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TOPIC HIGHLIGHT

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Molecular mechanisms involved in intestinal iron absorption

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Abstract

Iron is an essential trace metal in the human diet due to its obligate role in a number of metabolic processes. In the diet, iron is present in a number of different forms, generally described as haem (from haemoglobin and myoglobin in animal tissue) and non-haem iron (including ferric oxides and salts, ferritin and lactoferrin). This review describes the molecular mechanisms that co-ordinate the absorption of iron from the diet and its release into the circulation. While many components of the iron transport pathway have been elucidated, a number of key issues still remain to be resolved. Future work in this area will provide a clearer picture regarding the transcellular flux of iron and its regulation by dietary and humoral factors.

Key words: Haem; Non-haem iron; DMT1; IREG1; Dcytb; Hephaestin

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INTRODUCTION

Iron is an essential trace metal for all organisms. In humans it plays numerous biochemical roles, including oxygen binding in haemoglobin and as an important catalytic centre in many enzymes, for example the cytochromes. In normal healthy adults, some 0.5-2 mg of iron is lost each day due to blood loss and the constant exfoliation of iron-containing epithelial cells that line the gastrointestinal and urinary tracts, skin and hair. Therefore, the same amount of iron from dietary sources is required each day to replace the lost iron and maintain body iron homeostasis. Even though iron is an essential metal in human metabolism, it is highly toxic to cells and tissues if present in elevated levels. Perversely, humans do not possess the necessary machinery to rid the body of excess iron and, therefore, the absorptive process must be tightly regulated within defined physiological limits to avoid pathologies associated with both iron deficiency and overload.

Dietary iron is found in two basic forms, either as haem-found in meat and meat products-or non-haem iron-present in cereals, vegetables, pulses, beans, fruits *etc* in a number of forms ranging from simple iron oxides and salts to more complex organic chelates. Non-haem iron predominates in all diets comprising some 90%-95% of total daily iron intake. The absorption of both haem and non-haem iron takes place almost exclusively in the duodenum and the bioavailability of iron from these sources is influenced by a number of variables, e.g. the iron content of foods, the type of iron present, i.e. haem or non-haem, and other dietary constituents. Importantly, absorption is also regulated in line with metabolic demands that reflect the amount of iron stored in the body, and the requirements for red blood cell production. These humoral mechanisms are further reviewed in this volume[1].

Despite accounting for only 5%-10% of dietary iron in western countries, haem is the most bioavailable source of iron amounting to is some 20%-30%[2]. In contrast, the bioavailability of non-haem iron is low-only 1%-10% of the dietary load is absorbed-and is profoundly influenced by other dietary components that can enhance or inhibit non-haem iron bioavailability. The most potent enhancer is ascorbic acid (vitamin C), which acts by reducing ferric iron to the more soluble and absorbable ferrous form. Phytates found in cereal products and polyphenolic compounds found in all plant products are the most potent dietary inhibitors of non-haem iron absorption. However, it is important to note that on an equimolar basis the pro-absorptive action of dietary ascorbic acid can counteract the inhibitory effect of phytates and polyphenols[3].

