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Mechanisms of haem and non-haem iron absorption: Lessons from inherited disorders of iron metabolism

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Abstract

Our current state of knowledge of the mechanism and regulation of intestinal iron absorption has been critically dependent on the analysis of inherited disorders of iron homeostasis in both humans and other animal species. Mutations in DMT1 and Ireg1 have revealed that these molecules are major mediators of iron transport across the brush border and basolateral membranes of the enterocyte, respectively. Similarly, the iron oxidase hephaestin has been shown to play an important role in basolateral iron efflux. The analysis of a range of human iron loading disorders has provided very strong evidence that the products of the HFE, TfR2, hepcidin and hemojuvelin genes comprise integral components of the machinery that regulates iron absorption and iron traffic around the body. Engineered mouse strains have already proved very effective in helping to dissect pathways of iron homeostasis, and in the future they will continue to provide important insights into the absorption of both inorganic and haem iron by the gut.

Introduction

An adequate supply of iron is essential for good health. Iron is required for many enzymes that are critical for cellular function, and plays a fundamental role in oxygen carrying proteins such as haemoglobin and myoglobin. Iron can also be toxic when present in excess as it is able to catalyze the formation of reactive oxygen species. Because of this dual nature, the concentration of iron in the body must be kept within defined limits and there are precise mechanisms governing the regulation of iron homeostasis. In humans there is no active mechanism for the excretion of iron, so the amount of iron in the body is determined by the regulation of iron absorption in the proximal small intestine (Anderson & Vulpe 2002).

Iron is present in the diet as one of two forms – as inorganic iron or haem iron. Of these, inorganic iron is by far the most prevalent. It is the predominant form of iron in dietary components of plant origin and accounts for 80–90% of the iron in a standard diet (Hallberg 1981; Carpenter & Mahoney 1992), with the remaining 10% is as haem iron. This is derived primarily from haemoglobin and myoglobin and thus is mainly associated with meat intake. Despite its relative paucity, haem iron is absorbed far more efficiently than non-haem iron and may contribute up to 50% of the iron that actually enters the body (Hallberg 1981; Bezwoda *et al.* 1983; Carpenter & Mahoney 1992). This pathway assumes particular importance in many developed countries where the average meat consumption is relatively high.

The analysis of inherited disorders of iron metabolism in both humans and animals has in large part been responsible for the great advances in our understanding of the mechanisms of inorganic iron metabolism in recent years, and this approach is likely to be equally productive for the analysis of haem iron absorption. A summary of the pathways known to be involved in the absorption

of dietary iron and its regulation by body iron requirements is provided in Figure 1.

The pathway of inorganic iron absorption

Most inorganic iron in the diet is in the oxidized or ferric form, Fe(III), but this must first be reduced to Fe(II) before it can be taken up by the intestinal epithelial cells or enterocytes. A strong candidate for facilitating this reduction is the brush border iron reductase Dcytb (McKie *et al*. 2001). The resulting ferrous iron is then transported across the brush border membrane via divalent metal transporter 1 (DMT1) (Fleming *et al*. 1997; Gunshin *et al*. 1997) and into the enterocyte cytoplasm. If iron is required by the body it rapidly moves across the basolateral membrane and into the circulation. The basolateral transfer of iron is likely to be mediated by the membrane protein IREG1 (also known as ferroportin1) (Donovan *et al*. 2000; McKie *et al*. 2000). Iron efflux from the enterocyte also requires the action of an enzyme, the copper-containing iron oxidase hephaestin (Vulpe *et al*. 1999). While the pathways by which inorganic iron and haem iron are taken up from the intestinal lumen by the enterocytes vary, it has been proposed that their basolateral export from the enterocytes, i.e. the step involving hepaestin and IREG1, is common to iron from both sources.

