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Recent advances in mammalian haem transport

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Haem is a structural component of numerous cellular proteins and contributes greatly to iron metabolic processes in mammals. Haem-carrier protein 1 (HCP1) has recently been cloned and characterized as a putative transporter in the apical region of the duodenum, and is responsible for uptake of haem into the gut cells. Its expression is regulated pre- and post-translationally in hypoxic and iron-deficient mice, respectively. The identification of HCP1 has revealed the long-sought mechanism by which haem – an important source of dietary iron – is absorbed from the diet by the gut. Feline leukaemic virus receptor (FLCVR) and ABC transporter ABCG2, characterized in haematopoietic cells, have also recently been shown to export haem, particularly under stress. FLVCR protects developing erythroid cells from haem toxicity during the early stages of differentiation, and ABCG2 averts protoporphyrin accumulation (particularly under hypoxic conditions). These haem-efflux proteins are expressed in other cells and tissues including the intestine where they might function as apical haem exporters to prevent toxicity in the enterocytes.

Introduction

Haem (iron-protoporphyrin IX) contains a large proportion of the iron in mammals (~60%) and, therefore, plays a pivotal part in iron metabolism. Haem has vital roles as a component of haemoglobin, myoglobin, neuroglobin and cytoglobin and as a prosthetic group in many essential enzymes such as mitochondrial cytochromes, catalase, peroxidase and nitric oxide synthase. It regulates a variety of metabolic processes such as transcription, translation and cellular differentiation [1]. Haem itself is highly insoluble; free uncommitted pools (i.e. haem that is in transit between proteins, often called 'labile' haem) are potentially cytotoxic and could generate reactive oxygen species, hence the intracellular level of labile haem is maintained at a low concentration ($<10^{-9}$ M) [2]. Haem levels in cells are tightly controlled by transcriptional and translational regulation of genes encoding for enzymes such as δ–aminolaevulinic acid synthase and haem oxygenase 1 (HO-1), which are involved in its biosynthesis and catabolism, respectively. Moreover, haem-binding transcriptional factors such as Bach1 and neural PAS domain protein 2 (NPAS2) regulate the expression of genes involved in haemoprotein metabolism (reviewed in Ref. [3]). Because of its insolubility, haem is thought to be chelated transiently to amino acids, peptides or proteins while it is transported. An overview of the importance of haem in metabolism is summarized in Box 1. In recent years, haem-degradation products have been implicated as potential signalling molecules for several cellular processes, emphasizing an increasing appreciation of the importance of haem in regulation of physiological processes [4].

Transmembrane transport of haem is essential both for absorption in the gastrointestinal tract to maintain body iron homeostasis and for intracellular trafficking in other cells and tissues. Owing to its lipophilic nature, haem can diffuse into cell membranes [5], however, in several cases, transmembrane transport of haem is an energy-dependent process, which implies the presence of transport proteins [6,7]. This has now been shown to be the case and several of these haem transporters have been identified (Table 1). Here, we focus on these advances in haem transport.

Identification of an intestinal haem carrier

Haem transport in intestine and other cells

Haem iron from meat accounts for a small proportion of total iron found in human diets but, because it is better absorbed, dietary haem Fe has a great impact on iron nutrition. Advances in recent years have unravelled the molecular mechanism of non-haem Fe absorption, but explanations for the absorption of haem Fe have remained elusive until now. Although the solubility profile of non-haem Fe (often called 'inorganic Fe') and haem Fe are reciprocal with increasing pH, both species are highly absorbed in the proximal section of the gut [8]. Insoluble aggregates are prevented by chelation of haem to proteolytic digestion products in the slightly acidic region of the duodenum. Earlier studies proposed that haem, although not in competition with non-haem Fe, was acquired by the gut from food as an intact metalloporphyrin [9]. Haem was presumed to bind first to the brush border membrane of enterocytes then undergo internalization, finally appearing in the cytoplasm enclosed within vesicles [10]. Evidence of a brush-border haem receptor in pig [11] and human [7] intestine was presented in the early 1980s but the molecule involved was never identified. Parmley *et al.* [12] also showed by electron

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Box 1. Importance of haem in mammalian metabolism

- Haem has both vital and ubiquitous roles as a prosthetic group of enzymatic haemoproteins such as catalase, peroxidase, cytochrome c, cytochrome p450, nitric oxide synthase and NADPH oxidase.
- A regulatory haem pool is involved in transcriptional, translational and feedback regulatory mechanisms.
- Haem synthesis and recycling account for >80% of the metabolism of iron in mammals.
- Haem metabolism is involved in the pathophysiology of several disorders, for example, porphyria, sickle-cell disease and brain haemorrhage.
- Haem catabolites CO and Fe are themselves important as antioxidant, anti-inflammatory and cytoprotective signals [1–3].

