Iron Imports.
I. Intestinal iron absorption and its regulation

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Frazer, David M., and Gregory J. Anderson. Iron Imports. I. Intestinal iron absorption and its regulation. Am J Physiol Gastrointest Liver Physiol 289: G631–G635, 2005; doi:10.1152/ajpgi.00220.2005.—Our knowledge of how the body absorbs iron from the diet and how this process is controlled has increased at a rapid rate in recent years. The identification of key molecules, including the iron regulatory peptide hepcidin, and the analysis of how they are regulated and interact have led to the development of an integrated model for the control of iron absorption by body iron requirements. Research now focuses on the role of the liver as the primary regulator of iron absorption, and this review considers some of the recent highlights and controversies in this area.

hepcidin; hemouvelin; HFE; transferrin receptor 2

THE ABILITY OF IRON TO EITHER ACCEPT OR DONATE ELECTRONS HAS MADE IT AN ESSENTIAL ELEMENT FOR MOST FORMS OF LIFE, AS IT PLAYS A CRUCIAL ROLE IN A VARIETY OF PROCESSES SUCH AS OXYGEN TRANSPORT, ENERGY PRODUCTION, AND DNA SYNTHESIS. HOWEVER, THIS REDOX ACTIVITY CAN ALSO LEAD TO THE PRODUCTION OF OXYGEN FREE RADICALS, WHICH CAN DAMAGE VARIOUS CELLULAR COMPONENTS. FOR THIS REASON, ORGANISMS MUST TIGHTLY REGULATE THEIR IRON LEVELS TO PROVIDE ENOUGH FOR THEIR CELLULAR NEEDS WITHOUT DEVELOPING THE TOXICITY ASSOCIATED WITH IRON EXCESS. UNLIKE MANY OTHER NUTRIENTS, THE BODY LACKS A DEFINED MECHANISM FOR THE ACTIVE EXCRETION OF IRON, SO BODY IRON LEVELS MUST BE REGULATED AT THE POINT OF ABSORPTION, THE PROXIMAL SMALL INTESTINE. BECAUSE OF THE VITAL ROLE PLAYED BY IRON IN HUMAN HEALTH AND DISEASE, INTESTINAL IRON ABSORPTION AND ITS REGULATION HAVE BEEN THE FOCUS OF INTENSE RESEARCH FOR MANY DECADES. HOWEVER, IT IS ONLY IN THE PAST FEW YEARS WITH THE DISCOVERY OF A NUMBER OF KEY MOLECULES INVOLVED IN INTESTINAL IRON TRANSPORT THAT WE HAVE BEGUN TO UNDERSTAND THE PROCESS AT THE MOLECULAR LEVEL. THE CURRENT THEMES SERIES OF REVIEWS ENTITLED IRON IMPORTS FOCUSES ON THESE RECENT DISCOVERIES AND PROVIDES INSIGHT INTO HOW THEY ADVANCE OUR KNOWLEDGE OF BODY IRON HOMEOSTASIS. IN THIS REVIEW, WE GIVE A BRIEF OVERVIEW OF IRON ABSORPTION BEFORE FOCUSING ON SOME OF THE MOST IMPORTANT AND EXCITING RECENT DISCOVERIES. WE ALSO EXAMINE THE IMPLICATIONS OF THESE FINDINGS, WITH PARTICULAR EMPHASIS ON HOW THEY RELATE TO OUR RECENTLY PROPOSED MODEL FOR THE REGULATION OF IRON ABSORPTION, AS WELL AS HIGHLIGHTING SOME IMPORTANT QUESTIONS THAT REMAIN TO BE ANSWERED.

ABSORPTION OF DIETARY IRON

The absorption of dietary iron, which is present in either a heme or nonheme form, is carried out by mature villus enterocytes of the duodenum and proximal jejunum. Our knowledge of heme iron absorption is rudimentary, so this review will focus on the absorption of nonheme iron (for more details, see Ref 4). The first step in the absorption process is the uptake of iron from the lumen of the intestine across the apical membrane and into the enterocyte. This is mediated by the brush border iron transporter divalent metal transporter 1 (DMT1) which, as its name suggests, transports iron in the ferrous form. However, much of the iron that enters the lumen of the duodenum in the diet is in the oxidized or ferric form and, therefore, must be reduced before it can be taken up by enterocytes. The reduction of iron is likely to be carried out enzymatically by a brush border ferric reductase, and a strong candidate for this role is recently described Dcytb. Once inside the enterocyte, the intracellular trafficking of iron from the brush border membrane to the basolateral membrane is poorly understood. Intracellular iron may be bound to chaperone molecules to maintain its solubility, but to date none has been identified. Iron not transferred to the body is incorporated into the iron storage molecule ferritin and is lost when the cell is ultimately sloughed at the villus tip. The efflux of iron across the basolateral membrane and into the circulation is mediated by the iron transport protein ferroportin 1, which is also involved in the export of iron from other cell types, including macrophages (5). In addition to ferroportin 1, the basolateral efflux of iron from enterocytes requires the ferroxidase hephaestin. Although the exact role of this protein has not been defined, its ability to oxidize ferrous to ferric iron is predicted to be important for its function.