Haem iron absorption

In most non-vegetarian diets, more than one-third of the total daily iron intake is supplied by dietary haemoglobin and myoglobin, although haem iron accounts for only 10–15% of the dietary iron content (Hallberg 1981; Bezwoda *et al*. 1983; Carpenter & Mahoney 1992). Indeed, many studies using radiolabelled haem or haemoglobin have established that the intestinal absorption of haem is far more efficient than that of inorganic iron (e.g. Turnbull *et al*. 1962; Layrisse & Martinez-Torres 1972; Hallberg 1981; Bezwoda *et al*. 1983). Before haem iron can be utilized, haem must be released from proteins such as haemoglobin and myoglobin by proteolytic activity in the lumen of the stomach and small intestine (Conrad *et al*. 1966; 1967). While the chemical form of the resulting 'free' haem has not

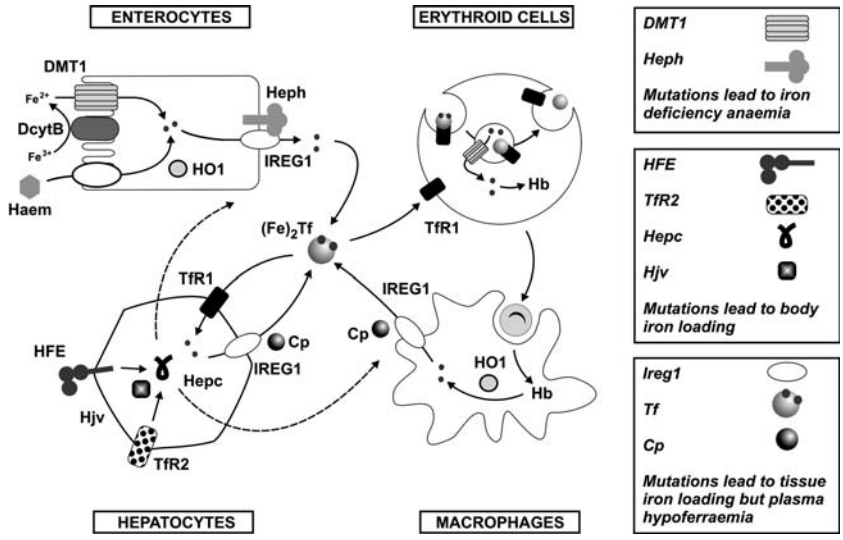

Figure 1. Major pathways of iron homeostasis and its regulation. Dietary iron in either the inorganic or haem form traverses first the brush border membrane then the basolateral membrane of intestinal enterocytes and binds to plasma transferrin. This diferric transferrin supplies iron to immature erythroid cells for haemoglobin synthesis as well as other body cells for their metabolic needs. At the end of their life senescent red cells are phagocytosed by macrophages and iron released from haem by haem oxygenase 1 is returned to the circulation. Excess iron is stored in the liver and this can also be utilized in times of need. The liver-derived peptide hepcidin acts to repress basolateral iron transport in the gut and iron release from macrophages and other cells. Hepcidin in turn respond to signals mediated by HFE, TfR2 and hemojuvelin. The panels on the right summarize the consequences of mutations in various iron related genes. Cp, ceruloplasmin; Dcytb, duodenal cytochrome b; DMT1, divalent metal transporter 1; $(Fe)_2Tf$, diferric transferrin; Hb, haemoglobin; heph, hephaestin; Hjv, hemojuvelin; HO1, haem oxygenase 1; TfR1, transferrin receptor 1; TfR2, transferrin receptor 2.

been well defined, it is likely to be stabilized by various dietary constituents, including haemoglobin degradation products. This is important as unstabilized haem readily forms dimers and higher aggregates in aqueous solution (Conrad *et al.* 1966). The haem moiety, with its bound iron, is then absorbed intact by the intestinal enterocytes (Hallberg 1981). The fact that iron is not released from haem prior to its cellular uptake explains why haem iron is less susceptible to variations in dietary composition than inorganic iron, which readily binds to a wide range of substances in the diet and which greatly reduces its bioavailability (Hallberg & Solvell 1967; Weintraub *et al.* 1968; Wheby *et al.* 1970; Levine *et al.* 1988).

