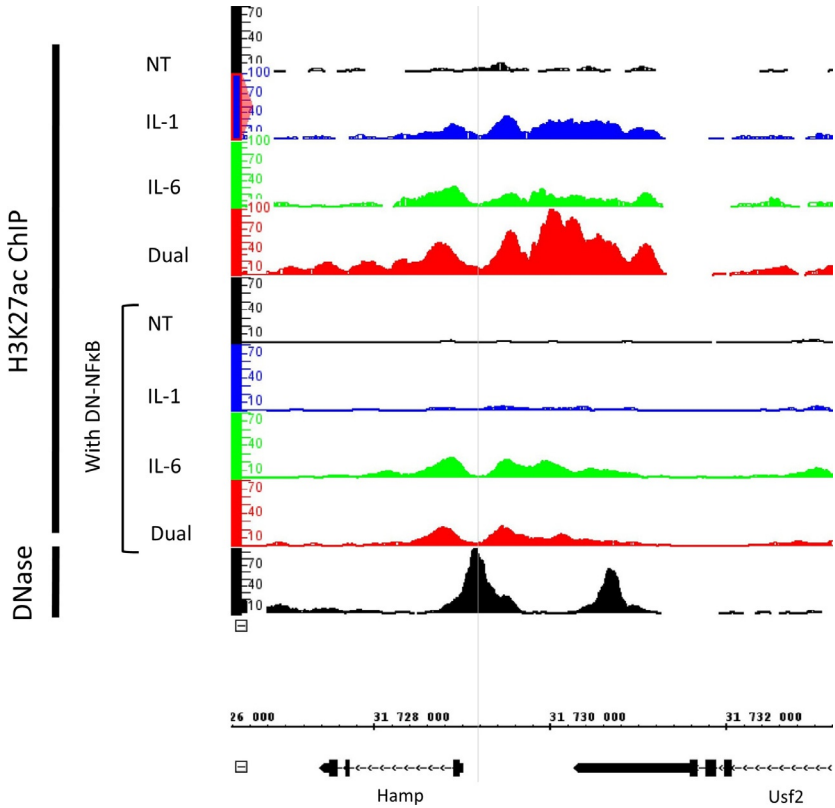


**Fig. 4** Genome browser tracks showing the changes in histone H3K27ac signal (a marker of enhancer activity) induced by cytokine treatment of mouse primary hepatocytes. The H3K27ac signal at the hepcidin locus is much higher when primary hepatocytes are treated with both cytokines (DUAL) than with each one separately, suggesting that IL-1 and IL-6 synergistically activate hepcidin transcription. Enhancer activity induced by IL-1 is negated in the presence of a dominant negative peptide inhibiting NF-κB activity (DN-NFκB), indicating that NF-κB is responsible for IL-1-mediated hepcidin expression. Synergistic induction of hepcidin by dual treatment also requires NF-κB signaling. Publically available H3K27ac ChIP-seq data were obtained from GSE96770, and DNAse hypersensitive sites from GSE72087.

Importantly, the observed cooperation between STAT3 and NF-κB signaling does not mean that activated STAT3 or NF-κB dimers have to bind directly to the hepcidin promoter. Rather, they could bind to and induce another target, preferably a transcription factor, which would in turn stimulate hepcidin transcription. C/EBPδ, whose gene expression is induced in hepatocytes both by IL-6 *via* Stat3 (Cantwell, Sterneck, & Johnson, 1998) and by IL-1 *via* NF-κB (Ali, Singh, Yildirim, & Ramji, 2010), was

recently reported to activate hepcidin transcription in hepatoma cell lines (Kanamori et al., 2017) and could be such an intermediate.

### 3.4 The BMP/SMAD pathway contributes to the maximal hepcidin induction by inflammation

Hepatocyte-specific disruption of Smad4 abrogates not only BMP but also IL6-mediated hepcidin induction, indicating that the BMP pathway also contributes to hepcidin upregulation by inflammation (Wang et al., 2005). This is further supported by the blunted hepcidin induction observed when mice with impaired Smad1/5/8 signaling are treated with LPS. Indeed, experiments on mice lacking both Hfe and Tfr2 (Wallace, McDonald, Ostini, & Subramaniam, 2011), Hjv or Bmp6 alone or in combination (Latour et al., 2017) or the Bmp type I receptor Alk3 (Mayeur, Lohmeyer, et al., 2014) have shown that the level of hepcidin production reached after the LPS challenge is highly dependent on the basal expression of hepcidin before stimulation. It is for instance highest in wild-type animals, intermediate in single Hjv or Bmp6 knockout mice, and lowest in double knockout animals (Latour et al., 2017). This is compatible with a synergistic effect of the inflammatory signaling on the BMP/SMAD signaling in regulating hepcidin during inflammation, a possibility also suggested by the mitigation of hepcidin induction by pharmacological intervention with dorsomorphin, LDN-193189, or other specific inhibitors of the BMP pathway in rodents treated with inflammatory agents (Steinbicker, Sachidanandan, et al., 2011; Theurl et al., 2011; Yu et al., 2008).

Intriguingly, Smad1/5/8 signaling in the mouse liver is activated by activin B as a consequence of LPS stimulation (Besson-Fournier et al., 2012; Besson-Fournier et al., 2017). However, although activin B induces Smad1/5/8 phosphorylation and hepcidin expression in primary hepatocytes (Besson-Fournier et al., 2012; Canali et al., 2016; Kanamori et al., 2016), it is not required for hepcidin induction by inflammation *in vivo* (Besson-Fournier et al., 2017). Even more puzzling is the lack of relationship between the level of activation of Smad1/5/8 signaling reached after LPS stimulation in the mouse liver and hepcidin expression (Latour et al., 2017). Further studies are needed to determine which liver cell populations contribute to the activation of Smad1/5/8 signaling and which ones contribute to hepcidin production during inflammatory stimuli. It is likely that these do not overlap, adding another level of complexity to the already complicated regulation of hepcidin by inflammation. A model for hepcidin regulation by inflammation, based on our current understanding, is shown in Fig. 5.

