Systemic Regulation of Intestinal Iron Absorption

Teresa M. Steele[1,2], David M. Frazer[1] and Gregory J. Anderson[1,2]

[1]Iron Metabolism Laboratory, Queensland Institute of Medical Research and
[2]School of Medicine, University of Queensland, QLD 4029

Iron plays an important role in a wide variety of metabolic processes and is an essential nutrient for almost all living organisms. However, in excess, iron is potentially toxic to cells due to its ability to catalyse the production of reactive oxygen species. Tight regulation of iron uptake and storage at both the cellular and whole body levels is therefore essential. For the maintenance of body iron homeostasis, there must be effective communication between the key sites of iron utilisation (e.g. the erythroid marrow), storage (e.g. the liver and reticuloendothelial system), and absorption in the small intestine. Since these sites are anatomically distant, the most likely form of communication is via a plasma-borne factor. Candidates for a circulating signal influencing iron absorption have been sought for some time, particularly in various plasma mediators of iron status and erythropoiesis e.g. transferrin (Tf), Tf-bound iron, non-transferrin bound iron, ferritin and erythropoietin. However, with recent advances in our understanding of iron metabolism, there is now significant evidence to suggest that the circulating liver-derived peptide hepcidin is the key systemic regulator of iron homeostasis.

Body Iron is Controlled at the Point of Absorption

Since it is not actively excreted, the amount of iron in the body must be controlled at the point of absorption in the small intestine. Iron absorption occurs mainly in the duodenum and upper jejunum, though small amounts may also be absorbed from the stomach, ileum and colon (1). At the cellular level, iron is absorbed through the differentiated epithelial cells of the mid to upper villus. Iron is provided to the body in various forms through the diet, but is primarily absorbed as either inorganic iron or as haem iron. The passage of iron through the enterocyte into the circulation is depicted in **Fig. 1**. The first step is termed brush border or mucosal uptake. In this step, dietary Fe^{3+} (ferric iron) is first reduced to Fe^{2+} (ferrous iron), most likely by duodenal cytochrome b (Dcyt$b$), a ferric reductase, making it available for transport across the brush border membrane by divalent metal transporter 1 (DMT1). The absorption of haem iron across the brush border occurs more efficiently but the mechanism is poorly characterised. Once inside the enterocyte, haem and non-haem iron enter a common transit pool, where iron may be chelated by low

Abbreviations:
Cp — ceruloplasmin
Dcyt$b$ — duodenal cytochrome b
DMT1 — divalent metal transporter 1
Hb — haemoglobin
Heph — hephaestin
HFE — haemochromatosis protein
HJV — hemojuvelin
Ireg1 — iron regulation protein 1
TfR1 — transferrin receptor 1
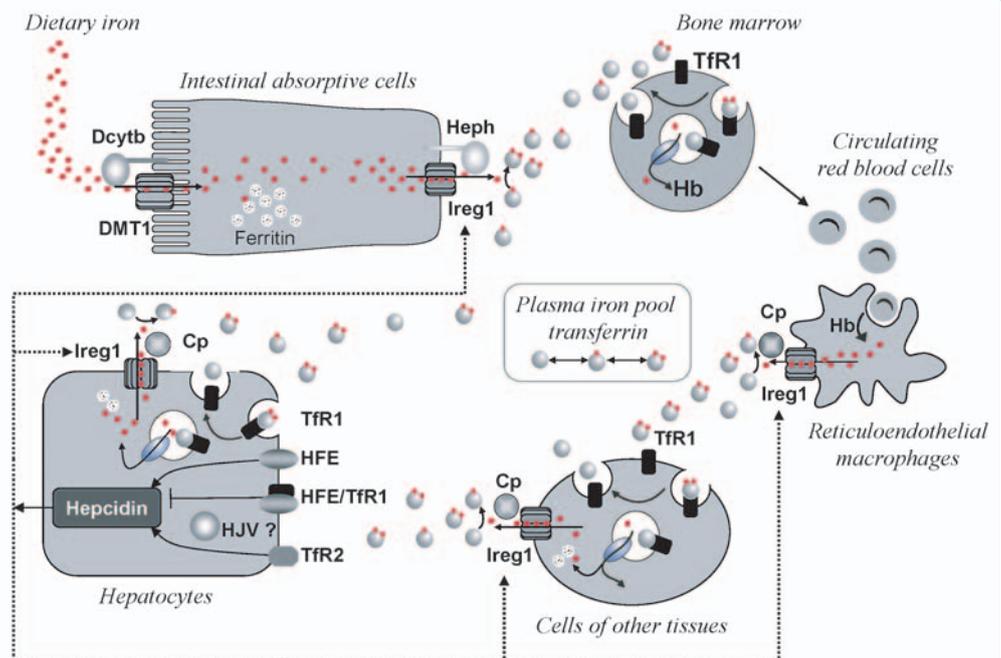TfR2 — transferrin receptor 2