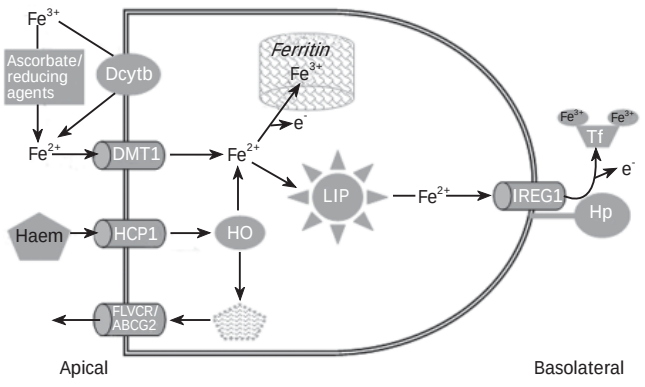

Figure 1 The cellular mechanisms involved in intestinal iron absorption. Dietary non-haem iron (mostly ferric) is reduced by the actions of the ferric reductase Dcytb and reducing agents in the diet to yield Fe^{2+}, which subsequently enters the enterocytes *via* DMT1. Haem is absorbed *via* HCP1, broken down by haem oxygenase 1 (HO) to liberate Fe^{2+} (this joins a common pool with iron from the non-haem pathway) and bilirubin (which might be removed from the cell by the efflux proteins FLVCR and ABCG2). If body iron stores are high, iron may be diverted into ferritin and lost when the cell is shed at the villus tip. Alternatively, iron passes into the labile iron pool (LIP) and is subsequently processed for efflux *via* IREG1 (as Fe^{2+}). The exiting iron is re-oxidised to Fe^{3+} through hephaestin (Hp) to enable loading onto transferrin (Tf).

MECHANISMS INVOLVED IN INTESTINAL IRON TRANSPORT

In recent years our understanding of the mechanisms involved in dietary iron absorption by duodenal enterocytes has increased dramatically. Both haem and non-haem iron are taken up in this proximal region of the small intestine, though their transport across the apical membrane of the enterocytes occurs through totally independent pathways (Figure 1).

Ferrous iron

The majority of dietary non-haem iron enters the gastrointestinal tract in the ferric form. However, Fe^{3+} is thought to be essentially non-bioavailable (see below) and, therefore, it must first be converted to ferrous iron prior to absorption. There are numerous dietary components capable of reducing Fe^{3+} to Fe^{2+}, including ascorbic acid[4], and amino acids such as cysteine[5] and histidine[6]. It is believed that the action of these dietary reducing agents takes place in the acidic environment of the gastric lumen. Indeed the essential requirement for an acid environment in iron absorption is exemplified by the fact that achlorhydria is commonly associated with iron deficiency anaemia[7,8]. However, ferric iron reaching the duodenal enterocytes may still be reduced by the cells endogenous reducing activity. A number of studies have demonstrated that the brush-border surface of duodenal enterocytes and cultured intestinal cells possess ferric reductase enzymic activity[9-11]. Using a subtractive cloning strategy designed to identify intestinal genes involved in iron absorption, Dcytb (for duodenal cytochrome b), a homologue of cytochrome b_{561}, was identified as the enzyme responsible for this process[12]. Like cytochrome b_{561}, Dcytb is a haem-containing protein with putative binding sites for ascorbate and semi-dehydroascorbate. The protein is expressed on the brush border membrane of duodenal enterocytes, the major site for the absorption of dietary iron. Moreover, antibodies raised against Dcytb block the endogenous ferric reductase activity of the duodenal brush border membrane, providing further evidence for a functional role in dietary iron processing[12].

Intriguingly, a recent study has reported that the targeted disruption of the *Cybrd1* gene in mice (which encodes Dcytb) does not lead to an iron deficient phenotype[13], implying that Dcytb is not necessary for intestinal iron absorption in mice. However, an important caveat to this study is the fact that mice are able to synthesize significant quantities of ascorbic acid-unlike humans who are reliant on dietary vitamin C to satisfy their requirements-and may, therefore, have less need for a duodenal surface ferric reductase.

Following reduction either by Dcytb or dietary reducing agents, the resulting Fe^{2+} becomes a substrate for the divalent metal transporter, DMT1-also known as the divalent cation transporter, DCT1[14], and natural resistance associated macrophage protein, Nramp2[15]. The relatively low pH of the proximal duodenum together with the acid microclimate present at the brush border membrane[16,17] stabilises iron in the ferrous form and provides a rich source of protons that are essential for driving iron uptake across the apical membrane of the intestinal epithelium[14,18].

The role of this transporter in intestinal iron homeostasis has been highlighted by a number of studies. Our work[18] and that of others[19] have shown that antibodies to DMT1 can significantly inhibit iron absorption. Furthermore, targeted disruption of DMT1 in mice has revealed the essential role of this transporter in both intestinal iron absorption and in erythroid precursor development[20]. In addition to these biochemical and molecular manipulations, two rodent models, the *mk/mk* mouse[15] and the Belgrade (*b*) rat[21], which posses a spontaneous mutation (G185R) in the DMT1 gene, exhibit defective intestinal iron uptake and microcytic anaemia. More recently a number of mutations in human DMT1 have also been identified[22-25] which in turn lead to the development of microcytic anaemia.