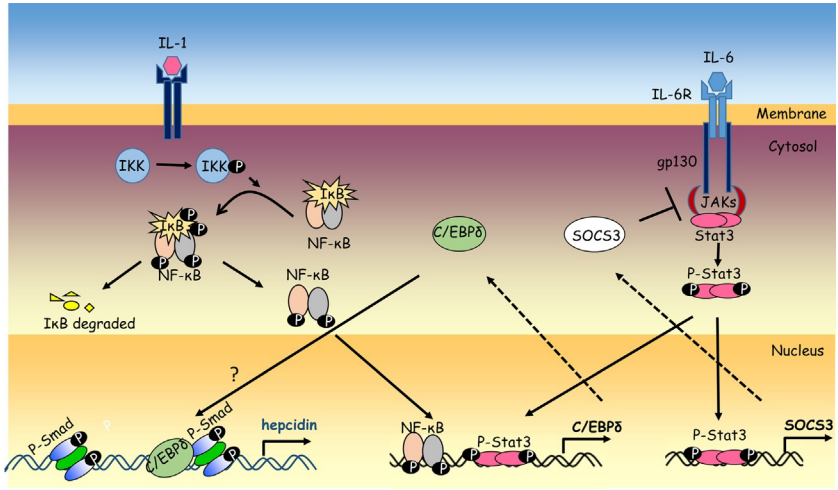


**Fig. 5** Proposed model for the regulation of hepcidin by inflammation. By activating the JAK/STAT pathway, IL-6 induces SOCS3 that participates in a rapid feedback loop to inhibit STAT3 activity. The induction of hepcidin mRNA is delayed in time, compared with SOCS3, which makes it less likely to be a direct target of activated STAT3. C/EBPδ, whose gene expression is induced in hepatocytes by both IL-6/Stat3 and IL-1/NF-κB pathways, was recently reported to activate hepcidin transcription in hepatoma cell lines and could be an intermediate between STAT3/NF-κB activation and hepcidin transcription. Importantly, the BMP/SMAD pathway contributes to the maximal induction of hepcidin by inflammation.

## 4. Hepcidin regulation by stress erythropoiesis and hypoxia

Iron is an important component of heme in hemoglobin and the production of red blood cells in the bone marrow is critically dependent on the bioavailability of iron. After blood loss or hemolysis the requirement for iron increases to allow for the replacement of lost red blood cells. Hepcidin must then be rapidly suppressed to allow increased iron absorption from the diet and the release of iron from body stores. First, EPO, a cytokine produced by the kidney in response to low oxygen levels, triggers the proliferation and terminal differentiation of erythroid progenitor cells. These precursors then release a soluble factor expected to communicate the increase in iron demand from the bone marrow to the liver and decrease hepcidin expression (Fig. 6).

Erythroferrone (ERFE), a member of the C1q/TNF-related protein family encoded by the *Fam132b* gene, possesses the main characteristics to be an erythroid regulator of hepcidin during stress erythropoiesis.

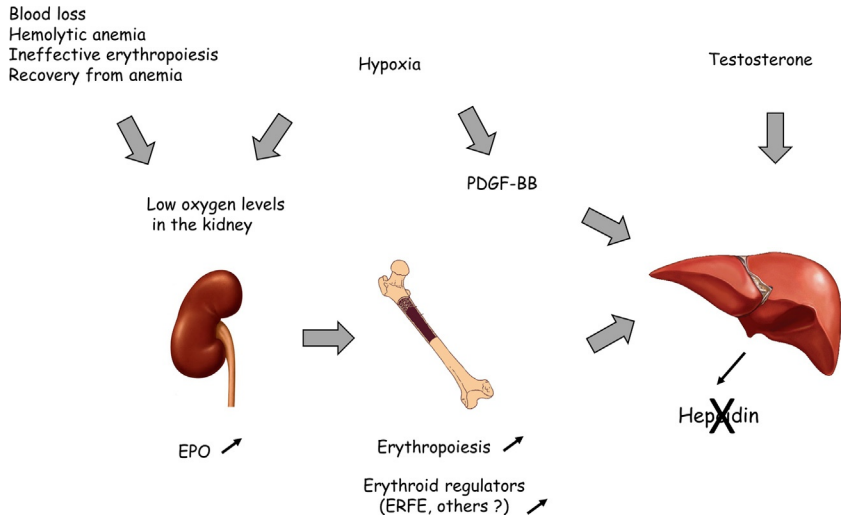


**Fig. 6** Negative regulators of hepcidin. Blood loss, hemolytic anemia and genetic diseases causing ineffective erythropoiesis produce anemia that is sensed by the kidney. The kidney then increases erythropoietin (EPO) production and EPO triggers bone marrow erythropoiesis. This increases erythroferrone (ERFE) production by erythroblasts. ERFE circulates in the plasma to cause hepatic suppression of hepcidin. Hypoxia may also increase EPO production and stimulate the EPO-ERFE axis. In addition, hypoxia increases platelet-derived growth factor BB (PDGF-BB), another suppressor of hepcidin. Finally testosterone, which increases hepatic EGF receptor expression, amplifies the inhibitory effect of EGF on hepcidin transcription. The exact mechanisms by which ERFE, PDGF-BB or EGF repress hepcidin in the hepatocyte remain to be delineated.

After phlebotomy or EPO injection to mice, *Fam132b* mRNA levels are greatly increased in the bone marrow and the spleen of these animals, which is accompanied by a large increase in serum Erfe concentrations. This increase in Erfe production is followed by the suppression of hepcidin. Furthermore, the treatment of mice or isolated hepatocytes with recombinant ERFE suppresses hepcidin mRNA, showing that ERFE acts directly on hepatocytes to regulate hepcidin expression. Anemia persists longer and recovery is delayed in *Fam132b* knockout mice, because these mice fail to suppress hepcidin as wild-type mice do (Kautz et al., 2014). ERFE also helps recovery of anemia in acute inflammation (Kautz, Jung, Nemeth, & Ganz, 2014). The mechanism of hepcidin inhibition by ERFE is still unclear but ERFE could act through the SMAD pathway because hepcidin is not suppressed by EPO in Smad1/5 double knockout mice (Wang et al., 2017).

Interestingly, matriptase-2, which is stabilized at the cell surface in iron deficiency (Zhao et al., 2015), has a dominant effect over ERFE. Indeed Erfe is unable to suppress the high hepcidin levels of *Tmprss6* knockout mice treated with EPO (Nai et al., 2016). The same inability to suppress hepcidin despite high Erfe levels is observed in *Tmprss6/Hfe/Tfr2* triple knockout and *Tmprss6/Tfr2* double knockout mice (Wallace et al., 2015). A parallel attenuation of BMP/SMAD signaling seems necessary for maximal hepcidin suppression in response to erythropoietic activity. Consistent with this, inactivation of *Tmprss6* in beta-thalassemia $Hbb^{th3/+}$ mice prevents iron overload and partially corrects the anemia phenotype (Guo, Casu, et al., 2013; Nai et al., 2012; Schmidt et al., 2013) whereas Erfe deletion in the same mice reduces iron overload without correcting anemia (Kautz et al., 2015).

In the long term, hepcidin eventually decreases even in *Fam132b* knockout mice which end up compensating their anemia, suggesting the existence of other erythroid regulators (U. Sardo, communication at the 2018 European Iron Club Meeting in Zurich). Two members of the TGF-β family, GDF15 (Tanno et al., 2007) and TWSG1 (Tanno et al., 2009), were postulated to function as putative erythroid regulators of hepcidin but their role could not be unequivocally confirmed, essentially because erythropoietic stress has no effect on these molecules in mice and suppression of hepcidin is observed even in the absence of Gdf15. Further studies are still necessary to clarify the receptor and signaling of Erfe and identify the so far unknown regulators which are capable of repressing hepcidin on the long term.

