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Interacting signals in the control of hepcidin expression

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Abstract The amount of iron in the plasma is determined by the regulated release of iron from most body cells, but macrophages, intestinal enterocytes and hepatocytes play a particularly important role in this process. This cellular iron efflux is modulated by the liver-derived peptide hepcidin, and this peptide is now regarded as the central regulator of body iron homeostasis. Hepcidin expression is influenced by systemic stimuli such as iron stores, the rate of erythropoiesis, inflammation, hypoxia and oxidative stress. These stimuli control hepcidin levels by acting through hepatocyte cell surface proteins including HFE, transferrin receptor 2, hemojuvelin, TMPRSS6 and the IL-6R. The surface proteins activate various cell signal transduction pathways, including the BMP-SMAD, JAK-STAT and HIF1 pathways, to alter transcription of *HAMP*, the gene which encodes hepcidin. It is becoming increasingly apparent that various stimuli can signal through multiple pathways to regulate hepcidin expression, and the interplay between positive and negative stimuli is critical in determining the net hepcidin level. The BMP-SMAD pathway appears to be particularly important and disruption of this pathway will abrogate the response of hepcidin to many stimuli.

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The iron cycle in mammals

The ability of iron to accept or donate electrons makes it essential for many of the biological reactions carried out by living systems. This same characteristic, however, allows free iron in solution to form highly reactive free radicals that can lead to cell damage. Therefore, appropriate regulation of systemic iron homeostasis is crucial for the survival and wellbeing of all complex organisms, including humans. The average adult male human contains approximately four grams of iron, approximately two-thirds of which is found in hemoglobin in circulating red blood cells (Brittenham 1994). Under normal conditions approximately 1–2 mg of iron per day enters the body via the enterocytes of the proximal small intestine. This newly absorbed dietary iron is released into the circulation and binds to the serum protein transferrin (Tf), each molecule of which can bind two atoms of iron. Approximately 3 mg of iron circulates bound to transferrin and is taken up by cells by transferrin receptor 1 (TfR1)-mediated endocytosis (Huebers and Finch 1987) where it can be incorporated into a wide range of intracellular proteins. Any excess iron is stored in iron storage protein ferritin.

Most of the transferrin bound iron in the circulation is destined for the developing erythrocytes of the bone marrow, where it is used in the production of hemoglobin. About 65–70% of body iron exists in this form in circulating red blood cells (Brittenham 1994). Old or damaged red blood cells are removed from the circulation by the macrophages of the reticuloendothelial (RE) system, where iron is released from hemoglobin and either stored in the intracellular iron storage protein ferritin, or released back into the circulation as transferrin-bound iron.

The movement of iron between these compartments is tightly regulated and can be modulated according to the body's iron requirements and a number of other signals. Individual cells maintain appropriate intracellular iron levels by altering the expression of TfR1 on the cell surface (Huebers and Finch 1987). The more iron each cell requires, the higher the expression of TfR1. While the uptake of iron by cells is predominantly controlled locally by intracellular iron levels, iron release, particularly from the cells of the RE system, liver and proximal small intestine, appears to be regulated by systemic signals. Evidence now suggests that the control of cellular iron efflux is the major regulatory point for the maintenance of systemic iron homeostasis (Frazer and Anderson 2003). The recognition that the liver-derived peptide hepcidin controls this vital process has been the cornerstone of advances in iron metabolism in recent years.

Hepcidin—a central regulator of iron homeostasis

Hepcidin was first discovered as an antimicrobial peptide in human blood ultrafiltrate (Krause et al. 2000) and urine samples (Park et al. 2001). The gene encoding hepcidin (*HAMP*) is very strongly expressed in the liver but weak expression has also been detected in heart, spinal cord, stomach, intestine, adipose tissue and lungs (Krause et al. 2000, Park et al. 2001, Pigeon et al. 2001, Bekri et al. 2006). The mature 25 amino acid peptide has eight cysteine residues forming four intramolecular disulfide bonds that are highly conserved among species from zebrafish to humans. Experiments with mouse models either lacking hepcidin expression or overexpressing the peptide, and studies in humans with mutations in the *HAMP* gene, have demonstrated that hepcidin is a negative regulator of cellular iron efflux (Nicolas et al. 2001, Nicolas et al. 2002b, Nicolas et al. 2003, Rivera et al. 2005a). Indeed mutation in HAMP in humans lead to a severe, early onset iron loading disorder known as juvenile hemochromatosis (JH) (Roetto et al. 2003).