The molecular identity of the functional DMT1 isoform in intestinal epithelial cells has been the subject of recent debate. At least four isoforms exist through alternate splicing in exon 16[26] and the presence of two transcription start sites-named exon 1A and 1B[27]. Exon 16 splicing gives rise to two variants which differ in their terminal 19-25 amino acids and their 3' untranslated sequence (UTR). Interestingly one of these variants contains an iron responsive element in its 3' UTR[26]. The significance of this is discussed later in this review. All four isoforms can be detected at varying levels in intestinal epithelial cells[27], but the exon 1A/IRE-containing variant has been suggested to be the major functional isoform in absorptive enterocytes.

Ferric iron

We stated earlier that Fe^{3+} is thought to be non-bioavailable. However, some workers have proposed that Fe^{3+} might be absorbed by intestinal enterocytes *via* a

mechanism that is distinct from DMT1 (reviewed in[28]). In this model, ferric iron, which is insoluble at physiological pH, is released from the food matrix in the acidic environment of the stomach, and is chelated by mucins on the duodenal brush border surface, which maintain the iron in the ferric state. Fe^{3+} enters the enterocyte across the apical membrane via interaction with β_3-integrin and mobilferrin (a calreticulin homologue). In the cytosol, this complex combines with flavin monooxygenase and β_2-microglobulin to form a larger conglomerate (approximately 520 kDa) known as paraferritin, which has ferric reductase activity resulting in the conversion of the absorbed Fe^{3+} to Fe^{2+}. Recent evidence suggests that the paraferritin complex may also contain DMT1[29], which may permit the delivery of ferrous iron to intracellular organelles.

Ferritin

In animal and plant tissues, the major iron storage protein is ferritin. Most nutrition texts focus only on haem and non-haem iron, and generally ignore the possibility that ferritin may be an important nutritional source of iron. While the issue of ferritin bioavailability is still controversial, a number of studies have shown that both plant and animal ferritin sources are absorbed in humans[30-32] with a bioavailability equivalent to that of ferrous sulphate. The ferritin iron uptake mechanism is yet to be determined. One possibility is that ferritin is broken down by protease activity in the upper gastrointestinal tract and the released iron is absorbed *via* the Dcytb/DMT1 route. However, studies have shown that ferritin is largely resistant to high temperature, low pH and protein denaturing agents[33,34]. Therefore, it is possible that ferritin may be absorbed intact and broken down intracellularly (in the lysosomes) to liberate its iron load. In support of this latter possibility, one study has reported that iron and ferritin protein are both taken up by the intestinal Caco-2 cell line[35]. Such a mechanism would require the presence of a ferritin receptor on the apical membrane of intestinal enterocytes. While the presence of ferritin receptors has been postulated on liver[36] and placental[37] plasma membranes, none has yet been identified in intestinal tissue. Taken together this evidence suggests that the molecular identity of at least one important intestinal iron transport gene may remain to be discovered.

Lactoferrin

In breast-fed infants, a major proportion of iron is bound to the human milk protein lactoferrin, an iron-binding protein capable of binding two ferric ions[38]. Specific receptors for lactoferrin have been identified on the brush border surface of foetal enterocytes[39] and subsequent studies have shown that these receptors mediate the uptake of lactoferrin-bound iron in intestinal epithelial cell cultures[40]. Interestingly, a recent study looking at ontogenic changes in the expression of iron transport proteins in mouse small intestine, has suggested that the lactoferrin receptor may be the principal iron transport pathway in early life[41]. Intriguingly, a recent human volunteer study has indicated that the nutritional importance of lactoferrin may not be limited to infants since it is also a bioavailable source of iron (with equivalent bioavailability to ferrous sulphate) in young adult females[42].