The main function of iron in hemoglobin is to bind oxygen. The levels of tissue oxygen thus depend on the availability of iron to form hemoglobin. It has long been known that hypoxic conditions increase erythropoiesis to enhance oxygen availability. Similarly to stress erythropoiesis, hypoxia also suppresses hepcidin. Platelet-derived growth factor BB (PDGF-BB) has been implicated in the regulation of hepcidin by hypoxia (Sonnweber et al., 2014). Human volunteers subjected to exercise under hypoxic conditions show elevated concentrations of PDGF-BB which inversely correlate with hepcidin levels. Mice injected with PDGF-BB suppress hepcidin and raise plasma iron. However, hypoxia is also a strong stimulus of EPO production and erythropoiesis (Liu, Davidoff, Niss, & Haase, 2012; Mastrogiannaki et al., 2012), which is associated with increased production of ERFE and other potential erythroid regulators. Further investigations are necessary to understand the relative contribution of these different factors to the hypoxic suppression of hepcidin.

## 5. Hepcidin regulation by sex hormones

Clinical data have shown that men and women exhibit important disparities in the progression of chronic liver diseases such as alcoholic liver disease, chronic hepatitis C, or genetic hemochromatosis, all of which have been reported associated with reduced hepcidin expression (Harrison-Findik, 2010), and gender-related variations in the regulation of iron metabolism have been suspected to contribute to these differences. In mice, whereas castration induced hepcidin in males, daily administration of testosterone to females significantly reduced hepcidin concentration and increased serum iron and transferrin saturation (Guo, Baschman, et al., 2013; Latour et al., 2014). Notably, administration of testosterone to females in a model of chronic inflammation reversed anemia, possibly through hepcidin suppression (Guo, Schmidt, Fleming, & Bhasin, 2016).

It has been shown that the suppressive effects of testosterone on hepcidin are not due to its ability to stimulate erythropoiesis but rather to its capacity to induce Egf receptor (Egfr) expression and activate Egfr signaling in the liver (Latour et al., 2014). Indeed, testosterone administration to female mice induced hepatic Egfr and selective inhibition of Egfr signaling in male mice significantly increased hepcidin expression. EGF has been shown to block iron-induced hepcidin mRNA in mice by suppressing BMP signaling upstream of the hepcidin promoter (Goodnough, Ramos, Nemeth, & Ganz, 2012). It is thus possible that, by increasing hepatic Egfr expression, testosterone amplifies the inhibitory effect of EGF on hepcidin transcription. It was speculated that this mechanism is a modifier of disease severity in conditions in which hepcidin production is already inadequate (Latour et al., 2014). The mechanisms linking testosterone-induced Egfr signaling to hepcidin suppression *in vivo* are not yet understood but could for instance involve the induction and phosphorylation of TGIF, a Smad co-repressor, by EGF (Liu, Hubchak, Browne, & Schnaper, 2014). The suppressive effect of testosterone might also be mediated by the association of androgen receptor with Smad1 and Smad4 that reduces their binding to BMP-response elements in the hepcidin promoter (Guo, Bachman, et al., 2013).

In humans, testosterone stimulates erythropoiesis in both sexes (Shahani, Braga-Basaria, Maggio, & Basaria, 2009) and excessive erythrocytosis is the most common adverse event associated with testosterone therapy in older men (Calof et al., 2005). Interestingly, the mechanisms by which

testosterone stimulates erythropoiesis do not involve stimulation of EPO secretion (Coviello et al., 2008) or erythroid progenitor cells (Kim et al., 2005) but suppression of hepcidin (Bachman et al., 2010). Another example of the suppressive role of testosterone on hepcidin in humans comes from investigations on women with polycystic ovary syndrome, a frequent androgen excess disorder, who present with mild iron overload (Escobar-Morreale, 2012). Notably, there is a negative correlation between serum hepcidin levels and serum free testosterone concentrations in these patients, suggesting that increased body iron stores are due to the down-regulation of hepcidin by testosterone (Luque-Ramirez, Alvarez-Blasco, Alpanes, & Escobar-Morreale, 2011).

Whereas testosterone clearly has suppressive effects on hepcidin, the effects of estrogens on hepcidin expression are less clear (Hou et al., 2012; Ikeda et al., 2012; Lehtihet et al., 2016; Yang, Jian, Katz, Abramson, & Huang, 2012; Zhen et al., 2013) and highlight the need for additional studies to delineate their possible role in hepcidin regulation.

## 6. Conclusions

The identification of hepcidin as the master regulator of systemic iron homeostasis has greatly advanced our understanding of iron biology. Hepcidin is produced predominantly in the liver but has effects on cells in distant locations, most notably enterocytes for the control of dietary iron absorption, hepatocytes for the control of iron storage, and macrophages for the control of iron release into the circulation. The regulation of hepcidin is complex, with a number of positive and negative regulators converging to fine-tune its expression. The best defined regulatory pathway of hepcidin is the BMP/SMAD pathway that mediates the effect of iron on hepcidin and also contributes to the maximal induction of hepcidin by inflammation. The IL-6/STAT3 pathway supposed to mediate the effect of inflammation on hepcidin still raises issues, particularly because, in the liver, the timing of hepcidin induction does not coincide with that of STAT3 activation. Finally, there remains a number of missing links to be discovered in the EPO/ERFE axis mediating hepcidin suppression by erythropoietic activity and the EGF/EGFR axis mediating hepcidin suppression by testosterone. Although there have been major breakthroughs in our understanding of hepcidin regulation in the last 17 years, numerous issues remain to be addressed, suggesting interesting areas for future research.

## References


Ali, S., Singh, N. N., Yildirim, H., & Ramji, D. P. (2010). Requirement for nuclear factor kappa B signalling in the interleukin-1-induced expression of the CCAAT/enhancer binding protein-delta gene in hepatocytes. *The International Journal of Biochemistry & Cell Biology*, *42*(1), 113–119. https://doi.org/10.1016/j.biocel.2009.09.018.

Anderson, G. J., & Frazer, D. M. (2006). Iron metabolism meets signal transduction. *Nature Genetics*, *38*(5), 503–504.

Andriopoulos, B., Jr., Corradini, E., Xia, Y., Faasse, S. A., Chen, S., Grgurevic, L., et al. (2009). BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. *Nature Genetics*, *41*(4), 482–487.

Armitage, A. E., Eddowes, L. A., Gileadi, U., Cole, S., Spottiswoode, N., Selvakumar, T. A., et al. (2011). Hepcidin regulation by innate immune and infectious stimuli. *Blood*, *118*(15), 4129–4139. https://doi.org/10.1182/blood-2011-04-351957.

Babitt, J. L., Zhang, Y., Samad, T. A., Xia, Y., Tang, J., Campagna, J. A., et al. (2005). Repulsive guidance molecule (RGMa), a DRAGON homologue, is a bone morphogenetic protein co-receptor. *The Journal of Biological Chemistry*, *280*(33), 29820–29827.

Babitt, J. L., Huang, F. W., Wrighting, D. M., Xia, Y., Sidis, Y., Samad, T. A., et al. (2006). Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. *Nature Genetics*, *38*(5), 531–539.