The amount of iron absorbed by enterocytes is influenced by a variety of factors including variations in body iron stores, changes to the rate of erythropoiesis, hypoxia, inflammation, and pregnancy. These factors result in changes in the duodenal expression of the major enterocyte iron transport molecules, particularly DMT1, Dcytb, and ferroportin 1, at both the mRNA level and protein level. How the body regulates the expression of these molecules in response to a stimulus to alter iron absorption is the major focus of current research in this field.

REGULATION OF INTESTINAL IRON ABSORPTION BY HEPcidIN

Perhaps the most important recent advance in our understanding of intestinal iron absorption has been the realization that the liver plays a central role in the regulation of this process. Although it has long been known that the liver is the main storage site for excess iron, the direct role played by the liver in regulating iron absorption has only become apparent with the discovery of the iron regulatory hormone hepcidin (16). This small antimicrobial peptide is secreted by hepatocytes...
cytes into the circulation, where it acts as an inhibitor of iron absorption and iron release from macrophages and other cell types. The production of hepcidin is decreased by stimuli known to increase iron absorption (e.g., iron deficiency and increased erythropoiesis) and increased under conditions where absorption is decreased (e.g., iron loading and inflammation) (11, 17). Hepcidin has recently been shown to interact directly with ferroportin 1 at the surface of HEK-293 cells, leading to its internalization and degradation (14). If this also occurs at the basolateral membrane of the enterocyte, it would explain the inhibition of iron absorption by hepcidin as a reduction in ferroportin 1 would decrease the transfer of iron to the body. However, the details of this pathway have yet to be resolved. For example, why are alterations in absorption associated with changes in ferroportin 1 mRNA as well as protein? It is possible that the binding of hepcidin to ferroportin 1 results in a signal to downregulate ferroportin 1 mRNA expression in addition to causing the internalization and degradation of the protein. Other receptors for hepcidin may also exist.

The mechanism by which the brush border iron uptake components DMT1 and Dcytb are regulated is not as clear. It has been suggested that hepcidin is able to alter the expression of these molecules directly, but this seems unlikely. We (3, 9) have previously shown that the expression of both DMT1 and Dcytb is strongly affected by the iron concentration within the enterocyte, whereas systemic signals from the body appear to preferentially modulate the export of iron across the basolateral membrane. This led us to suggest that systemic signals to alter iron absorption initially affect the basolateral export of iron, which leads to alterations in enterocyte iron levels and subsequently to changes in the expression of the brush border uptake components. The effect of hepcidin on ferroportin 1 expression is consistent with this model. The iron-dependent regulation of DMT1 may be mediated by iron regulatory proteins (IRPs). These molecules bind to iron responsive elements (IREs) in mRNA transcripts of various genes and affect their expression. This binding is dependent on intracellular iron levels. The major splice variant of DMT1 expressed in enterocytes contains an IRE motif, and the binding of IRPs to DMT1 mRNA when intracellular iron levels are low appears to stabilize this message, increasing the level of DMT1 protein. Whereas Dcytb is regulated by intracellular iron levels in the same direction as DMT1, the Dcytb message does not contain an IRE motif and cannot be directly regulated by IRP activity. Further studies are required to determine the regulatory mechanism involved.

Another consequence of the discovery of hepcidin was that it provided compelling evidence against the long-supported crypt hypothesis for the regulation of iron absorption. Since the 1960s, it has been proposed that signals from the body to alter iron absorption were detected by nascent enterocytes in the crypts of the duodenum, thus programming them to absorb more or less iron after they had matured (for a review, see Ref. 8). This hypothesis was based on the presence of a lag period of several days between a stimulus to alter iron absorption and the actual change in absorption. The lag period was interpreted as the time required for the programmed crypt cells to mature and become absorptive enterocytes on the villus. We (10) have recently demonstrated using a rat model of stimulated erythropoiesis that this lag period reflects the time required by the body to detect the need for a change in absorption and alter hepcidin expression and that once this has occurred changes in iron absorption follow rapidly. In fact, an injection of hepcidin into mice results in decreases in serum iron levels within 4 h (22). We (7) have also demonstrated a close temporal relationship between the expression of hepcidin and duodenal iron transporters in rats switched from an iron-replete diet to an iron-deficient diet, with no evidence for a several-day lag period. The demonstration that hepcidin is the principal molecule regulating absorption (23) and the finding that it interacts with ferroportin 1 (14), which is only expressed in mature enterocytes, provide very strong evidence that signals from the body can be detected by mature villus enterocytes. Collectively, these data provide convincing evidence that the intestinal crypt cells do not play a primary role in the regulation of iron absorption.