Haem

Non-vegetarian diets provide an additional and important source of iron in the form of haem (largely from haemoglobin and myoglobin). While haem comprises only approximately 10% of dietary iron intake, because it is more bioavailable than non-haem iron, it may contribute as much as half of the total iron absorbed from western meat-rich diets[43]. Despite the wealth of information available on the uptake of non-haem iron, the mechanisms involved in haem iron absorption are only just beginning to emerge. Early work on intestinal absorption suggested that haem binds to the duodenal brush border membrane and is absorbed as an intact molecule[43,44]. In support of this proposed mechanism, workers have reported the existence of haem binding proteins on pig enterocytes[45,46] and intestinal Caco-2 cells[47]. More recently, a number of candidate haem binding proteins have been identified in the intestinal epithelial cells including the ATP-binding cassette protein, ABCG2[48], the feline leukaemia virus C receptor protein, FLVCR[49] and the haem carrier protein, HCP1[50]. Of these candidate haem transporters, ABCG2 and FLVCR mediate haem efflux and only HCP1 acts as a haem import protein. The high duodenal expression of HCP1 suggests that it may be the protein involved in haem uptake from the diet. However, the precise role of HCP1 in iron metabolism remains to be fully elucidated. This issue has been complicated by recent data indicating that HCP1 may also function as a proton-coupled folate transporter, independent from its haem transporting properties[51].

Following absorption, haem is detectable in membrane-bound vesicles within the cytoplasm[52,53]. Within these vesicles, it is thought that the iron contained with the protoporphyrin ring is excised by the action of haem oxygenase 1[54] yielding ferrous iron which enters a common intracellular pool along with the iron absorbed via the non-haem transport pathways. Digestion appears to be complete within the enterocytes since a number of studies have failed to detect intact haem efflux across the basolateral membrane[44,47]. One intriguing possibility is that the efflux proteins ABCG2 and FLVCR, also expressed in the duodenum, may act to remove bilirubin formed as a by-product of haem degradation from the enterocytes.

Intracellular storage and translocation of iron

At this stage, the absorbed iron has two fates depending on the body's requirements. If the body stores are replete, and there is no increased erythropoietic drive, a significant amount of newly absorbed iron will be stored in the enterocytes as ferritin. Because duodenal enterocytes turnover very rapidly (their lifespan is approximately 3-4 d) and the majority of enterocytes contributing to absorption lie in the mid/upper villus region, this intracellular ferritin iron is quickly lost into the intestinal lumen as the ageing cells are sloughed off at the villus tip. Interestingly, in human subjects there is a positive correlation between

dietary iron bioavailability and faecal ferritin content which supports the above mechanism[55,56]. Indeed it is likely that this is a very important mechanism for controlling the release of iron into the circulation.

The mechanism by which iron is translocated from the apical pole of the enterocytes so that it becomes available for export across the basolateral membrane is poorly understood. A body of evidence has emerged from studies in Caco-2 cells for a vesicular transport pathway that co-ordinates the transcellular movement of iron. Central to this mechanism is apo-transferrin (apo-Tf) which when added at the basolateral surface of the Caco-2 cell monolayer stimulates transepithelial iron flux in a dose-dependent manner[57-59]. Interestingly, in Caco-2 cells apo-Tf and Fe-Tf, once taken up from the basolateral medium, appear to be directed into distinct endosomal vesicular fractions[59,60]. The apo-Tf containing endosomes are routed towards the apical pole of the cell where they co-localise with vesicles containing DMT1[61]. It is proposed that the iron entering the cell along with DMT1 is transferred to apo-Tf within these endocytic vesicles, and is subsequently exocytosed into the basolateral medium as Fe-Tf. Using a combination of biochemical inhibitors to disrupt this vesicular network, it is estimated that this pathway may count for as much as 50% of the transepithelial iron flux in Caco-2 cells[62,63].