Babitt, J. L., Huang, F. W., Xia, Y., Sidis, Y., Andrews, N. C., & Lin, H. Y. (2007). Modulation of bone morphogenetic protein signaling in vivo regulates systemic iron balance. *The Journal of Clinical Investigation*, *117*(7), 1933–1939.

Bachman, E., Feng, R., Travison, T., Li, M., Olbina, G., Ostland, V., et al. (2010). Testosterone suppresses hepcidin in men: A potential mechanism for testosterone-induced erythrocytosis. *The Journal of Clinical Endocrinology and Metabolism*, *95*(10), 4743–4747.

Besson-Fournier, C., Latour, C., Kautz, L., Bertrand, J., Ganz, T., Roth, M. P., et al. (2012). Induction of activin B by inflammatory stimuli up-regulates expression of the iron-regulatory peptide hepcidin through Smad1/5/8 signaling. *Blood*, *120*(2), 431–439.

Besson-Fournier, C., Gineste, A., Latour, C., Gourbeyre, O., Meynard, D., Martin, P., et al. (2017). Hepcidin upregulation by inflammation is independent of Smad1/5/8 signaling by activin B. *Blood*, *129*(4), 533–536. https://doi.org/10.1182/blood-2016-10-748541.

Bode, J. G., Albrecht, U., Haussinger, D., Heinrich, P. C., & Schaper, F. (2012). Hepatic acute phase proteins—Regulation by IL-6- and IL-1-type cytokines involving STAT3 and its crosstalk with NF-kappaB-dependent signaling. *European Journal of Cell Biology*, *91*(6–7), 496–505. https://doi.org/10.1016/j.ejcb.2011.09.008.

Calof, O. M., Singh, A. B., Lee, M. L., Kenny, A. M., Urban, R. J., Tenover, J. L., et al. (2005). Adverse events associated with testosterone replacement in middle-aged and older men: A meta-analysis of randomized, placebo-controlled trials. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *60*(11), 1451–1457.

Camaschella, C., Roetto, A., Cali, A., De Gobbi, M., Garozzo, G., Carella, M., et al. (2000). The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. *Nature Genetics*, *25*(1), 14–15.

Campbell, J. S., Prichard, L., Schaper, F., Schmitz, J., Stephenson-Famy, A., Rosenfeld, M. E., et al. (2001). Expression of suppressors of cytokine signaling during liver regeneration. *The Journal of Clinical Investigation*, *107*(10), 1285–1292. https://doi.org/10.1172/JCI11867.

Canali, S., Core, A. B., Zumbrennen-Bullough, K. B., Merkulova, M., Wang, C. Y., Schneyer, A. L., et al. (2016). Activin B induces noncanonical SMAD1/5/8 signaling via BMP type I receptors in hepatocytes: Evidence for a role in hepcidin induction by inflammation in male mice. *Endocrinology*, *157*(3), 1146–1162. https://doi.org/10.1210/en.2015-1747.

Canali, S., Wang, C. Y., Zumbrennen-Bullough, K. B., Bayer, A., & Babitt, J. L. (2017). Bmp2 controls iron homeostasis in mice independent of Bmp6. *American Journal of Hematology*, *92*(11), 1204–1213. https://doi.org/10.1002/ajh.24888.

Canali, S., Zumbrennen-Bullough, K. B., Core, A. B., Wang, C. Y., Nairz, M., Bouley, R., et al. (2017). Endothelial cells produce bone morphogenetic protein 6 required for iron homeostasis in mice. *Blood*, *129*(4), 405–414. https://doi.org/10.1182/blood-2016-06-721571.

Cantwell, C. A., Sterneck, E., & Johnson, P. F. (1998). Interleukin-6-specific activation of the C/EBPdelta gene in hepatocytes is mediated by Stat3 and Sp1. *Molecular and Cellular Biology*, *18*(4), 2108–2117.

Chung, B., Verdier, F., Matak, P., Deschemin, J. C., Mayeux, P., & Vaulont, S. (2010). Oncostatin M is a potent inducer of hepcidin, the iron regulatory hormone. *The FASEB Journal*, *24*(6), 2093–2103.

Corradini, E., Meynard, D., Wu, Q., Chen, S., Ventura, P., Pietrangelo, A., et al. (2011). Serum and liver iron differently regulate the bone morphogenetic protein 6 (BMP6)-SMAD signaling pathway in mice. *Hepatology*, *54*(1), 273–284. https://doi.org/10.1002/hep.24359.

Corradini, E., Rozier, M., Meynard, D., Odhiambo, A., Lin, H. Y., Feng, Q., et al. (2011). Iron regulation of hepcidin despite attenuated Smad1,5,8 signaling in mice without transferrin receptor 2 or Hfe. *Gastroenterology*, *141*(5), 1907–1914. https://doi.org/10.1053/j.gastro.2011.06.077.

Coviello, A. D., Kaplan, B., Lakshman, K. M., Chen, T., Singh, A. B., & Bhasin, S. (2008). Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. *The Journal of Clinical Endocrinology and Metabolism*, *93*(3), 914–919.

Croker, B. A., Krebs, D. L., Zhang, J. G., Wormald, S., Willson, T. A., Stanley, E. G., et al. (2003). SOCS3 negatively regulates IL-6 signaling in vivo. *Nature Immunology*, *4*(6), 540–545. https://doi.org/10.1038/ni931.

D'Alessio, F., Hentze, M. W., & Muckenthaler, M. U. (2012). The hemochromatosis proteins HFE, TfR2, and HJV form a membrane-associated protein complex for hepcidin regulation. *Journal of Hepatology*, *57*(5), 1052–1060. https://doi.org/10.1016/j.jhep.2012.06.015.

Derynck, R., & Zhang, Y. E. (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature*, *425*(6958), 577–584. https://doi.org/10.1038/nature02006.

Donovan, A., Brownlie, A., Zhou, Y., Shepard, J., Pratt, S. J., Moynihan, J., et al. (2000). Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature*, *403*(6771), 776–781.

Ehlting, C., Lai, W. S., Schaper, F., Brenndorfer, E. D., Matthes, R. J., Heinrich, P. C., et al. (2007). Regulation of suppressor of cytokine signaling 3 (SOCS3) mRNA stability by TNF-alpha involves activation of the MKK6/p38MAPK/MK2 cascade. *Journal of Immunology*, *178*(5), 2813–2826.

Enns, C. A., Ahmed, R., Wang, J., Ueno, A., Worthen, C., Tsukamoto, H., et al. (2013). Increased iron loading induces Bmp6 expression in the non-parenchymal cells of the liver independent of the BMP-signaling pathway. *PLoS One*, *8*(4), e60534. https://doi.org/10.1371/journal.pone.0060534.

Escobar-Morreale, H. F. (2012). Iron metabolism and the polycystic ovary syndrome. *Trends in Endocrinology and Metabolism*, *23*(10), 509–515.

Feder, J. N., Gnirke, A., Thomas, W., Tsuchihashi, Z., Ruddy, D. A., Basava, A., et al. (1996). A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genetics*, *13*(4), 399–408.