MOLECULES INVOLVED IN THE REGULATION OF HEPATIC HEPcidIN EXPRESSION

Although the discovery of hepcidin and its effects on intestinal iron absorption represent major advances in our understanding of body iron homeostasis, the question of how hepcidin expression is altered in response to body iron requirements still remains. In recent years, however, the analysis of patients with inherited iron loading disorders has led to the identification of three molecules that play important roles in this regulatory pathway.

The first of these to be described was HFE, which is mutated in the most common form of the iron overload disorder hemochromatosis (6). Affected patients have increased iron absorption despite adequate or high body iron stores, indicating an inability to appropriately limit absorption. This observation, coupled with the widespread support for the crypt programming model, led most researchers to focus on the intestinal crypts as the site of HFE action. However, in 2003, our laboratory (2) reported that mutations in HFE led to inappropriately low hepcidin levels in both humans and mice, suggesting that HFE is involved in the regulation of hepcidin expression. Because hepcidin expression is essentially restricted to the liver, this provided strong evidence that HFE was exerting its effects in hepatic cells and not in intestinal crypts. Furthermore, Nicolas et al. (18) showed that iron loading in HFE-null mice could be corrected by constitutively expressing hepcidin, confirming that HFE acts upstream of hepcidin in the iron regulatory pathway and strengthening the case that the liver was the most likely site of HFE activity. Subsequent studies showed that HFE is most strongly expressed in hepatocytes, as indeed is hepcidin, suggesting that it is in these cells that HFE is exerting its effect on hepcidin expression (25).

Also strongly expressed in hepatocytes is transferrin receptor 2 (TfR2). This protein is a homolog of the classical TfR1 and can take up transferrin-bound iron from the circulation via receptor-mediated endocytosis. Mutations in TfR2, although much less common than those in HFE, cause an iron loading disease with symptoms very similar to those of HFE-associated hemochromatosis (8). Further studies have shown that the disruption of TfR2 leads to the same inappropriately low hepcidin levels linked with the disruption of HFE, suggesting that TfR2 and HFE are part of the same regulatory pathway (15).

The most recently discovered member of this regulatory network is hemojuvelin. This molecule is expressed in hepato-
tocytes and is disrupted in most cases of juvenile hemochromatosis, a rare condition that results in rapid iron loading to a much more severe extent than that associated with mutations in HFE or TfR2 (20). Juvenile hemochromatosis patients do not produce detectable levels of hepcidin despite their high iron load, suggesting that hemojuvelin is absolutely required for the production of hepcidin and, along with HFE and TfR2, lies upstream of hepcidin in the pathway regulating iron homeostasis.

**A MODEL FOR THE MOLECULAR BASIS OF HEPCIDIN REGULATION**

Precisely how these molecules monitor body iron requirements and direct the regulation of hepcidin expression is not yet known. Any regulatory pathway must allow the hepatocytes to detect events occurring at distant sites in the body, such as alterations in iron demands of developing erythroid cells in the bone marrow. Transferrin saturation has previously been suggested as a regulator of iron absorption, and recent studies from our laboratory suggest that diferric transferrin in the circulation may relay body iron demand back to the liver. We (7, 10) have shown a close correlation between the level of diferric transferrin and hepatic hepcidin mRNA expression in rats after hemolysis or a switch from a control to an iron-deficient diet. Circulating levels of diferric transferrin would make an ideal indicator of body iron demand because the protein is preferentially taken up by cells that require iron. Therefore, when cellular iron demand increases, diferric transferrin levels would decrease, and vice versa.

We (8) have recently proposed a detection mechanism for diferric transferrin that involves both HFE and TfR2 on the hepatocyte plasma membrane. HFE and transferrin bind to overlapping sites on TfR1. In our model, we proposed that diferric transferrin would outcompete HFE for TfR1 binding such that higher diferric transferrin levels would lead to an increased amount of free HFE on the cell surface. Evidence for such a competition has been reported recently using tagged HFE constructs transfected into cell lines (12). Under normal conditions, HFE was found both on the plasma membrane and in TfR1-containing endosomes; however, when treated with diferric transferrin, HFE was found only on the plasma membrane, indicating that diferric transferrin had outcompeted HFE for TfR1 binding. We suggested that unbound HFE on the cell surface is able to stimulate a signal transduction pathway that leads to an increase in the expression of hepcidin. This would explain the decrease in hepcidin expression that occurs when HFE is disrupted in hemochromatosis.