While on the face of it, the above studies provide compelling evidence for a co-ordinated mechanism for the transcellular routing of iron, a number of caveats must be taken into consideration. (1) This model requires the expression of both DMT1 on the apical surface and transferrin receptors (TfR) on the basolateral membrane of the same enterocytes. While this requirement holds for Caco-2 cells[64], the evidence from rat and mouse intestine suggests that TfR are predominantly expressed in the proliferating crypt and lower villus enterocytes[65-69] while dietary iron uptake through apical membrane DMT1 occurs in the upper villus enterocytes[70-73]. (2) Recent studies have shown that intestinal-specific inactivation of IREG1 (the basolateral iron transporter) results in anaemia confirming the essential role of this pathway in iron homeostasis[74]. (3) Caco-2 cells, while an excellent model of the intestinal epithelium, exhibit some non-enterocyte properties including the ability to synthesize and secrete transferrin[75-76]. Taken together, all of these studies highlight the need for further investigation into the transcellular iron transport mechanisms and their role in maintaining body iron homeostasis.

Iron export

Efflux of iron across the basolateral surface of enterocytes is achieved through the co-ordinated action of a transport protein IREG1[77]-also known as ferroportin[78] and MTP1[79]-and a ferrioxidase, hephaestin[80]. Studies in which IREG1 was expressed in *Xenopus laevis* oocytes indicate that this is a unidirectional efflux transporter of ferrous iron[77,78]. Interestingly, this efflux function is up-regulated in the presence of ceruloplasmin, a copper binding ferrioxidase, plus transferrin to bind the newly liberated iron[77]. This suggests that while ferrous iron is released through IREG1 it must be oxidised to ferric iron to facilitate its loading onto transferrin for onward transport in the circulation. Interestingly studies with the yeast ceruloplasmin homologue, Fet3p, have highlighted the requirement for ferrioxidase activity in iron accumulation by transferrin[81,82].

As stated above, the use of knockout mice has elegantly demonstrated the essential role of IREG1 in basolateral iron efflux[74]. In addition, a second genetic mutant mouse model-the sex-linked anaemia (sla) mouse-has highlighted the critical requirement for oxidation of iron leaving the enterocytes for normal iron homeostasis. The sla mouse develops a moderate to severe microcytic hypochromic anaemia[83]. It has been shown subsequently that these mice exhibit normal uptake of iron into enterocytes[84], but the subsequent release of iron into the circulation is diminished[85]. As a result, iron accumulates in enterocytes, and is lost when these cells are sloughed at the villus tip[86]. While the *in vitro* studies described earlier[77] used the ferrioxidase activity of ceruloplasmin to drive iron efflux, in the intestine the oxidation of iron is achieved by a ceruloplasmin homologue, hephaestin, which is also a multicopper ferrioxidase[87]. In the sla mouse, the hephaestin gene is defective leading to a truncated form of the protein[80], which is mislocalised within the enterocytes[88] and has reduced ferrioxidase activity[87].

REGULATION OF INTESTINAL IRON TRANSPORT

The regulation of intestinal iron absorption is complex and relies on mechanisms which sense dietary iron content as well as iron storage levels in the body and erythropoietic iron requirements (Figure 2). The iron regulatory hormone hepcidin is likely to be an important intermediate in signalling the storage and erythroid requirements and this aspect of iron homeostasis will be dealt with in an accompanying review[1].

Basal transporter expression

In the healthy physiological state intestinal transporter expression will reflect body iron status exemplified by the circulating levels of iron bound to transferrin. Cells in the duodenal crypts of Lieberkühn express both Hfe[89,90], the protein mutated in more than 80% of haemochromatosis patients[91], and TfR on their basolateral surface. It is believed that Hfe binds to TfR regulating the rate at which transferrin-bound iron can enter the cell[92,93]. One suggestion is that the cellular iron concentration established as a result of this interaction ultimately determines the basal level of expression of the proteins involved in iron absorption in the mature absorptive cells in the upper third of the villus. Importantly, in a modification to this hypothesis, we propose that in response to humoral signals, such as hepcidin, iron transport[94] and transporter expression[95] in mature epithelial cells, can be modified rapidly without the need to re-programme the crypt cell sensing mechanism.