The pathway by which haem iron is absorbed is less well defined than that of inorganic iron, however, in the small intestine there is clear evidence for a haem binding protein. Grasbeck *et al.* (1979; 1982) used ^{14}C-labelled haem to identify a haem-binding protein in pig intestine with a dissociation constant of 10^{-6} –10^{-7} M. The protein required detergent extraction for solubilization, and haem binding was saturable and sensitive to trypsin treatment, characteristics consistent with an integral membrane protein. Haem binding was found to be highest in duodenum and progressively lower distally. Roberts *et al.* (1993) also described specific haem binding to isolated brush border membranes from the rat intestine. Evidence for the specific uptake of haem has also been found by examining the uptake of the fluorescent haem analogue zinc mesoporphyrin by the human intestinal cell line Caco2 (Worthington *et al.* 2001). More recently several candidate haem-binding proteins have been identified in the small intestine (Krishnamurthy *et al.* 2004; McKie *et al.* unpublished observations) and the role of these in dietary haem absorption is currently being evaluated. Haem binding/transport has also been reported for a variety of other organisms and cell types (Smith 1990), and most notably by erythroleukemia cells (Galbraith *et al.* 1985; Majuri 1989).

The pathway of haem uptake by enterocytes has also been examined morphologically in both rats and dogs using electron microscopy (Parmley *et al.* 1981; Wyllie & Kaufman 1982). Haem was detected by autoradiography after labelling with ^{59}Fe, or by using its endogenous peroxidase activity. Haem was first observed on the apical surface of the enterocytes, then sequentially in pits/caveolae between the microvilli, in membrane-bound tubules in the apical cytoplasm, and finally in secondary lysosomes. The precise details of this pathway and the identity of the organelles involved have yet to be defined. Within the epithelial cell, iron is released from the protoporphyrin moiety, most likely by haem oxygenase 1 (HO-1) (Weintraub *et al.* 1968; Raffin *et al.* 1974; Hartmann & Bissell 1982). Indeed, HO inhibitors are able to inhibit the uptake of haem iron (Boni *et al.* 1993). The protoporphyrin is then degraded and the iron is proposed to join the same pathway as inorganic iron. Several studies have followed the uptake of haem containing ^{59}Fe or ^{55}Fe and have not been able to detect intact haem in the mesenteric circulation (Wheby *et al.* 1970; Worthington *et al.* 2001; Smith *et al.* unpublished observations), consistent with its degradation in the enterocyte. In addition, the finding that inorganic iron is able to reduce the absorption of haem iron, and vice versa (Hallberg & Solvell 1967), supports the concept that the two forms of iron share some components of their absorption pathway.

The regulation of iron absorption

Iron absorption is a tightly regulated process which responds to changes in body iron demand (Anderson & Vulpe 2002). The regulation of inorganic iron absorption has been studied extensively and, if haem iron absorption shares part of the absorption pathway of inorganic iron, then some regulation of haem absorption would also be expected. Most studies have shown that iron deficiency is able to stimulate the absorption of both haem iron and inorganic iron, although the stimulation is less for haem iron (Turnbull *et al.* 1962; Bannerman 1965; Hallberg & Solvell 1967; Wheby *et al.* 1970; Raffin *et al.* 1974; Hallberg *et al.* 1979; Wyllie & Kaufman 1982; Roberts *et al.* 1993). Enhanced erythropoiesis is another potent stimulus of iron absorption. Weintraub *et al.* (1965) found that haem iron absorption did not change after bleeding in rats despite a threefold increase in inorganic iron absorption, but a subsequent study in dogs by the same group (Weintraub *et al.* 1968) did observe an increase, and human blood donors also have increased haem absorption (Hallberg & Solvell 1967). These studies highlight the fact that there are species

differences in the absorption of haem iron. While the basic pathways followed by haem as it crosses the intestinal epithelium appear to be similar in the species studied, there are quantitative differences in the relative contribution of haem iron to the total amount of iron absorbed, with haem iron absorption being most important in humans (Bannerman 1965; Weintraub *et al.* 1965; Hallberg & Solvell 1967; Wheby *et al.* 1970).