Hepcidin interacts directly with ferroportin1 (FPN) at the cell surface of HEK293 cells in culture, causing the internalization and subsequent degradation of the FPN protein (Nemeth et al. 2004a, b, De Domenico et al. 2007). This loss of ferroportin from the cell surface reduces iron export from the cells, leading to intracellular iron accumulation. Although it is highly likely that this process also occurs in vivo, there has been little direct confirmatory evidence of this. However, support for this model is provided by studies showing that (1) radiolabelled hepcidin injected into mice accumulates in FPN-rich tissues (Rivera et al. 2005b); (2) human patients with mutations in FPN that impair its interaction with hepcidin develop tissue iron overload (Drakesmith et al. 2005), and (3) hepcidin administered to mice leads to a reduction in intestinal iron absorption (Laftah et al. 2004, Chaston et al. 2008).

Systemic signals that regulate hepcidin

The factors that regulate iron homeostasis also modulate hepcidin levels. Changes in body iron stores, the rate of erythropoiesis, inflammation and hypoxia all influence iron absorption in the gut and iron release from cells, and these are the major systemic factors that regulate *HAMP* mRNA levels in the liver (Fig. 1).

Hepcidin levels are increased in response to oral and parenteral iron loading and decreased under iron deficient conditions (Frazer et al. 2002). The regulation of hepcidin by body iron acts as a feedback mechanism to allow sufficient iron to enter the plasma when demand is high, but to limit iron release into the plasma in times of iron sufficiency. The hepcidin response to changes in body iron levels is incompletely understood. Since hepcidin expression is largely restricted to the liver (Krause et al. 2000), it is highly likely that the hepatocyte is the site of action of the regulatory stimulus, and the expression of a range of upstream regulators of hepcidin in hepatocytes is consistent with this view. Whether

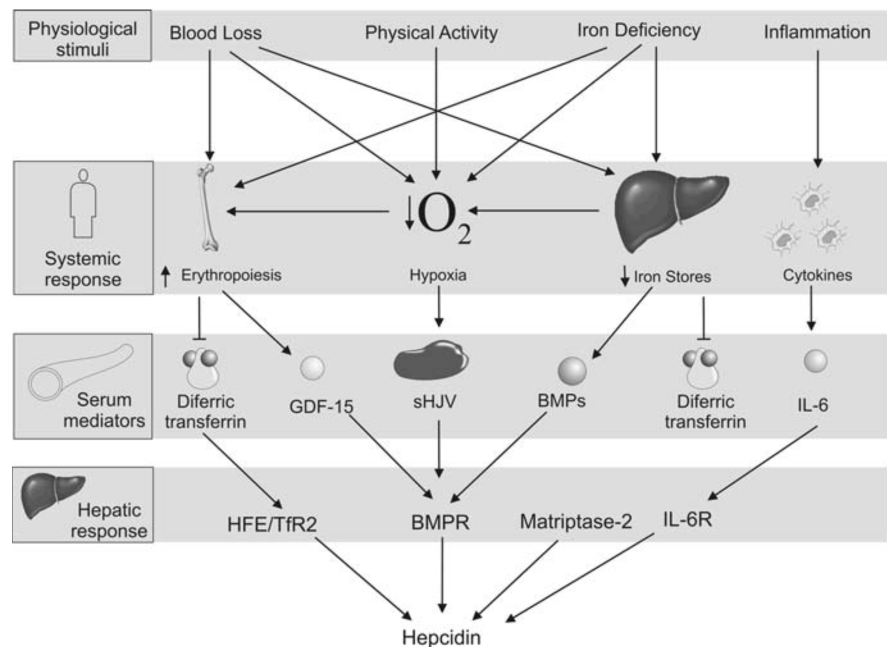

Fig. 1 Systemic regulation of hepcidin. A range of physiological stimuli act in an integrated way to alter expression of the hepcidin gene in the liver. Stimulated erythropoiesis, hypoxia and decreased body iron levels will decrease hepcidin expression, whereas increased body iron and inflammation will stimulate production of the peptide. These systemic signals are received by proteins on the surface of hepatocytes, and these in turn activate signal transduction pathways that lead to changes in *HAMP* gene expression