Fig. 1. Regulation of intestinal iron absorption and body iron homeostasis.
Variations in body iron demand manifest themselves as changes in the level of diferric transferrin in the plasma. These changes are recognised in the liver (and likely by the hepatocyte) by the HFE/TfR1 complex and TfR2, and these molecules in turn signal alterations in the expression of the regulatory peptide hepcidin. Hepcidin is secreted into the circulation and acts on the intestinal enterocytes, macrophages, and probably other body cells, to modulate iron efflux.

Table 1. Some genetic disturbances of iron homeostasis.

Disease	Gene affected	Species	Phenotype
Haemochromatosis	HFE	Human	Iron loading; mainly parenchymal cells
Haemochromatosis	TfR2	Human	Iron loading; mainly parenchymal cells
Haemochromatosis	Ireg1	Human	Iron loading (mainly macrophages e.g. Kupffer cells); early mild anaemia
Juvenile Haemochromatosis	Hemojuvelin	Human	Severe iron loading; mainly parenchymal cells
Juvenile Haemochromatosis	Hepcidin	Human	Severe iron loading; mainly parenchymal cells
Atransferrinaemia	Transferrin	Human/Mouse	Severe iron loading
Aceruloplasminaemia	Ceruloplasmin	Human	Iron loading; early mild anaemia
Hereditary hyperferritinemia	L-ferritin	Human	Iron loading
Fredreich Ataxia	Frataxin	Human	Iron loading (mitochondria)
Refractory-microcytic anaemia	DMT1	Human/Mouse/Rat	Iron deficiency anaemia
Sex-linked anaemia	Hephaestin	Mouse	Iron deficiency anaemia

molecular weight compounds or bound to a protein ligand such as ferritin. The second stage of iron absorption is termed basolateral or serosal transfer, where iron is transported from the enterocytes into the intestinal capillaries across the basolateral membrane, most likely via the iron transporter iron regulated protein 1 (Ireg1 or ferroportin 1). The ferroxidase hephaestin (Heph) is also essential for basolateral transfer. Iron that is not transferred across the basolateral membrane of the enterocyte is lost after 1-2 days when the epithelial cells are sloughed at the villus tip. Once absorbed, Fe^{3+} is bound to circulating plasma Tf which transports it around the body to various tissues. The transferrin-bound iron is delivered to the cells after binding to Tf receptors (TfR1 and, in some tissues, TfR2 as well) on the cell surface. Iron may also be released from these cells and re-enter the pool of transferrin-bound iron after export by Ireg1 and oxidation to Fe^{3+} by the ferroxidase ceruloplasmin (Cp).