At the molecular level, the key systemic regulator of iron absorption is the liver-derived peptide hepcidin (Frazer & Anderson 2003). Hepcidin appears to be a repressor of iron absorption and recent evidence has suggested that it decreases basolateral iron export from enterocytes by facilitating the internalization of Ireg1 (Nemeth *et al.* 2004b). The expression of hepcidin is decreased when body iron requirements are high, such as when iron stores decrease or the rate of erythropoiesis increases, and increased when iron requirements decline (Frazer *et al.* 2002). How changes in iron requirements are sensed by the body is unclear, although the liver seems to play a central role. Data that have emerged in recent years have also shown that HFE, TfR2 and hemojuvelin are all involved in the expression of hepcidin and these may form part of the sensing mechanism.

Inherited disorders of iron metabolism

If a molecule plays an important role in iron homeostasis then it might be predicted that a mutation leading to modification of the function of that molecule would result in a distinctive iron-related phenotype. This is indeed the case and the role of almost all of the molecules known to be important in iron transport and its regulation has been confirmed by the analysis of inherited disorders of iron metabolism. Traditionally these conditions mainly comprise human diseases of iron homeostasis, although mutations in key iron metabolism genes have occasionally arisen in various animal species as well. In addition to these there is a growing number of induced mutations in experimental rodents. These include mutants induced by chemical or radiation treatment, as well as the more contemporary knockout and transgenic mouse strains. Summaries of mutations in some molecules and the resulting phenotypes are provided in Table 1 and Figure 1.

Inherited disorders of iron deficiency

Inherited disorders of iron metabolism leading to iron deficiency are rare in humans and this may attest to the essentiality of the metal. However, there are a number of reports of inherited refractory anaemias which cannot be accounted for by environmental factors or co-existing disease that likely represent defects in iron transport (Anderson & McLaren 2000). To date, in only one case has such a condition been attributed to a mutation in any of the known molecules of iron transport, with Mims *et al.* (2004) recently describing a patient carrying a mutation in DMT1 who exhibited a severe hypochromic, microcytic anaemia. A similar phenotype has been observed in the *mk* mouse, the Belgrade rat, and a mutant Zebrafish strain (Fleming *et al.* 1997; 1998; Donovan *et al.* 2002). In each case this anaemia is attributed to a mutation in DMT1. In fact, the positional cloning of the gene affected in the *mk* mouse was one approach that led to the original identification of DMT1 (Fleming *et al.* 1997). These studies provide very strong evidence that DMT1 plays a critical role in intestinal iron absorption. Extensive evidence that has arisen since the DMT1 gene was cloned has indicated that it is the molecule primarily responsible for the uptake of inorganic iron from the diet (Morgan & Oates 2002), but whether it also may play a role in the absorption of haem iron remains to be determined.

Another mouse strain with an inherited microcytic, hypochromic anaemia is the *sla* mouse (Russell 1979). Using a positional candidate approach, the gene defective in *sla* mice was identified as the ceruloplasmin homolog hephaestin (Vulpe *et al.* 1999). While many aspects of the biology of hephaestin remain to be clarified, analysis of the phenotype of the *sla* mouse has shown that hephaestin is an iron oxidase that plays a key role in the export of iron from the intestinal enterocytes into the circulation. More recently we have also used this mouse strain to demonstrate that molecules involved in the transport of iron across the brush border can be regulated by the local enterocyte iron concentration, while basolateral membrane iron transport molecules re-

Table 1. Inherited defects of iron metabolism in selected genes.