hepatocyte iron levels per se play a primary role or whether an external signal is involved is unclear, but current evidence would suggest that the latter is more likely. Diferric Tf has emerged as a possible extra-hepatic signal for hepcidin regulation in response to changes in body iron load, but other factors could certainly be involved (Frazer and Anderson 2003).

Approximately 65–70% of body iron is found in erythrocytes in the form of hemoglobin and this represents the largest sink for iron in the body (Brittenham 1994). Consequently, body iron demand is closely linked to the rate of erythropoiesis. For example, when erythropoiesis is stimulated, following blood loss or hemolysis, hepcidin expression is suppressed (Nicolas et al. 2002a, Frazer et al. 2004b). This increases cellular iron release, making available iron stored in the macrophages of the reticuloendothelial system and hepatocytes of the liver. At the same time, an increase in iron release from intestinal enterocytes allows iron taken up from the diet to enter the circulation to replenish the body's iron stores, and provides an increased iron flow into the plasma and to the developing red cells. This regulation of *HAMP* mRNA levels is independent of direct erythropoietin effects, and this has been supported by the observation that suppression of erythropoiesis by irradiation or by post-transfusion polycythemia leads to increased hepcidin levels (Pak et al. 2006, Vokurka et al. 2006). Erythropoiesis could also alter hepcidin expression by affecting iron supply or through hypoxia, or via other mechanisms. Recent evidence has suggested that there may be an erythropoiesis-specific factor that affects hepcidin expression. An increase in the rate of erythropoiesis leads an expansion of the erythroid compartment and erythroblast maturation. A recent report has shown that growth differentiation factor 15 (GDF15), a member of the transforming growth factor-beta superfamily, showed increased expression and secretion during erythroblast maturation (Tanno et al. 2007). GDF15 suppresses hepcidin expression in vitro, but the suppression appears to be modest. Furthermore, GDF15 and hepcidin levels do not correlate following the recovery from erythropoietic stem cell transplants (Kanda et al. 2008), suggesting that GDF15 may not be critical for hepcidin regulation.

A reduction in amount of circulating hemoglobin, e.g. from blood loss, leads not only in increased erythropoiesis, but also to decreased delivery of oxygen to the tissues, resulting in hypoxia. Hypoxia stimulates both erythropoiesis and iron supply to the plasma, and thus is associated with reduced hepcidin production. Animals placed in a hypoxic chamber show a drop in hepcidin levels within 2–4 days and an increase in luminal iron uptake in the small

intestine (Nicolas et al. 2002a). In this case, the in vivo effects of hypoxia on hepcidin expression are more likely to be secondary to stimulated erythropoiesis due to the delay in the hepcidin response. However, evidence for a direct effect of low oxygen on hepcidin expression has come from the demonstration that hypoxia downregulates *HAMP* mRNA levels in human hepatoma cell lines (Nicolas et al. 2002a, Peyssonnaux et al. 2007). In vivo, both mechanisms are likely to be operating.

It has been long known that inflammation, whether acute or chronic, perturbs iron homeostasis. Under inflammatory conditions, iron absorption declines and iron is sequestered in macrophages, with the consequence that the plasma iron level is decreased, resulting in hypoferremia (Rivera et al. 2005b). If this condition persists chronically, it may lead to anemia, hence it is often called the anemia of chronic disease or anemia of chronic inflammation. Inflammation positively regulates hepcidin expression and this provides a mechanism for the hypoferremia. One of the major mediators of the inflammatory response is the cytokine IL-6. IL-6 infusion in humans or administration to experimental animals leads to an increase in hepcidin production and decrease in serum iron levels within a few hours (Nemeth et al. 2004a). A time course analysis in human subjects injected with LPS revealed a strong temporal correlation between increases in serum IL-6 and urinary hepcidin, and the decrease in serum iron (Kemna et al. 2005). Similarly, IL-6, other pro-inflammatory cytokines like IL-1α and IL-1β, stimulate hepcidin in primary hepatocytes and hepatoma cell lines (Lee et al. 2004, Lee et al. 2005).