Absorption is Altered in Response to Changes in Body Iron Requirements

There are a number of factors that regulate intestinal iron absorption, including the systemic stimuli of inflammation, hypoxia and pregnancy. However, the two most prominent factors are the level of iron stores (mainly represented by iron in the liver and reticuloendothelial macrophages) and the iron requirements of the developing erythroid mass. Quantitatively, the most significant pool of iron in the body lies in the red blood cells. In the marrow, iron is used both to meet the metabolic requirements of the developing erythrocytes and for incorporation into new haemoglobin molecules. At the end of their life, senescent red cells are taken up by the reticulo-endothelial (RE) macrophages where the iron is either stored or released (presumably via Ireg1) back to the plasma transferrin pool. When iron requirements are increased, iron is donated to the plasma transferrin pool from storage sites or by increasing absorption through the villus enterocytes.

The analysis of inherited disorders of iron homeostasis has led to the identification of several molecules that play key roles in the regulation of iron traffic (**Table 1**). For example, the iron overload disorder hereditary haemochromatosis (HH) results from mutations in the genes encoding HFE (commonly), TfR2 (rarely) or Ireg1 (rarely). A more severe form of the disease, juvenile haemochromatosis (JH), has been linked with mutations in hepcidin or hemojuvelin (HJV). Several studies have shown that HFE (a MHC Class I-like protein) appears essential for hepcidin regulation (2, 3) and that it is able to bind to TfR1 at a site which overlaps with the Tf binding site (4). The current hypothesis for systemic regulation of iron homeostasis suggests that hepcidin is regulated by HFE and TfR2 in response to changes in Tf saturation, with HJV playing a modulatory role. Hepcidin then acts on its target cells, the enterocytes and reticuloendothelial macrophages, to modulate iron efflux.

Hepcidin

Hepcidin was originally isolated from human urine as an antimicrobial peptide (5), then later identified as a molecule upregulated during states of iron loading and downregulated during iron deficiency (6). A strong relationship between the regulation of iron homeostasis and hepcidin expression has been confirmed in two mouse models. In the first, the absence of hepcidin expression resulted in a severe iron loading phenotype (7). In the second, overexpression of hepcidin led to severe iron deficiency anemia and death shortly after birth (8). The relationship between hepcidin and iron homeostasis was confirmed in humans with the discovery of mutations in hepcidin in some patients with JH (9). Since its identification in 2001, hepcidin has been strongly implicated by multiple studies as a humoral factor playing a key role in the regulation of iron homeostasis.

How is Hepcidin Regulated?

Systemic stimuli for hepcidin regulation include altered iron requirements of the body (10), inflammation (11, 12), hypoxia (11) and pregnancy (13). However, the precise pathways that lead to altered hepcidin expression in response to such stimuli remain unclear. We hypothesised that diferric Tf plays a key role linking body iron requirements to hepcidin regulation by competing with HFE for binding to TfR1 at the hepatocyte cell surface (14). This hypothesis suggests that increased body iron levels lead to an increase in the concentration of diferric Tf which out-competes HFE for binding to TfR1. An increased amount of unbound HFE at the cell surface then signals to the nucleus and causes increased hepcidin production (10). In iron deficiency, where Tf saturation is decreased, there is an increase in HFE binding to TfR1 at the hepatocyte cell surface. A decrease in the level of free HFE on the plasma membrane leads to a reduction in the HFE-mediated signal for hepcidin expression. In support of this hypothesis, it is well known that Tf saturation, and therefore diferric Tf levels, are positively correlated with body iron status. We found that a decrease in the level of diferric transferrin preceded a decrease in hepcidin expression when body iron requirements were rapidly increased by phenylhydrazine-induced hemolysis (15). Further evidence in support of diferric Tf as a key signalling molecule has come from the demonstration that patients with mutations in TfR2, a Tf-binding cell surface molecule, develop iron overload (16) and have reduced hepcidin levels. We have thus proposed that hepcidin is regulated in response to changes in plasma diferric Tf levels by a dual pathway involving HFE and TfR2 (14).

Since the publication of this hypothesis in 2003, the gene mutated in most cases of JH (17) has been identified as HJV. Hepcidin levels are reduced in these patients, implying that HJV plays an upstream role in hepcidin regulation and, or synthesis. However, little is known about HJV and how it fits into the hepcidin regulatory pathway remains unclear.