Gene	Function of protein	Species	Origin of mutation[a]	Phenotype
Ceruloplasmin	Systemic iron oxidase	Human	Spontaneous	Plasma hypoferraemia
		Mouse	Engineered	with tissue iron loading
Dcytb	Iron reductase		None known	
DMT1	Ferrous iron importer	Human	Spontaneous	Iron deficiency anaemia
		Mouse	Spontaneous	
		Rat	Induced	
		Zebrafish	Induced	
H-Ferritin	Iron storage	Human	Spontaneous (in 5′ UTR)	Iron loading
L-Ferritin	Iron storage	Human	Spontaneous (in 5′ UTR)	Bilateral cataracts
Haem oxygenase 1	Degradation of haem	Mouse	Engineered	Plasma hypoferraemia with tissue iron loading
Hemojuvelin	Regulation of iron homeostasis	Human	Spontaneous	Iron loading
Hepcidin	Systemic regulator of iron metabolism	Human	Spontaneous	Iron loading
		Mouse	Engineered	
Hephaestin	Intestinal iron oxidase	Mouse	Induced	Iron deficiency anaemia
HFE	Regulator of iron homeostasis	Human	Spontaneous	Iron loading
		Mouse	Engineered	
Ireg1	Ferrous iron exporter	Human	Spontaneous	Plasma hypoferraemia
		Zebrafish	Induced	with tissue iron loading
Transferrin	Plasma iron transport protein	Human	Spontaneous	Plasma hypoferraemia
		Mouse	Spontaneous	with tissue iron loading
Transferrin receptor 1	Uptake of transferrin	Mouse	Engineered	Severe anaemia and other abnormalities
Transferrin receptor 2	Uptake of transferrin;	Human	Spontaneous	Iron loading
	Regulator of iron homeostasis	Mouse	Engineered	

[a]Spontaneous mutations arose *de novo*; Induced mutations were detected following treatment of animals with radiation or chemicals; Engineered mutations were generated using knockout/transgenic mouse technology.

spond primarily to systemic stimuli (Chen *et al.* 2003). A protein involved in iron export from the enterocyte should affect inorganic and haem iron absorption similarly if iron from both sources share the same efflux pathway. Although this has yet to be investigated exhaustively, preliminary studies by our group suggest that this is the case.

Since Ireg1 is also involved in export from enterocytes, one would expect that mutations in Ireg1 leading to loss of function would result in an iron deficient phenotype. In Zebrafish with Ireg1 mutations this is the case (Donovan *et al.* 2000). Human subjects with mutations in this molecule show iron deficiency early in the disease, but paradoxically, these patients ultimately show signs of iron loading and this is the cause of the clinical consequences of the disease (Pietrangelo 2004a, b). This paradox is explained with the recognition that Ireg1 is involved in the efflux of iron not only from enterocytes but from most other body cells as well. As a result, iron recirculation through the reticuolendothelial macrophages is impaired in affected individuals and the internal organs become iron loaded at the expense of plasma iron.

Transferrin has in the past been considered to play an important role in the passage of iron across the intestinal epithelium, although the accumulated evidence now suggests that such a role is highly unlikely (Anderson *et al.* 1990). In fact, rare patients with congenital transferrin deficiency actually hyperabsorb iron (Bernstein 1987; Hayashi *et al.* 1993). This non-transferrin bound iron is very rapidly taken up by tissues, particularly the liver, and individuals become iron loaded. However, they also develop a significant anaemia as iron supply to the immature erythroid cells, which are highly dependent on diferric transferrin as their iron source, is severely reduced. Mutations in another plasma protein, ceruloplasmin, also lead to iron loading of the tissues in the

face of systemic anaemia (Gitlin 1998) but through a different mechanism. Ceruloplasmin has long been known to be required for iron release from cells, and particularly macrophages, so it plays a critical role in the recycling of iron from phagocytosed senescent red cells. If there is a defect leading to a reduction in functional ceruloplasmin, this recycling of iron is very inefficient (Harris *et al*. 1999). Interestingly, ceruloplasmin does not appear to be required for iron release by intestinal enterocytes (Brittin & Chee 1966), and that role falls to its membrane-bound homolog hephaestin.