Interactions between signals that alter hepcidin levels

The factors that regulate hepcidin expression have been studied extensively at the individual level. However, in an in vivo situation, the net levels of hepcidin are determined by the complex interplay of all these factors combined (Fig. 1). Depending on the specific situation, different stimuli will predominate. This is perhaps best illustrated by several examples.

β-thalassemia results from reduced globin chain synthesis and this in turn leads to an increase in the rate of erythropoiesis that is secondary to the breakdown of defective red cells. Increased erythropoiesis means increased intestinal iron absorption, and progressive iron loading is characteristic of this disease. In both mice and humans with this disorder, hepcidin levels are initially low, despite increased iron stores, and thus the erythropoietic stimulus is predominating (Adamsky et al. 2004, Papanikolaou et al. 2005). However, as the disease advances, the effect of the increasing iron stores become relatively stronger and hepcidin levels increase (Gardenghi et al. 2007). A similar situation is found in iron loaded experimental animals subjected to an erythropoietic stimulus, such as chemically-induced hemolysis. Hepcidin is initially high due to the iron overload, but it is reduced when erythropoiesis is strongly stimulated (Nicolas et al. 2002a). As a final example, mice lacking the *Hfe* gene mimic the human iron loading disease hemochromatosis, yet have relatively low hepcidin levels because of the Hfe disruption (Ahmad et al. 2002). Despite their iron loading, such animals increase their hepcidin normally in response to an inflammatory stimulus (Frazer et al. 2004a, Lee et al. 2004). These studies demonstrate the importance that the balance of competing stimuli plays in hepcidin regulation. Some of the molecular mechanisms underlying these responses will be considered in more detail below.

How does hepcidin respond to external signals?

While the major systemic factors that alter hepcidin expression are well established, the mechanism by which hepcidin production is regulated at the molecular level is less well known and is a topic of intense research at present. Many of the advances in this area have come from the examination of inherited iron loading disorders in humans and mice, so it is appropriate to consider these in the first instance.

HFE is the gene mutated in the most common form of hereditary hemochromatosis (Feder et al. 1996). The disease is characterised by iron loading in the parenchymal cells of various tissues, secondary to increased iron absorption and increased iron release from reticuloendothelial cells. This occurs despite adequate or elevated iron stores, and reflects the fact that hecpidin levels are inappropriately low when HFE is dysfunctional (Ahmad et al. 2002, Bridle et al. 2003). By crossing animals that overexpress

hepcidin with those lacking Hfe, the iron loading defect can be overcome (Nicolas et al. 2003), and this provides experimental evidence that the reduced hepcidin expression in the absence of Hfe is driving the iron loading. The HFE protein is a nonclassical MHC class I molecule that is expressed at low levels in most tissues, but it is most strongly expressed in the hepatocytes of the liver, the site of synthesis of hepcidin (Feder et al. 1996). Indeed animals lacking Hfe expression solely in hepatocyes show an iron loading phenotype (Vujic Spasic et al. 2008), whereas those lacking Hfe in either the small intestine or in macrophages have a wild-type phenotype (Vujic Spasic et al. 2007, Vujic Spasic et al. 2008). Similarly, HFE hemochromatosis patients who have undergone orthotopic liver transplantation do not demonstrate reaccumulation of excess liver iron (Bralet et al. 2004). These data confirm that the hepatocyte is the physiologically relevant site of HFE action and that HFE acts upstream of hepcidin in the liver (Fig. 2).