The pathway described above may not be the only way in which hepcidin is regulated. During an acute phase response (APR), for example, a change in hepcidin expression precedes any alteration in Tf saturation (18). Induction of acute or chronic inflammation by the administration of lipopolysaccharide (6), Freund's complete adjuvant (18) or turpentine (11) causes a rapid increase in hepcidin expression (within eight hours) and a subsequent decrease in both intestinal iron absorption and iron release from the reticuloendothelial system resulting in depleted plasma iron levels. Hepcidin is essential for this response since hypoferraemia does not follow an acute phase stimulus in hepcidin deficient mice (11). We have recently shown that regulation of hepcidin in the APR is independent of HFE (12). There are, therefore, at least two independent regulatory pathways for hepcidin expression. Following an acute phase stimulus, hepcidin expression is induced by IL-6 (19) as part of the classical inflammatory response.

How Does Hepcidin Repress Iron Absorption?

For many years it has been considered that the intestinal crypt cell is programmed by body iron requirements to absorb more or less iron after it migrates up the villus and matures (1). However, the recent advances in our understanding of the biology of hepcidin suggest that such a scenario is unlikely. It is now considered that hepcidin, secreted from the liver, exerts a direct effect on iron export from mature intestinal enterocytes because the time between a change in hepcidin expression and the subsequent change in iron transport is too short to support the crypt cell maturation model (14). Our laboratory found a close inverse relationship between changes in hepcidin expression and changes in the level of intestinal iron transport molecules (Dcytb, DMT1 and Ireg1) after switching rats from a control diet to an iron deficient diet (10). We confirmed this close temporal relationship by examining hepcidin and iron transporter gene expression after induction of an acute phase response (18) or following phenylhydrazine-induced haemolysis (15). We and others (20) have since shown a direct effect of hepcidin on transporter expression *in vitro*, and a decrease in DMT1 and Ireg1 mRNA and protein expression when the human intestinal epithelial cell line Caco-2 is incubated with hepcidin.

The details of how hepcidin interacts with enterocytes and other cell types to alter iron transport are not known, but a growing body of evidence suggests that it is likely to repress iron release from these cells. Since Ireg1 is the major cellular iron export protein, an effect of hepcidin on the expression, activity or cellular localisation of Ireg1 might be predicted. Supporting this proposal, early kinetic studies indicated that the rate-limiting step for iron absorption was iron release from the enterocytes (1). In addition, recent molecular studies have shown that Ireg1 expression is responsive to changes in systemic iron levels, but not to variations in the enterocyte iron concentration, whereas the brush border transport system responds rapidly to changes in enterocyte iron content (14). These data are consistent with the proposal that basolateral export of iron from the intestinal epithelial cells represents the primary site at which iron absorption is regulated, whereby the locally responsive brush border transport components act to buffer the body against the absorption of excessive iron.

Conclusion

The marriage of contemporary genetic and molecular techniques with clinical and physiological studies has led to enormous advances in our understanding of intestinal iron absorption in recent years. The discovery of hepcidin as a central regulator of body iron homeostasis and the identification of a number of its target iron transport molecules have been key developments. Future challenges will centre on the detailed biochemical analysis of these molecules and the application of this analysis to develop therapeutic agents for human disorders where iron metabolism is disturbed.

References

1. Anderson, G.J. and Vulpe, C.D. (2002) in **Molecular and Cellular Iron Transport** (Templeton, D.M., ed), pp. 559-598, Marcel Dekker

2. Bridle, K.R., Frazer, D.M., Wilkins, S.J., Dixon, J.L., Purdie, D.M., Crawford, D.H., Subramaniam, V.N., Powell, L.W., Anderson, G.J., and Ramm, G.A. (2003) *Lancet* **361**, 669-673

3. Nicolas, G., Viatte, L., Lou, D.Q., Bennoun, M., Beaumont, C., Kahn, A., Andrews, N.C., and Vaulont, S. (2003) *Nat. Genet.* **34**, 97-101