Feng, Q., Migas, M. C., Waheed, A., Britton, R. S., & Fleming, R. E. (2012). Ferritin upregulates hepatic expression of bone morphogenetic protein 6 and hepcidin in mice. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *302*(12), G1397–G1404. https://doi.org/10.1152/ajpgi.00020.2012.

Finberg, K. E., Heeney, M. M., Campagna, D. R., Aydinok, Y., Pearson, H. A., Hartman, K. R., et al. (2008). Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). *Nature Genetics*, *40*(5), 569–571. https://doi.org/10.1038/ng.130.

Finberg, K. E., Whittlesey, R. L., Fleming, M. D., & Andrews, N. C. (2010). Down-regulation of Bmp/Smad signaling by Tmprss6 is required for maintenance of systemic iron homeostasis. *Blood*, *115*(18), 3817–3826. https://doi.org/10.1182/blood-2009-05-224808.

Frydlova, J., Prikryl, P., Truksa, J., Falke, L. L., Du, X., Gurieva, I., et al. (2016). Effect of erythropoietin, iron deficiency and iron overload on liver matriptase-2 (TMPRSS6) protein content in mice and rats. *PLoS One*, *11*(2), e0148540. https://doi.org/10.1371/journal.pone.0148540.

Ganz, T. (2013). Systemic iron homeostasis. *Physiological Reviews*, *93*(4), 1721–1741. https://doi.org/10.1152/physrev.00008.2013.

Ganz, T. (2018). Iron and infection. *International Journal of Hematology*, *107*(1), 7–15. https://doi.org/10.1007/s12185-017-2366-2.

Gao, J., Chen, J., Kramer, M., Tsukamoto, H., Zhang, A. S., & Enns, C. A. (2009). Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. *Cell Metabolism*, *9*(3), 217–227.

Gardenghi, S., Renaud, T. M., Meloni, A., Casu, C., Crielaard, B. J., Bystrom, L. M., et al. (2014). Distinct roles for hepcidin and interleukin-6 in the recovery from anemia in mice injected with heat-killed *Brucella abortus*. *Blood*, *123*(8), 1137–1145. https://doi.org/10.1182/blood-2013-08-521625.

Goldstein, I., Paakinaho, V., Baek, S., Sung, M. H., & Hager, G. L. (2017). Synergistic gene expression during the acute phase response is characterized by transcription factor assisted loading. *Nature Communications*, *8*(1), 1849. https://doi.org/10.1038/s41467-017-02055-5.

Goodnough, J. B., Ramos, E., Nemeth, E., & Ganz, T. (2012). Inhibition of hepcidin transcription by growth factors. *Hepatology*, *56*(1), 291–299.

Goswami, T., & Andrews, N. C. (2006). Hereditary hemochromatosis protein, HFE, interaction with transferrin receptor 2 suggests a molecular mechanism for mammalian iron sensing. *The Journal of Biological Chemistry*, *281*(39), 28494–28498.

Guo, W., Bachman, E., Li, M., Roy, C. N., Blusztajn, J., Wong, S., et al. (2013). Testosterone administration inhibits hepcidin transcription and is associated with increased iron incorporation into red blood cells. *Aging Cell*, *12*(2), 280–291. https://doi.org/10.1111/acel.12052.

Guo, S., Casu, C., Gardenghi, S., Booten, S., Aghajan, M., Peralta, R., et al. (2013). Reducing TMPRSS6 ameliorates hemochromatosis and beta-thalassemia in mice. *The Journal of Clinical Investigation*, *123*(4), 1531–1541. https://doi.org/10.1172/JCI66969.

Guo, W., Schmidt, P. J., Fleming, M. D., & Bhasin, S. (2016). Effects of testosterone on erythropoiesis in a female mouse model of anemia of inflammation. *Endocrinology*, *157*(7), 2937–2946. https://doi.org/10.1210/en.2016-1150.

Gutschow, P., Schmidt, P. J., Han, H., Ostland, V., Bartnikas, T. B., Pettiglio, M. A., et al. (2015). A competitive enzyme-linked immunosorbent assay specific for murine hepcidin-1: Correlation with hepatic mRNA expression in established and novel models of dysregulated iron homeostasis. *Haematologica*, *100*(2), 167–177. https://doi.org/10.3324/haematol.2014.116723.

Harrison-Findik, D. D. (2010). Gender-related variations in iron metabolism and liver diseases. *World Journal of Hepatology*, *2*(8), 302–310.

He, G., & Karin, M. (2011). NF-kappaB and STAT3—Key players in liver inflammation and cancer. *Cell Research*, *21*(1), 159–168. https://doi.org/10.1038/cr.2010.183.

Healey, E. G., Bishop, B., Elegheert, J., Bell, C. H., Padilla-Parra, S., & Siebold, C. (2015). Repulsive guidance molecule is a structural bridge between neogenin and bone morphogenetic protein. *Nature Structural & Molecular Biology*, *22*(6), 458–465. https://doi.org/10.1038/nsmb.3016.

Hou, Y., Zhang, S., Wang, L., Li, J., Qu, G., He, J., et al. (2012). Estrogen regulates iron homeostasis through governing hepatic hepcidin expression via an estrogen response element. *Gene*, *511*(2), 398–403. https://doi.org/10.1016/j.gene.2012.09.060.

Ikeda, Y., Tajima, S., Izawa-Ishizawa, Y., Kihira, Y., Ishizawa, K., Tomita, S., et al. (2012). Estrogen regulates hepcidin expression via GPR30-BMP6-dependent signaling in hepatocytes. *PLoS One*, *7*(7), e40465. https://doi.org/10.1371/journal.pone.0040465.

Isaacs, M. J., Kawakami, Y., Allendorph, G. P., Yoon, B. H., Izpisua Belmonte, J. C., & Choe, S. (2010). Bone morphogenetic protein-2 and -6 heterodimer illustrates the nature of ligand-receptor assembly. *Molecular Endocrinology*, *24*(7), 1469–1477. https://doi.org/10.1210/me.2009-0496.

Kanamori, Y., Sugiyama, M., Hashimoto, O., Murakami, M., Matsui, T., & Funaba, M. (2016). Regulation of hepcidin expression by inflammation-induced activin B. *Scientific Reports, 6*, 38702. https://doi.org/10.1038/srep38702.

Kanamori, Y., Murakami, M., Sugiyama, M., Hashimoto, O., Matsui, T., & Funaba, M. (2017). Interleukin-1beta (IL-1beta) transcriptionally activates hepcidin by inducing CCAAT enhancer-binding protein delta (C/EBPdelta) expression in hepatocytes. *The Journal of Biological Chemistry*, *292*(24), 10275–10287. https://doi.org/10.1074/jbc.M116.770974.

Kanda, J., Uchiyama, T., Tomosugi, N., Higuchi, M., Uchiyama, T., & Kawabata, H. (2009). Oncostatin M and leukemia inhibitory factor increase hepcidin expression in hepatoma cell lines. *International Journal of Hematology*, *90*(5), 545–552. https://doi.org/10.1007/s12185-009-0443-x.