HFE is presumed to play a role in monitoring body iron status and then directing the appropriate hepcidin response, but how does it do this? The HFE protein has been shown to interact with TfR1 at a site that overlaps with the binding site for Tf (Parkkila et al. 1997, Feder et al. 1998, Lebron et al. 1998, Bennett et al. 2000). Diferric Tf can displace HFE from TfR1 due to its higher binding affinity. High diferric Tf would "free up" HFE and enable it to exert a positive effect on hepcidin synthesis (Fig. 2). A recent study has provided experimental support for this by generating mutant mouse strains that either promote or prevent the HFE/TfR1 interaction. Mice with constitutive HFE/TfR1 interaction had low hepcidin levels and developed iron overload, whereas mice carrying a mutation that interferes with the HFE/TfR1 binding developed iron deficiency and inappropriately high hepcidin expression (Schmidt et al. 2008). It has been suggested that HFE not bound to TfR1 exerts its effects by binding to TfR2 (Schmidt et al. 2008), but this has yet to be proven.

Mutations in the gene encoding TfR2 lead to body iron loading with symptoms very similar to, but somewhat more severe than, those of HFE-associated hemochromatosis (Camaschella et al. 2000). Furthermore, TfR2 mutations lead to the same inappropriately low hepcidin levels seen when HFE is disrupted (Nemeth et al. 2005), suggesting that TfR2 and HFE may be part of the same regulatory pathway. When TfR2 and HFE are overexpressed in the same cell, they are able to interact (Goswami and Andrews 2006). Studies like these form the basis of the suggestion that HFE and TfR2 might regulate hepcidin expression as a complex. However, such a complex may not be essential, as even in the absence of HFE or TfR2, hepcidin can still be regulated in response to changes in iron status to some degree (Gehrke et al. 2005). Despite this, there is good evidence that TfR2 could participate in transducing iron-related signals. Diferric Tf stabilizes the TfR2 protein (Johnson and Enns 2004, Robb and Wessling-Resnick 2004), and thus under high iron conditions, higher TfR2 levels on the cell surface would be consistent with hepcidin upregulation. Furthermore, when diferric Tf binds to TfR2 the ERK1/ERK2 and p38 MAP kinase pathways are activated and these can induce hepcidin expression (Calzolari et al. 2006) (Fig. 2).

The level of circulating diferric Tf is a likely means by which information about body iron stores and demand is communicated to the hepcidin regulatory machinery (Frazer and Anderson 2003), although other signals may also be involved. Tf iron saturation reflects the sum of iron entering the serum from the gut, macrophages and liver, and iron leaving the serum for utilization by various cell types. When cellular iron demand is high or supply is low, circulating diferric Tf levels will decrease, while the opposite occurs when iron demand is low or supply is high. A close correlation between diferric Tf levels and hepatic hepcidin mRNA expression in rats has been demonstrated following hemolysis (Frazer et al. 2004b) or the switch from a control to an iron deficient diet (Frazer et al. 2002). Recent evidence from the hemoglobin deficit (*hbd*) mouse also supports a role for diferric Tf in hepcidin regulation. The gene affected in these mice is Sec15l1 (Lim et al. 2005) which, when disrupted, alters the recycling of TfR1-containing endosomes (Zhang et al. 2006). This leads to a decrease in TfR1-mediated iron uptake, limiting the iron supply to the bone marrow and resulting in anemia. The reduction in iron uptake leads to an increase in the level of diferric Tf in the circulation of *hbd* mice (Wilkins et al. 2006) and this increase parallels an increase in hepcidin expression, despite the anemia in the mice.

The BMP/SMAD pathway and hepcidin regulation

While mutations in the gene encoding hepcidin can explain some cases of juvenile hemochromatosis (JH), most cases of this severe form of iron loading can be attributed to mutations in the HFE2 gene (which encodes hemojuvelin; HJV) (Roetto et al. 1999, Papanikolaou et al. 2004). HJV is a glycophosphatidylinositol (GPI)-linked membrane protein that is expressed at high levels in skeletal muscle and heart, to a moderate extent in liver, and at low levels in some other tissues (Papanikolaou et al. 2004). JH patients with HJV mutations and *Hjv* knockout mice essentially have no hepcidin expression despite their iron loading (Papanikolaou et al. 2004, Huang et al. 2005), indicating that hemojuvelin is essential for the production of hepcidin and that it is an upstream regulator of the peptide.