4. West, A.P.Jr., Giannetti, A.M., Herr, A.B., Bennett, M.J., Nangiana, J.S., Pierce, J.R., Weiner, L.P., Snow, P.M., and Bjorkman, P.J. (2001) *J. Mol. Biol.* **313**, 385-397

5. Park, C.H., Valore, E.V., Waring, A.J., and Ganz, T. (2001) *J. Biol. Chem.* **276**, 7806-7810

6. Pigeon, C., Ilyin, G., Courselaud, B., Leroyer, P., Turlin, B., Brissot, P., and Loreal, O. (2001) *J. Biol. Chem.* **276**, 7811-7819

7. Nicolas, G., Bennoun, M., Devaux, I., Beaumont, C., Grandchamp, B., Kahn, A., and Vaulont, S. (2001) *Proc. Nat. Acad. Sci. USA* **98**, 8780-8785

8. Nicolas, G., Bennon, M., Porteu, A., Mativet, S., Beumont, C., Grandchamp, B., Sirito, M., Sawadogo, M., Kahn, A., Vaulont, S. (2002) *Proc. Nat. Acad. Sci. USA* **99**, 4596-4601

9. Roetto, A., Papanikolaou, G., Politou, M., Alberti, F., Girelli, D., Christakis, J., Loukopoulos, D., and Camaschella, C. (2003) *Nat. Genet.* **33**, 21-22

10. Frazer, D.M., Wilkins, S.J., Becker, E.M., Vulpe, C.D., McKie, A.T., Trinder, D., and Anderson, G.J. (2002) *Gastroenterology* **123**, 835-844

11. Nicolas, G., Chauvet, C., Viatte, L., Danan, J.L., Bigard, X., Devaux, I., Beaumont, C., Kahn, A., and Vaulont, S. (2002) *J. Clin. Invest.* **110**, 1037-1044

12. Frazer, D.M., Wilkins, S.J., Millard, K.N., McKie, A.T., Vulpe, C.D., and Anderson, G.J. (2004) *British J. Haematol.* **126**, 434-436

13. Millard, K.N., Frazer, D.M., Wilkins, S.J., and Anderson, G.J. (2004) *Gut* **53**, 655-660

14. Frazer, D.M., and Anderson, G.J. (2003) *Blood Cells Mol. Dis.* **30**, 288-297

15. Frazer, D.I., Ingles, H.R., Wilkins, S.J., Millard, K.N., Steele, T.M., Anderson, G.J. *Gut* in press

16. Roetto, A., Totaro, A., Piperno, A., Piga, A., Longo, F., Garozzo, G., Cali, A., De Gobbi, M., Gasparini, P., and Camaschella, C. (2001) *Blood* **97**, 2555-2560

17. Papanikolaou, G., Samuels, M.E., Ludwig, E.H., MacDonald, M.L., Franchini, P.L., Dube, M.P., Andres, L., MacFarlane, J., Sakellaropoulos, N., Politou, M., Nemeth, E., Thompson, J., Risler, J.K., Zaborowska, C., Babakaiff, R., Radomski, C.C., Pape, T.D., Davidas, O., Christakis, J., Brissot, P., Lockitch, G., Ganz, T., Hayden, M.R., and Goldberg, Y.P. (2004) *Nat. Genet.* **36**, 77-82

18. Anderson, G.J., Frazer, D.M., Wilkins, S.J., Becker, E.M., Millard, K.N., Murphy, T.L., McKie, A.T., and Vulpe, C.D. (2002) *Biochem. Soc. Trans.* **30**, 724-726

19. Nemeth, E., Valore, E.V., Territo, M., Schiller, G., Lichtenstein, A., and Ganz, T. (2003) *Blood* **101**, 2461-2463

20. Yamaji, S., Sharp, P., Ramesh, B., and Srai, S.K. (2004) *Blood* in press