Kautz, L., Meynard, D., Monnier, A., Darnaud, V., Bouvet, R., Wang, R. H., et al. (2008). Iron regulates phosphorylation of Smad1/5/8 and gene expression of Bmp6, Smad7, Id1, and Atoh8 in the mouse liver. *Blood*, *112*(4), 1503–1509. https://doi.org/10.1182/blood-2008-03-143354.

Kautz, L., Meynard, D., Besson-Fournier, C., Darnaud, V., Al Saati, T., Coppin, H., et al. (2009). BMP/Smad signaling is not enhanced in Hfe-deficient mice despite increased Bmp6 expression. *Blood*, *114*(12), 2515–2520. https://doi.org/10.1182/blood-2009-02-206771.

Kautz, L., Jung, G., Nemeth, E., & Ganz, T. (2014). Erythroferrone contributes to recovery from anemia of inflammation. *Blood*, *124*(16), 2569–2574. https://doi.org/10.1182/blood-2014-06-584607.

Kautz, L., Jung, G., Valore, E. V., Rivella, S., Nemeth, E., & Ganz, T. (2014). Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nature Genetics*, *46*(7), 678–684. https://doi.org/10.1038/ng.2996.

Kautz, L., Jung, G., Du, X., Gabayan, V., Chapman, J., Nasoff, M., et al. (2015). Erythroferrone contributes to hepcidin suppression and iron overload in a mouse model of beta-thalassemia. *Blood*, *126*(17), 2031–2037. https://doi.org/10.1182/blood-2015-07-658419.

Kemna, E., Pickkers, P., Nemeth, E., van der Hoeven, H., & Swinkels, D. (2005). Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. *Blood*, *106*(5), 1864–1866.

Kim, S. W., Hwang, J. H., Cheon, J. M., Park, N. S., Park, S. E., Park, S. J., et al. (2005). Direct and indirect effects of androgens on survival of hematopoietic progenitor cells in vitro. *Journal of Korean Medical Science*, *20*(3), 409–416.

Kim, A., Fung, E., Parikh, S. G., Valore, E. V., Gabayan, V., Nemeth, E., et al. (2014). A mouse model of anemia of inflammation: Complex pathogenesis with partial dependence on hepcidin. *Blood*, *123*(8), 1129–1136. https://doi.org/10.1182/blood-2013-08-521419.

Koch, P. S., Olsavszky, V., Ulbrich, F., Sticht, C., Demory, A., Leibing, T., et al. (2017). Angiocrine Bmp2 signaling in murine liver controls normal iron homeostasis. *Blood*, *129*(4), 415–419. https://doi.org/10.1182/blood-2016-07-729822.

Krijt, J., Fujikura, Y., Ramsay, A. J., Velasco, G., & Necas, E. (2011). Liver hemojuvelin protein levels in mice deficient in matriptase-2 (Tmprss6). *Blood Cells, Molecules & Diseases*, *47*(2), 133–137. https://doi.org/10.1016/j.bcmd.2011.04.009.

Kubo, M., Hanada, T., & Yoshimura, A. (2003). Suppressors of cytokine signaling and immunity. *Nature Immunology*, *4*(12), 1169–1176. https://doi.org/10.1038/ni1012.

Latour, C., Kautz, L., Besson-Fournier, C., Island, M. L., Canonne-Hergaux, F., Loreal, O., et al. (2014). Testosterone perturbs systemic iron balance through activation of epidermal growth factor receptor signaling in the liver and repression of hepcidin. *Hepatology*, *59*(2), 683–694. https://doi.org/10.1002/hep.26648.

Latour, C., Besson-Fournier, C., Meynard, D., Silvestri, L., Gourbeyre, O., Aguilar-Martinez, P., et al. (2016). Differing impact of the deletion of hemochromatosis-associated molecules HFE and transferrin receptor-2 on the iron phenotype of mice lacking bone morphogenetic protein 6 or hemojuvelin. *Hepatology*, *63*(1), 126–137. https://doi.org/10.1002/hep.28254.

Latour, C., Besson-Fournier, C., Gourbeyre, O., Meynard, D., Roth, M. P., & Coppin, H. (2017). Deletion of BMP6 worsens the phenotype of HJV-deficient mice and attenuates hepcidin levels reached after LPS challenge. *Blood*, *130*(21), 2339–2343. https://doi.org/10.1182/blood-2017-07-795658.

Lee, P., Peng, H., Gelbart, T., & Beutler, E. (2004). The IL-6- and lipopolysaccharide-induced transcription of hepcidin in HFE-, transferrin receptor 2-, and beta 2-microglobulin-deficient hepatocytes. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(25), 9263–9265.

Lee, P., Peng, H., Gelbart, T., Wang, L., & Beutler, E. (2005). Regulation of hepcidin transcription by interleukin-1 and interleukin-6. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(6), 1906–1910.

Lehtihet, M., Bonde, Y., Beckman, L., Berinder, K., Hoybye, C., Rudling, M., et al. (2016). Circulating hepcidin-25 is reduced by endogenous estrogen in humans. *PLoS One*, *11*(2), e0148802. https://doi.org/10.1371/journal.pone.0148802.

Lesbordes-Brion, J. C., Viatte, L., Bennoun, M., Lou, D. Q., Ramey, G., Houbron, C., et al. (2006). Targeted disruption of the hepcidin 1 gene results in severe hemochromatosis. *Blood*, *108*(4), 1402–1405.

Liu, Q., Davidoff, O., Niss, K., & Haase, V. H. (2012). Hypoxia-inducible factor regulates hepcidin via erythropoietin-induced erythropoiesis. *The Journal of Clinical Investigation*, *122*(12), 4635–4644. https://doi.org/10.1172/JCI63924.

Liu, X., Hubchak, S. C., Browne, J. A., & Schnaper, H. W. (2014). Epidermal growth factor inhibits transforming growth factor-beta-induced fibrogenic differentiation marker expression through ERK activation. *Cellular Signalling*, *26*(10), 2276–2283. https://doi.org/10.1016/j.cellsig.2014.05.018.

Luque-Ramirez, M., Alvarez-Blasco, F., Alpanes, M., & Escobar-Morreale, H. F. (2011). Role of decreased circulating hepcidin concentrations in the iron excess of women with the polycystic ovary syndrome. *The Journal of Clinical Endocrinology and Metabolism*, *96*(3), 846–852.

Mastrogiannaki, M., Matak, P., Mathieu, J. R., Delga, S., Mayeux, P., Vaulont, S., et al. (2012). Hepatic hypoxia-inducible factor-2 down-regulates hepcidin expression in mice through an erythropoietin-mediated increase in erythropoiesis. *Haematologica*, *97*(6), 827–834. https://doi.org/10.3324/haematol.2011.056119.