HJV is a member of the repulsive guidance molecule (RGM) family of proteins that function as co-receptors for Bone Morphogenetic Protein (BMP) signalling. HJV can bind to type I BMP receptors and, upon stimulation with BMPs (such as BMP2, 4 or 9), it can enhance the phosphorylation of SMAD1/5/8 (Babitt et al. 2006) (Fig. 2). These activated SMADs can in turn bind to SMAD4 and the complex moves to the nucleus where it can stimulate hepcidin expression. As further support for a role of the BMP pathway in the regulation of hepcidin expression, mice with a liver-specific knockout of the *SMAD4* gene develop iron overload and express little, if any, hepcidin (Wang et al. 2005). In addition, hepcidin expression in these mice cannot be stimulated by iron loading or inflammation, as it can in wild-type mice (Wang et al. 2005), suggesting that SMAD4, and possibly the entire BMP pathway, is essential for hepcidin production in response to these stimuli. The induction of hepcidin expression by BMPs appears to be independent of HFE, TfR2 and IL-6, as a study by Truksa et al has shown that hepatocytes from knockout mice lacking these molecules have a normal hepcidin response to BMP 2, 4 and 9 (Truksa et al. 2006). The strongest stimulation was seen with BMP-9, which is predominantly expressed in the liver (Truksa et al. 2006), and this suggests a possible autocrine or paracrine role for this BMP in hepcidin regulation.

An intriguing aspect of HJV is its strong expression in skeletal and cardiac muscle. JH patients and *Hjv* knockout mice do not show any skeletal muscle defects, which rules out the possibility that HJV has a primary role in muscle development. However, skeletal muscle is a large tissue (approximately one-third of the body weight) and is a significant consumer of iron to form myoglobin. This suggests that HJV and muscle may play a critical role in iron homeostasis. Support for such a role comes from studies that show that HJV is regulated at the post-transcriptional level. HJV protein is expressed in two isoforms: a secreted full length molecule (sHJV) that is processed by furin (a proprotein convertase) and a membrane bound heterodimer that is formed after autocatalytic cleavage (Kuninger et al. 2006, Lin et al. 2008, Silvestri et al. 2008). sHJV has been detected in the plasma and a number of studies have now shown that it acts as a repressor of BMP signaling by competing out the membrane-associated form of HJV (Lin et al. 2005, Babitt et al. 2007, Lin et al. 2008). Thus any stimulus that leads to increased sHJV production could lead to a reduction in hepcidin expression. Importantly, the generation of sHJV appears to be increased by iron treatment and hypoxia (Lin et al. 2005, Zhang et al. 2007, Silvestri et al. 2008), both stimuli that lead to reduced hepcidin production and increased iron flow into the plasma.

The furin promoter possesses hypoxia-responsive elements, binding sites for the hypoxia-inducible factor-1 (HIF-1) transcription complex, and levels of furin mRNA are markedly increased by hypoxia (McMahon et al. 2005). It has been reported that under hypoxic conditions increased furin levels enhance HJV shedding and this might be a physiologic mechanism that takes place in cells expressing endogenous HJV (Silvestri et al. 2008). Exercise has also been shown to increase HIF1α levels and its DNA binding capacity (Ameln et al. 2005, Lundby et al. 2006). Thus HIF/furin–induced s-HJV release will suppress hepcidin production to meet the increased iron requirement during hypoxia or exercise. In addition, the *HAMP* gene itself contains hypoxia response elements in its promoter (Peyssonnaux et al. 2007), and thus its expression can be reduced directly by hypoxia. Together, these mechanisms of hepcidin regulation ensure an adequate iron supply to meet the demands of hemoglobin and myoglobin production.