Primary iron overload diseases

Inherited disorders of iron metabolism in humans for the most part consist of iron overload diseases or haemochromatosis (HC). Mutations in HFE, TfR2, hepcidin, hemojuvelin, Ireg1, transferrin, ceruloplasmin and ferritin all lead to some form of iron loading. Of these, mutations in the *HFE* gene are by far the most common and HFE-associated HC is regarded as the most prevalent autosomally inherited genetic disease of Caucasians (Busfield *et al*. 2002). Defects in HFE, TfR2, hepcidin and hemojuvelin lead to phenotypically very similar forms of iron loading and this provides strong evidence that these proteins form part of the same regulatory pathway (Frazer & Anderson 2003). In each of these conditions, the iron loading results from an increase in iron absorption that in turn reflects the inability of the normal regulatory machinery to accurately reflect body iron requirements. Thus the regulatory pathway perceives the body as iron deficient despite excessive iron accumulation and iron absorption continues.

Patients with HFE-associated HC absorb excessive amounts of iron from birth and this leads to progressive iron accumulation throughout life in many tissues, but notably the liver' heart and pancreas (Anderson & Powell 2002; Pietrangelo 2004b). While a normal adult male may have approximately 1 g of storage iron in his body, in HC patients this may reach 20 or 30 g or more before clinical symptoms become apparent. In the early stages of the disease these symptoms are quite non-specific e.g. lethargy, but in more advanced disease hepatic fibrosis and cirrhosis, diabetes, arthropathy, cardiac problems and pituitary dysfunction may occur. If the disease remains untreated, end stage liver disease, hepatocellular carcinoma or cardiomyopathy may result. Iron initially is deposited in the parenchymal cells of the liver and other organs, but in advanced iron loading, other cell types may also be affected (Pietrangelo 2004b). The phenotype of patients with iron overload due to mutations in TfR2 is very similar to that of patients with HFE-associated HC (Camaschella *et al*. 2000) and such individuals normally present in the fourth of fifth decade of life. However, while patients carrying mutations in hemojuvelin or hepcidin show a similar histological pattern of iron distribution, they present with much more severe disease, usually in the second or third decade of life (Roetto *et al*. 2003; Papanikolaou *et al*. 2004). For this reason such forms of iron loading have been referred to as juvenile HC.

The inability of HC patients to effectively regulate their iron absorption has been recognised for many years, and the consensus of a number of earlier studies is that it is the basolateral efflux of iron from the enterocytes that is inappropriately increased in affected individuals (Powell *et al*. 1970; McLaren *et al*. 1991). These studies also showed that both inorganic and haem iron absorption were increased in HC (Lynch *et al*. 1989), a feature that would be expected if iron from both dietary sources shares the same efflux pathway from enterocytes as predicted. Recent molecular studies have provided strong support for dysregulated basolateral iron transport in HC. The most significant finding is that hepcidin levels in patients with HFE-associated HC are very low (Bridle *et al*. 2003). In a normal individual an increase in body iron levels would lead to increased hepcidin expression and this in turn would decrease iron absorption by reducing basolateral iron efflux. Such an increase in hepcidin does not occur in HC patients and this explains why their iron absorption continues in the face of body iron loading. These studies, and the finding that HFE is most strongly expressed in the liver, have also indicated that the main role of HFE is to act in the liver as part of body's mechanism for sensing iron requirements (Frazer & Anderson 2003). Previous studies had suggested HFE exerted its primary role in the small intestine. Recent studies have also shown that hepcidin levels are reduced in patients with mutations in TfR2 and hemojuvelin (Nemeth *et al*. 2004a; Papanikolaou *et al*. 2004), supporting

the contention that these molecules are part of the same regulatory pathway. The relationship between these molecules will be an important focus of future research in this field.