Mayeur, C., Leyton, P. A., Kolodziej, S. A., Yu, B., & Bloch, K. D. (2014). BMP type II receptors have redundant roles in the regulation of hepatic hepcidin gene expression and iron metabolism. *Blood*, *124*(13), 2116–2123. https://doi.org/10.1182/blood-2014-04-572644.

Mayeur, C., Lohmeyer, L. K., Leyton, P., Kao, S. M., Pappas, A. E., Kolodziej, S. A., et al. (2014). The type I BMP receptor Alk3 is required for the induction of hepatic hepcidin gene expression by interleukin-6. *Blood*, *123*(14), 2261–2268. https://doi.org/10.1182/blood-2013-02-480095.

McKie, A. T., Weher, K., Brennan, K., Farzaneh, F., Peters, T. J., Hentze, M. W., et al. (1999). A novel IRE containing mRNA predominantly expressed in duodenum and placenta encodes a basolateral membrane protein associated with iron transport. In *Bioiron'99, Sorrento, May 23–28*.

Meynard, D., Kautz, L., Darnaud, V., Canonne-Hergaux, F., Coppin, H., & Roth, M. P. (2009). Lack of the bone morphogenetic protein BMP6 induces massive iron overload. *Nature Genetics*, *41*(4), 478–481.

Mueller, T. D. (2015). RGM co-receptors add complexity to BMP signaling. *Nature Structural & Molecular Biology*, *22*(6), 439–440. https://doi.org/10.1038/nsmb.3037.

Nai, A., Pagani, A., Mandelli, G., Lidonnici, M. R., Silvestri, L., Ferrari, G., et al. (2012). Deletion of TMPRSS6 attenuates the phenotype in a mouse model of beta-thalassemia. *Blood*, *119*(21), 5021–5029. https://doi.org/10.1182/blood-2012-01-401885.

Nai, A., Rubio, A., Campanella, A., Gourbeyre, O., Artuso, I., Bordini, J., et al. (2016). Limiting hepatic Bmp-Smad signaling by matriptase-2 is required for erythropoietin-mediated hepcidin suppression in mice. *Blood*, *127*(19), 2327–2336. https://doi.org/10.1182/blood-2015-11-681494.

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B. K., et al. (2004). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *The Journal of Clinical Investigation*, *113*(9), 1271–1276.

Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. B., Donovan, A., Ward, D. M., et al. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*, *306*(5704), 2090–2093.

Nicolas, G., Bennoun, M., Devaux, I., Beaumont, C., Grandchamp, B., Kahn, A., et al. (2001). Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(15), 8780–8785.

Nicolas, G., Bennoun, M., Porteu, A., Mativet, S., Beaumont, C., Grandchamp, B., et al. (2002). Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(7), 4596–4601.

Nicolas, G., Chauvet, C., Viatte, L., Danan, J. L., Bigard, X., Devaux, I., et al. (2002). The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *The Journal of Clinical Investigation*, *110*(7), 1037–1044.

Nili, M., Shinde, U., & Rotwein, P. (2010). Soluble repulsive guidance molecule c/hemojuvelin is a broad spectrum bone morphogenetic protein (BMP) antagonist and inhibits both BMP2- and BMP6-mediated signaling and gene expression. *The Journal of Biological Chemistry*, *285*(32), 24783–24792. https://doi.org/10.1074/jbc.M110.130286.

Nohe, A., Hassel, S., Ehrlich, M., Neubauer, F., Sebald, W., Henis, Y. I., et al. (2002). The mode of bone morphogenetic protein (BMP) receptor oligomerization determines different BMP-2 signaling pathways. *The Journal of Biological Chemistry*, *277*(7), 5330–5338. https://doi.org/10.1074/jbc.M102750200.

Papanikolaou, G., Samuels, M. E., Ludwig, E. H., MacDonald, M. L., Franchini, P. L., Dube, M. P., et al. (2004). Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. *Nature Genetics*, *36*(1), 77–82.

Park, C. H., Valore, E. V., Waring, A. J., & Ganz, T. (2001). Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *The Journal of Biological Chemistry*, *276*(11), 7806–7810.

Pigeon, C., Ilyin, G., Courselaud, B., Leroyer, P., Turlin, B., Brissot, P., et al. (2001). A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *The Journal of Biological Chemistry*, *276*(11), 7811–7819.

Ramos, E., Kautz, L., Rodriguez, R., Hansen, M., Gabayan, V., Ginzburg, Y., et al. (2011). Evidence for distinct pathways of hepcidin regulation by acute and chronic iron loading in mice. *Hepatology*, *53*(4), 1333–1341. https://doi.org/10.1002/hep.24178.

Rausa, M., Pagani, A., Nai, A., Campanella, A., Gilberti, M. E., Apostoli, P., et al. (2015). Bmp6 expression in murine liver non parenchymal cells: A mechanism to control their high Iron exporter activity and protect hepatocytes from Iron overload? *PLoS One*, *10*(4), e0122696. https://doi.org/10.1371/journal.pone.0122696.

Rishi, G., & Subramaniam, V. N. (2017). The liver in regulation of iron homeostasis. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *313*(3), G157–G165. https://doi.org/10.1152/ajpgi.00004.2017.

Rishi, G., Crampton, E. M., Wallace, D. F., & Subramaniam, V. N. (2013). In situ proximity ligation assays indicate that hemochromatosis proteins Hfe and transferrin receptor 2 (Tfr2) do not interact. *PLoS One*, *8*(10), e77267. https://doi.org/10.1371/journal.pone.0077267.

Roetto, A., Papanikolaou, G., Politou, M., Alberti, F., Girelli, D., Christakis, J., et al. (2003). Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. *Nature Genetics*, *33*(1), 21–22.

Roy, C. N., Mak, H. H., Akpan, I., Losyev, G., Zurakowski, D., & Andrews, N. C. (2007). Hepcidin antimicrobial peptide transgenic mice exhibit features of the anemia of inflammation. *Blood*, *109*(9), 4038–4044. https://doi.org/10.1182/blood-2006-10-051755.

Schmidt, P. J., & Fleming, M. D. (2012). Transgenic HFE-dependent induction of hepcidin in mice does not require transferrin receptor-2. *American Journal of Hematology*, *87*(6), 588–595. https://doi.org/10.1002/ajh.23173.

Schmidt, P. J., Toran, P. T., Giannetti, A. M., Bjorkman, P. J., & Andrews, N. C. (2008). The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. *Cell Metabolism*, 7(3), 205–214.

Schmidt, P. J., Toudjarska, I., Sendamarai, A. K., Racie, T., Milstein, S., Bettencourt, B. R., et al. (2013). An RNAi therapeutic targeting Tmprss6 decreases iron overload in Hfe(-/-) mice and ameliorates anemia and iron overload in murine beta-thalassemia intermedia. *Blood*, *121*(7), 1200–1208. https://doi.org/10.1182/blood-2012-09-453977.

Shahani, S., Braga-Basaria, M., Maggio, M., & Basaria, S. (2009). Androgens and erythropoiesis: Past and present. *Journal of Endocrinological Investigation*, *32*(8), 704–716.