HJV has also been shown to interact with neogenin (Zhang et al. 2005), a netrin receptor involved in neuronal development, but the role of neogenin in

regulating hepcidin expression in liver cells is not clear. Xia et al reported that neither overexpression nor knockdown of neogenin alters HJV-mediated BMP signalling or hepcidin expression in a hepatoma cell line (Xia et al. 2008). However, Zhang et al. showed that knockdown of endogenous neogenin in muscle cells suppresses HJV shedding and that overexpression of neogenin in liver cells markedly enhances this process (Zhang et al. 2007). These data suggest that membrane HJV shedding is mediated by neogenin, but further information is required.

Other regulators of hepcidin expression

As noted above, inflammation is able to strongly stimulate hepcidin expression and this induction is responsible for the hypoferremia that accompanies inflammatory episodes. Although several proinflammatory cytokines have been shown to increase hepcidin expression, IL-6 has been the best studied. IL-6 signals via the JAK/STAT signalling pathway and the *HAMP* promoter contains binding sites for the phosphorylated STAT3 dimer (Wrighting and Andrews 2006, Verga Falzacappa et al. 2007) (Fig. 2). The demonstration that the induction of hepcidin during inflammation is equally robust in wild-type, *Hfe* knockout and *Tfr2* knockout mice suggests that proinflammatory cytokines stimulate hepcidin independently of these molecules (Frazer et al. 2004a, Lee et al. 2004). However, the inactivation of SMAD4 in the liver prevents upregulation of hepcidin by the inflammatory cytokine IL-6 (Wang et al. 2005), suggesting that the two pathways must converge at some point at or before the involvement of SMADs. In support of this, it has recently been shown that mutation of a critical BMP-response element in the *HAMP* promoter severely impairs hepcidin expression in response to IL-6 (Verga Falzacappa et al. 2008). It has also been demonstrated that dorsomorphin, a selective inhibitor of BMP-responsive SMAD phosphorylation, blocked the IL-6 mediated induction of hepcidin (Yu et al. 2008). Thus current evidence suggests that an intact BMP/SMAD pathway is required for a normal hepcidin response to inflammation, and this highlights an important interaction between these key pathways for hepcidin regulation.

The most recently described player in the hepcidin regulatory pathway is the membrane-bound serine protease matriptase-2 (encoded by the *TMPRSS6* gene) (Fig. 2). *TMPRSS6* was identified as the gene affected in cases of refractory iron deficiency anemia in humans, and also in the *mask* mouse mutant, a strain of mice with an inherited hypochromic, microcytic anemia (Du et al. 2008, Finberg et al. 2008). Subsequently other studies have confirmed the human results (Guillem et al. 2008, Melis et al. 2008), and the *Tmprss6* gene has been disrupted in mice to confirm the iron deficiency phenotype (Folgueras et al. 2008). Of particular interest is the demonstration that hepcidin levels are inappropriately high when *TMPRSS6* is mutated (Du et al. 2008, Folgueras et al. 2008), suggesting that matripase-2 acts as a repressor of hepcidin expression under normal conditions. Furthermore, overexpression of TMPRSS6 suppresses *HAMP* promoter activity and the protease domain is required for a full effect (Du et al. 2008). It is not yet known whether matriptase-2 is associated with any of the previously described pathways of hepcidin regulation. The protease activity certainly could be involved in processing one of the intermediates involved in these pathways, but since matriptase-2 plays a repressive rather than an activating role, it may represent a novel regulatory pathway. This area will certainly be a very active one in the forthcoming years.

Conclusion

Hepcidin has emerged as the master regulator of body iron homeostasis and has been a major research focus in mammalian iron metabolism in recent years. Despite many important advances in the area, much remains to be learned. The BMP-SMAD signalling cascade (Fig. 2) has been shown to be central to hepcidin regulation and it likely plays an important role in maintaining basal hepcidin expression. This pathway may also be involved in the response of hepcidin to changes in iron status or hypoxia, and this effect may be mediated by soluble hemojuvelin. It is thought that HFE and TfR2 are intimately involved in relaying iron-related signals to hepcidin, but how they do so and whether their action involves the BMP-SMAD pathway are important unresolved questions. Inflammatory cytokines such as IL-6 can influence hepcidin expression through the JAK-STAT pathway, but this stimulation is dependent of an intact BMP-SMAD pathway as well. This highlights the interrelatedness of