As noted above, mutations in Ireg1 can also lead to iron loading (known as Type 4 HC or ferroportin disease), although anaemia is often present in the early stages of the disease. Overall the prevalence of mutations in Ireg1 is quite low, but they appear to account for many cases of non-HFE HC in some areas, such as southern Europe (Pietrangelo 2004a). The iron overload phenotype in patients with Type 4 HC appears to result from reduced function of Ireg1 in extraintestinal tissues and will not be considered further here.

While the analysis of inherited disorders of iron overload has already taught us a great deal about the regulation of intestinal iron absorption many of the details have yet to be elucidated. This research will be aided considerably by the availability of a number of important mouse models of iron overload. For example, several engineered mouse strains lacking HFE, TfR2 or hepcidin expression have been developed, as have several strains in which HFE has been mutated (Nicolas *et al*. 2001; Fleming *et al*. 2002; Pietrangelo 2003; Wallace *et al*. 2004). The major histological features of the corresponding human disease are reproduced in each of these strains and the animals have already been used extensively to look at aspects of the phenotype under controlled conditions on uniform genetic backgrounds. Other strains in which hemojuvelin and Ireg1 have been disrupted are currently being developed.

Increased iron absorption and disorders of erythropoiesis

Erythropoiesis is a major stimulus for iron absorption and in diseases where erythropoiesis is markedly elevated increased iron absorption may result. A number of disorders of haemoglobin production fall into this category, including homozygous β-thalassaemia, compound heterozygosity for β-thalassaemia and haemoglobin E, and haemoglobin H disease (Gordeuk *et al*. 1994). Absorption is increased in these conditions as the demand for iron by the erythroid marrow exceeds the rate at which it can be recycled through the reticuloendothelial system. Other disorders of red cell production that are associated with ineffective erythropoiesis and increased iron absorption are the sideroblastic anaemias (Sheth & Brittenham 2000) and hereditary pyruvate kinase deficiency (Andersen *et al*. 2004). These disorders and their corresponding animal models are valuable not so much to help define the process of iron passage across the intestinal epithelium *per se*, but to examine how erythroid iron demand influences absorption. Since stimulated erythropoiesis leads to an increase in the absorption of both inorganic and haem iron, the analysis of these disorders has widespread application.

Other disorders

The potential of inherited disorders of iron metabolism to make further significant contributions to our understanding of iron physiology remains high. For example, there are a number of such disorders where the affected gene has yet to be identified. Most of the cases of inherited refractory anaemias that have been described in humans have not yet been attributed to any of the molecules known to be involved in iron absorption or its regulation. In addition, there are a number of murine anaemias where the defective gene is unknown (e.g. the haemoglobin deficit or *hbd* mouse (Bannerman et al. 1986)). Iron absorption is a complex physiological process and it is highly likely that a number of important molecules involved have yet to be identified.

In addition to novel molecules, the role of existing protein requires clarification. For example, while there is strong circumstantial evidence that haem oxygenase 1 is involved in the degradation of haem in the enterocyte and the release of haem-bound iron prior to its passage into the portal circulation, definitive proof of this is lacking. Analysis of the phenotype of the HO1 knockout mouse (Poss & Tonegawa 1997) should provide the necessary clarification in this area.

Conclusions

The significant advances that have been made in understanding the absorption of inorganic iron in recent years have been largely underpinned by the

investigation of inherited disorders of iron homeostasis. Such studies have led to the identification of a number of the key molecules involved in both the passage of iron across the intestinal epithelium and the regulation of this pathway. Furthermore, the ability to examine the phenotypes resulting from mutations in these molecules has been of enormous value in understanding their physiological roles. However, little has been learned about the mechanism of haem iron absorption despite the disproportionately high contribution haem iron makes to iron nutrition, and this remains one of the most significant unresolved issues of body iron homeostasis. While some of the genetic models described in this review are highly relevant to the analysis of haem iron absorption, many of the required studies have yet to be carried out, and engineered mouse strains with defects in molecules specifically involved in haem transport need to be developed. This remains a major challenge for the future.

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