Silvestri, L., Pagani, A., Nai, A., De Domenico, I., Kaplan, J., & Camaschella, C. (2008). The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. *Cell Metabolism*, *8*(6), 502–511. https://doi.org/10.1016/j.cmet.2008.09.012.

Smith, C. L., Arvedson, T. L., Cooke, K. S., Dickmann, L. J., Forte, C., Li, H. Y., et al. (2013). IL-22 regulates iron availability in vivo through the induction of hepcidin. *Journal of Immunology*, *191*(4), 1845–1855. https://doi.org/10.4049/jimmunol.1202716.

Sonnweber, T., Nachbaur, D., Schroll, A., Nairz, M., Seifert, M., Demetz, E., et al. (2014). Hypoxia induced downregulation of hepcidin is mediated by platelet derived growth factor BB. *Gut*, *63*(12), 1951–1959. https://doi.org/10.1136/gutjnl-2013-305317.

Steinbicker, A. U., Bartnikas, T. B., Lohmeyer, L. K., Leyton, P., Mayeur, C., Kao, S. M., et al. (2011). Perturbation of hepcidin expression by BMP type I receptor deletion induces iron overload in mice. *Blood*, *118*(15), 4224–4230.

Steinbicker, A. U., Sachidanandan, C., Vonner, A. J., Yusuf, R. Z., Deng, D. Y., Lai, C. S., et al. (2011). Inhibition of bone morphogenetic protein signaling attenuates anemia associated with inflammation. *Blood*, *117*(18), 4915–4923.

Tanno, T., Bhanu, N. V., Oneal, P. A., Goh, S. H., Staker, P., Lee, Y. T., et al. (2007). High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. *Nature Medicine*, *13*(9), 1096–1101. https://doi.org/10.1038/nm1629.

Tanno, T., Porayette, P., Sripichai, O., Noh, S. J., Byrnes, C., Bhupatiraju, A., et al. (2009). Identification of TWSG1 as a second novel erythroid regulator of hepcidin expression in murine and human cells. *Blood*, *114*(1), 181–186. https://doi.org/10.1182/blood-2008-12-195503.

Theurl, I., Schroll, A., Sonnweber, T., Nairz, M., Theurl, M., Willenbacher, W., et al. (2011). Pharmacologic inhibition of hepcidin expression reverses anemia of chronic inflammation in rats. *Blood*, *118*(18), 4977–4984.

Trinder, D., & Baker, E. (2003). Transferrin receptor 2: A new molecule in iron metabolism. *The International Journal of Biochemistry & Cell Biology*, *35*(3), 292–296.

Truksa, J., Peng, H., Lee, P., & Beutler, E. (2006). Bone morphogenetic proteins 2, 4, and 9 stimulate murine hepcidin 1 expression independently of Hfe, transferrin receptor 2 (Tfr2), and IL-6. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(27), 10289–10293.

Valera, E., Isaacs, M. J., Kawakami, Y., Izpisua Belmonte, J. C., & Choe, S. (2010). BMP-2/6 heterodimer is more effective than BMP-2 or BMP-6 homodimers as inductor of differentiation of human embryonic stem cells. *PLoS One*, *5*(6), e11167. https://doi.org/10.1371/journal.pone.0011167.

Verga Falzacappa, M. V., Vujic Spasic, M., Kessler, R., Stolte, J., Hentze, M. W., & Muckenthaler, M. U. (2007). STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. *Blood*, *109*(1), 353–358.

Wallace, D. F., & Subramaniam, V. N. (2015). Analysis of IL-22 contribution to hepcidin induction and hypoferremia during the response to LPS in vivo. *International Immunology*, *27*(6), 281–287. https://doi.org/10.1093/intimm/dxu144.

Wallace, D. F., Summerville, L., Crampton, E. M., Frazer, D. M., Anderson, G. J., & Subramaniam, V. N. (2009). Combined deletion of Hfe and transferrin receptor 2 in mice leads to marked dysregulation of hepcidin and iron overload. *Hepatology*, *50*(6), 1992–2000. https://doi.org/10.1002/hep.23198.

Wallace, D. F., McDonald, C. J., Ostini, L., & Subramaniam, V. N. (2011). Blunted hepcidin response to inflammation in the absence of Hfe and transferrin receptor 2. *Blood*, *117*(10), 2960–2966. https://doi.org/10.1182/blood-2010-08-303859.

Wallace, D. F., Secondes, E. S., Rishi, G., Ostini, L., McDonald, C. J., Lane, S. W., et al. (2015). A critical role for murine transferrin receptor 2 in erythropoiesis during iron restriction. *British Journal of Haematology*, *168*(6), 891–901. https://doi.org/10.1111/bjh.13225.

Wang, R. H., Li, C., Xu, X., Zheng, Y., Xiao, C., Zerfas, P., et al. (2005). A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. *Cell Metabolism*, *2*(6), 399–409.

Wang, H., Lafdil, F., Kong, X., & Gao, B. (2011). Signal transducer and activator of transcription 3 in liver diseases: A novel therapeutic target. *International Journal of Biological Sciences*, *7*(5), 536–550.

Wang, C. Y., Core, A. B., Canali, S., Zumbrennen-Bullough, K. B., Ozer, S., Umans, L., et al. (2017). Smad1/5 is required for erythropoietin-mediated suppression of hepcidin in mice. *Blood*, *130*(1), 73–83. https://doi.org/10.1182/blood-2016-12-759423.

Wrighting, D. M., & Andrews, N. C. (2006). Interleukin-6 induces hepcidin expression through STAT3. *Blood*, *108*(9), 3204–3209.

Yang, Q., Jian, J., Katz, S., Abramson, S. B., & Huang, X. (2012). 17beta-estradiol inhibits iron hormone hepcidin through an estrogen responsive element half-site. *Endocrinology*, *153*(7), 3170–3178. https://doi.org/10.1210/en.2011-2045.

Yu, P. B., Hong, C. C., Sachidanandan, C., Babitt, J. L., Deng, D. Y., Hoyng, S. A., et al. (2008). Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nature Chemical Biology*, *4*(1), 33–41.

Zhang, A. S., Gao, J., Koeberl, D. D., & Enns, C. A. (2010). The role of hepatocyte hemojuvelin in the regulation of bone morphogenic protein-6 and hepcidin expression in vivo. *The Journal of Biological Chemistry*, *285*(22), 16416–16423. https://doi.org/10.1074/jbc.M110.109488.

Zhao, N., Nizzi, C. P., Anderson, S. A., Wang, J., Ueno, A., Tsukamoto, H., et al. (2015). Low intracellular iron increases the stability of matriptase-2. *The Journal of Biological Chemistry*, *290*(7), 4432–4446. https://doi.org/10.1074/jbc.M114.611913.

Zhen, A. W., Nguyen, N. H., Gibert, Y., Motola, S., Buckett, P., Wessling-Resnick, M., et al. (2013). The small molecule, genistein, increases hepcidin expression in human hepatocytes. *Hepatology*, *58*(4), 1315–1325. https://doi.org/10.1002/hep.26490.