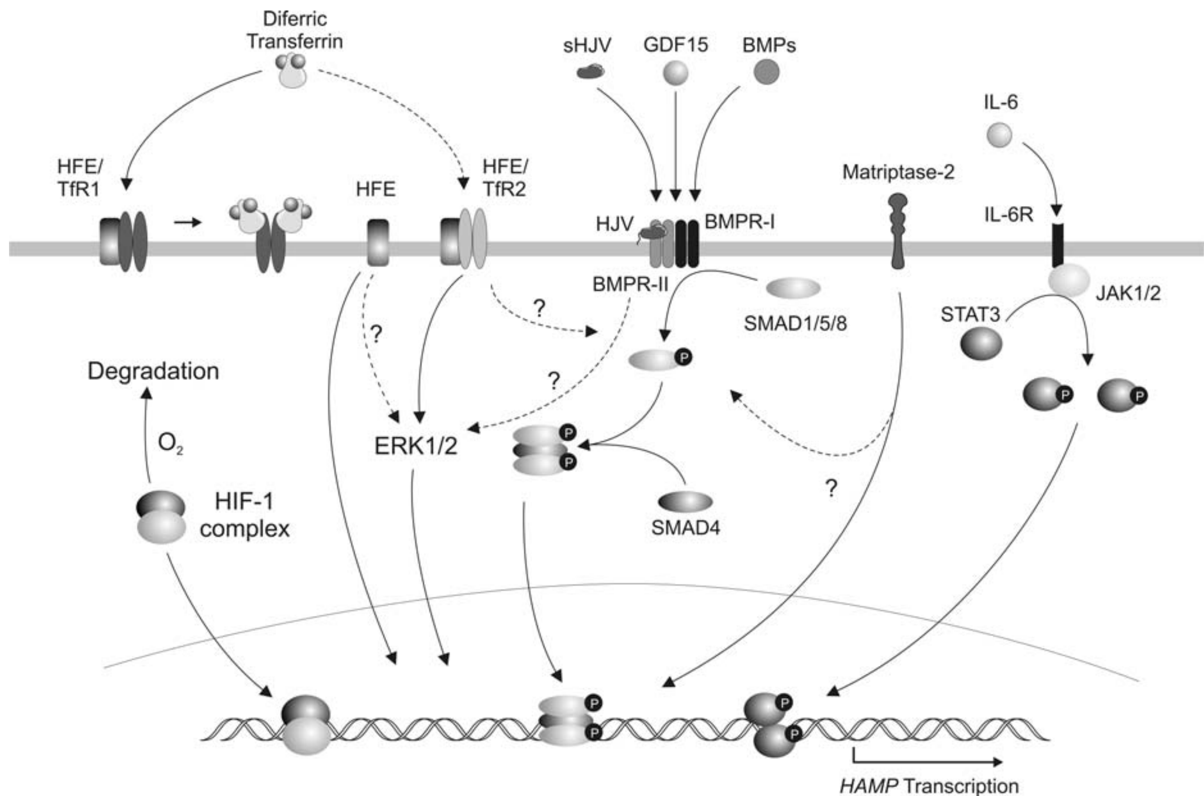

Fig. 2 Signal transduction pathways in the regulation of hepcidin. A range of signal transduction pathways are now known to influence the expression of the *HAMP* gene. The BMP-SMAD pathway has proved to be particularly important, and when this pathway is disrupted hepcidin does not respond appropriately to a range if stimuli. The JAK-STAT pathway is necessary for the *HAMP* response to proinflammatory cytokines such as IL-6, whereas the direct effects of hypoxia are mediated by the HIF1 complex. Precisely how HFE, TfR2 and matriptase-2 alter *HAMP* expression remains to be determined

the various pathways and it is becoming increasingly difficult to consider any one pathway in isolation. In addition, further members of the regulatory network continue to be described, such as the membrane-bound protease and hepcidin repressor matriptase-2. Future studies will help resolve precisely how these proteins function and will define the complexities of their interplay in this essential regulatory system.

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