The role of dietary iron

Rapid regulation of intestinal transporter expression in

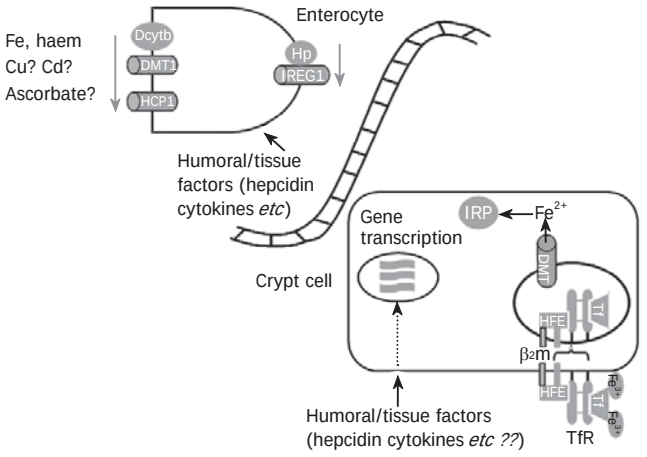

Figure 2 The regulation of intestinal iron transport. The duodenal crypt-villus axis represents a differentiation pathway that can be influenced by the dietary and humoral signals that ultimately regulate iron absorption. Immature proliferating cells in the crypt take up transferrin from the circulation *via* transferrin receptors (TfR). This process is governed by interactions between Hfe, $\beta_2$microglobulin and TfR. The crypt cells are thus sensitive to the circulating levels and the iron-saturation of Tf. Iron regulatory proteins (IRP) recognise the cellular iron content and regulate iron responsive mRNAs posttranscriptionally. In addition, transcription control of iron metabolism may be exerted by a number of humoral and tissue-derived factors, such as hepcidin or cytokines interacting with their individual specific receptors. Together, these processes set the basal level of iron transporter protein expression in enterocytes as they mature and travel up the villus. Importantly, this basal level of transporter expression can be modified rapidly in mature enterocytes in response to changes in the levels of dietary factors (e.g. the iron content of the diet, other metals *etc*) and humoral and local tissue mediators (especially hepcidin and pro-inflammatory cytokines). This combination of basal programming in the crypts and fine tuning in villus enterocytes permits tight control of intestinal iron transport.

response to dietary factors is probably sensed by the villus enterocytes. More than half a century ago the "mucosal block" hypothesis was formulated following studies which demonstrated that a large oral dose of iron could reduce the absorption of a smaller dose administered several hours later[96,97]. It was argued that due to the short time interval between doses, the initial dose must be having a direct effect on the mature enterocytes rather than the crypt cells. Whether such a phenomenon occurs with meaningful dietary iron levels is not clear but this may be a considerable problem with supplemental iron levels[98]. Using the Caco-2 cell model, we have addressed the issue of whether non-haem iron can regulate iron transporter expression within a timescale and at concentrations that are consistent with digestion and absorption of a meal. We found that DMT1 (the IRE-containing isoforms) protein expression on the apical surface of these cells is decreased by iron concentrations as low as 20 μmol/L[64]. The decrease in DMT1 transporter expression was rapid, occurring within 1-4 h following the exposure to iron[99]. Further analysis revealed that DMT1 protein was internalised and targeted towards a late endosomal/lysosomal compartment. Interestingly, these iron-dependent effects were restricted to the apical uptake pathway-IREG1 protein expression was unaltered-and were fully reversible (DMT1 protein levels returned to their original basal levels within 4-8 h) following the removal of iron[99]. Our findings in this pertinent cell culture model are consistent with those observed in rats following oral gavage with an iron bolus[100-102] suggesting that the redistribution of DMT1 between different cellular compartments may be important physiologically for optimising iron absorption from a meal so that it matches better the body's metabolic requirements.