TfR2 could play a similar role in regulating the expression of hepcidin in response to circulating diferric transferrin levels. In

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**Fig. 1. Model for the regulation of intestinal iron absorption.** Changes in body iron usage alter the amount of diferric transferrin in the circulation (A) as this molecule is preferentially taken up by cells requiring iron. The change in diferric transferrin concentration is detected by transferrin receptor (TfR)2 and hemochromatosis protein (HFE)/TfR1 on the plasma membrane of hepatocytes and leads to changes in the expression of hepcidin (B). Hepatocyte iron stores also affect hepcidin production by altering the amount of TfR1 on the plasma membrane. Circulating hepcidin interacts with ferroportin 1 at the basolateral membrane of the villus enterocytes of the small intestine, causing the iron transporter to be internalized and degraded and reducing iron export (C). The accumulation of iron within the cell decreases the expression of divalent metal transporter 1 (DMT1) and Dcytb at the brush border. Any changes in iron absorption affect diferric transferrin levels, thereby completing a negative feedback loop that regulates body iron homestasis.
this case, however, there is evidence suggesting that the TIR2 protein is stabilized by the binding of diferric transferrin (13). If a signal to increase hepcidin expression is produced by TIR2, the stabilization of this molecule by diferric transferrin would maintain this signal. A lowering of diferric transferrin levels would decrease this signal, reducing hepcidin expression and increasing iron absorption.

Hepatic iron stores could also play a part in the regulation of hepcidin by altering the surface expression of TIR1 in hepatocytes (8). Low intracellular iron levels increase the level of TIR1 expression at the cell surface, making more of this molecule available to interact with HFE. The increased levels of TIR1 would also effectively outcompete TIR2 for diferric transferrin binding because TIR2 has an ~25-fold lower affinity for diferric transferrin than TIR1. The combined effect would be a decrease in the signal to produce hepcidin when hepatocyte iron stores are low. The opposite would occur when iron stores are high as the cell surface expression of TIR1 decreases. The expression of hepcidin, therefore, would be regulated by the combined effects of diferric transferrin levels, which indicate body iron usage and hepatocyte iron stores.

The presence of two parallel pathways (one requiring HFE and the other TIR2) for the regulation of hepcidin expression explains the phenotypes observed when the various molecules are disrupted. Mutations in either HFE or TIR2 produce a relatively mild iron overload, with hepcidin levels lower than expected but still detectable. A study (1) in knockout mice showed that animals with disrupted HFE retained the ability to regulate their iron absorption, although the level of iron absorbed was inappropriately high for their iron stores. This residual regulation has also been observed in humans and may be mediated by the remaining intact pathway allowing some regulation of hepcidin to occur. Mutations in hemojuvelin result in much more severe iron loading in which iron absorption cannot be downregulated, implying that hemojuvelin is essential for hepcidin production. We (8) previously predicted from our model that mutations in both HFE and TIR2 would result in a more severe phenotype, similar to that seen with mutations in hepcidin or hemojuvelin. Recently, a patient with juvenile hemochromatosis due to mutations in both HFE and TIR2 was reported, supporting our hypothesis (21).

The role played by hemojuvelin in the regulation of hepcidin is more speculative. Surprisingly, it is most highly expressed in skeletal muscle and the heart, although no specific defect in these tissues has been detected in patients with disrupted hemojuvelin (20). Lower levels have been detected in the liver and presumably it is here that hemojuvelin is acting to regulate hepcidin expression. Most of what we do know about hemojuvelin comes from the study of the repulsive guidance molecule (RGM) family of proteins, of which hemojuvelin is a member (also known as RGMc). Thus hemojuvelin, at least in cell culture models, is a GPI membrane-anchored protein that is located on the extracellular surface of the plasma membrane (19). A recent study (24) has shown that hemojuvelin can interact with bone morphogenetic protein receptors and enhance the signal produced by ligand binding, as can the other members of the RGM family. It is unlikely that hemojuvelin plays this role in vivo as bone morphogenetic proteins are involved in embryonic development and there is no evidence that this is disrupted in juvenile hemochromatosis patients. It does, however, raise the possibility that hemojuvelin is performing a similar function in hepatocytes by initiating or enhancing the signal transduction pathway regulating hepcidin in response to diferric transferrin levels, possibly by interacting directly with HFE, TIR1, or TIR2.

CONCLUSIONS

On the basis of recent data, it is possible to present a working hypothesis for the regulation of intestinal iron absorption (Fig. 1), although many questions remain to be answered. For example: Is diferric transferrin the primary factor communicating body iron requirements to the hepatocytes? How do HFE, TIR2, and hemojuvelin on the surface of hepatocytes affect the level of hepcidin expression in the nucleus? How does the binding of hepcidin to ferroportin 1 lead to a reduction in the level of ferroportin 1 mRNA? This model does, however, provide a starting point from which these questions can be addressed. Some of these issues will be explored in more detail in other articles in this series.

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