Iron regulatory proteins and iron responsive elements

In addition to trafficking of iron transport proteins, a number of intestinal iron metabolism genes can be regulated post-transcriptionally through interactions between cytosolic iron regulatory proteins (IRP) which bind to iron responsive elements (IRE), stem loop structures in either the 3' or 5' untranslated region (UTR) of several mRNA species, under conditions of cellular iron deficiency. TfR mRNA contains five IREs in its 3' UTR, and is stabilised following IRP binding as this blocks a target site for endonuclease activity[103-106]. Interestingly, two isoforms of DMT1 contain a single IRE in the 3'UTR[26]. While the DMT1 IRE can bind IRP *in vitro*[107,108], its role in regulating DMT1 expression remains to be fully determined.

In contrast to the reported role of the 3'IRE sequences in conferring mRNA stability, the expression of mRNAs possessing 5'IREs, such as ferritin, are poorly translated with cellular iron low. But, expression is increased by high iron levels in duodenal enterocytes[109]. This is because IRP/IRE binding blocks the association of the eukaryotic initiation factor complex to the 43S ribosomal unit[110]. Interestingly, IREG1 mRNA contains a single IRE in the 5'UTR[77,79]. However, the role of IRP/IRE interactions in the regulation of IREG1 transporter expression is even more controversial than its role in regulating DMT1 expression. Evidence suggests that the response to changes in iron status is tissue-specific-IREG1 expression in the liver[79] and lung[111] and in macrophages[112] is up-regulated by high iron whereas in the intestine expression is elevated by iron deficiency[77]. This may indicate that transcriptional, translational and post-translational processing of IREG1 varies between cell types[113]. Clearly, the mechanisms involved in iron-dependent regulation of IREG1 in the intestine require further attention.

Local tissue factors

While the majority of this review has focussed on the transport pathways in the enterocytes it is important to bear in mind that the intestinal cell population is a highly heterogeneous affair. It is likely, therefore, that cross-talk between the epithelial cells and other cell types, such as macrophages, neutrophils, intraepithelial lymphocytes *etc* will be important in the overall regulation of intestinal iron transport. One intriguing hypothesis in this regard is the possible physiological role of the pro-inflammatory cytokine TNFα in regulating intestinal iron transport. TNFα is not only synthesized by peripheral blood monocytes and macrophages in response to inflammatory stimuli, but is also released by intraepithelial lymphocytes (IEL) that reside within the intestine in response to high iron intakes[114]. These findings led to the formation of a hypothesis (named the piggyback-sensor model[115])

which suggested that interaction between Hfe in the developing enterocytes with specific epitopes on the IELs was essential for regulating local TNFα production. Once released, TNFα leads to iron deposition within intestinal enterocytes *via* a TNF receptor 2-dependent mechanism[116]. Further studies by our group[117] and others[118,119] have shown that TNFα has a direct influence on intestinal iron transporter expression and iron transport. These studies have opened the way for further investigations into the role of cell to cell cross-talk and the role of local tissue factors in regulating intestinal iron transport.

SUMMARY AND FUTURE DIRECTIONS

Clearly, our understanding of the molecular components of the intestinal iron transport pathway has increased exponentially in the last decade. However, there are still a number of important questions that remain unanswered: (1) How is iron shunted across the enterocytes from the apical pole to the basolateral membrane? There is some evidence for the presence of a tubulovesicular pathway. But, this work has largely been carried out in cell lines, and needs to be explored further in "normal" intestinal tissue. A role for calreticulin, a proposed component of the paraferritin pathway, remains a possibility though is still unproven. (2) What are the relative contributions of ferritin (and possibly lactoferrin) to iron nutrition? Could these iron sources be exploited for new supplemental therapies to treat iron deficiency? (3) Is there a role for cross-talk between enterocytes and other intestinal cell types in the local regulation on intestinal iron transport? If so, what are the cellular mechanisms involved? Are local tissue factors (such as TNFα) relevant physiologically in the control of iron absorption? (4) Hepcidin - how does it regulate intestinal iron transport? Is it an important physiological regulatory or is its main role in iron-related pathologies such as iron deficiency anaemia, haemochromatosis and anaemia of chronic disease?

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