microscopy the uptake of haem in the apical brush-border membrane of rat. Cultured intestinal cells such as Caco-2, as well as other non-intestinal cells (K562 and HepG2), were shown to take up haem by energy-dependent mechanisms [6,7]. Therefore, uptake of haem by cells is a general process that is not confined to the intestine. Moreover, other reports (see, for example, Ref. [5]) suggested that haem traversed epithelial cells by a passive transcellular mechanism. Liem *et al*. [13] reported that haem intercalates into the hydrophobic environment of the lipid bilayer. After endocytosis (passive pinocytosis or active receptor mediation), the iron porphyrin ring is catabolized by an inducible haem oxygenase, HO-1, to yield ferrous iron, CO and biliverdin, which is reduced to bilirubin. The Fe produced enters the inorganic iron pool of the enterocytes, and is handled by an iron efflux mechanism (Figure 1). Non-haem ^{59}Fe instead of haem was found at the basolateral side of the duodenum [14] after absorption of ^{59}Fe–haem. The molecular mechanism of haem trafficking from the apical tubule cavity (or from the haem receptor) to the haem degradation site has yet to be defined [14,15].

A different hypothesis was put forward earlier based on the idea that haemoprotein in meat is degraded into low-molecular-weight non-haem compounds before its absorption in the duodenum [16]. These studies were conducted in rats and meat digestion products were characterized by chromatography and spectrophotometry. Although it is conceivable that a proportion of haemoprotein in meat is degraded upon proteolysis, the form of iron that was absorbed in the gut of the rats was not determined. Moreover, chemical analysis of luminal digesta cannot account for absorbed components of the diet [17]. By contrast, electron microscopy techniques have demonstrated the unchanged haem porphyrin ring in micro-endocytic vesicles at the base of the microvilli and within tubulovesicular structures in the apical cytoplasm of mucosal cells [12].

Haem-carrier protein 1

The recent cloning and identification [18] of a gene encoding a novel plasma membrane protein, haem-carrier protein 1 (HCP1), might now shed some light on the mystery of how haem enters the enterocyte. HCP1 was isolated from mouse duodenum by subtractive suppression hybridization. The murine HCP1 cDNA spans five exons (1942 base pairs), is located on chromosome ch11B5 and encodes a 54-kDa protein with 459 amino acids. The human gene (2097 base pairs) on ch1711.1 encodes a protein of 446 amino acids. HCP1 is highly hydrophobic and contains nine predicted transmembrane domains. The primary amino acid sequence of HCP1 is markedly similar (22% similarity) to the bacterial metal tetracycline transporters. The latter contain 12 transmembrane (TM) domains and are members of the major facilitator superfamily. A highly conserved motif GxxSDRxGRR, present between TM2 and TM3 of all metal-tetracycline transporters and other multi-drug-resistance transporters, is found in all HCP1 sequences of mouse, rat, man, rabbit and zebrafish [18]. It is of interest that other members of this family include transporters of ferric siderophores, ferric chelates, vitamin B_{12} and haem. However, HCP1 does not contain any known haem-binding motifs such as the Cys-Xaa-Xaa-Cys-His found in c-type cytochromes [19]. As with most genes involved in intestinal Fe transport, HCP1 is highly expressed in the proximal intestine (mainly duodenum) but is also found in other tissues. Transcripts of the gene have been found in the liver and kidney, which strongly suggests a role in these tissues [18]. HCP1 mRNA seems to be regulated by hypoxia and hypotransferrinaemia, whereas HCP1 protein is also post-translationally regulated by iron levels in duodenum. The regulation of haem absorption by iron

Table 1. Function and tissue localization of putative haem-transport-associated proteins

Protein	Localization	Function	Refs
HCP1	Mainly duodenum, some in the liver and kidney	Duodenal apical haem transporter, possible haem scavenging or recovery	[18]
FLVCR	Haematopoietic cells, liver, kidney and pancreas	Haem efflux in erythroid progenitor cells	[43]
ABCG2	Haematopoietic cells, fetal liver, pancreas, kidney and intestine	Haem or porphyrin efflux	[55]
SOUL	Eye	Undefined (putative haem-binding protein)	[34]
ABCme	Erythroid cells and inner mitochondria membrane	Possible transport of haem from mitochondria to cytosol	[37]
ABC3, ABC7, ABCme	Mitochondria	Transport of haem derivatives, FeS-cluster assembly	[37]
Haemopexin receptor LRP or CD91	Hepatocyte	Haem endocytosis from circulation	[66]
Haptoglobulin receptor, CD163	Macrophages	Haemoglobin clearance from circulation	[63]
Megalin and cubilin receptors	Kidney	Renal haemoglobin reabsorption	[68,69]

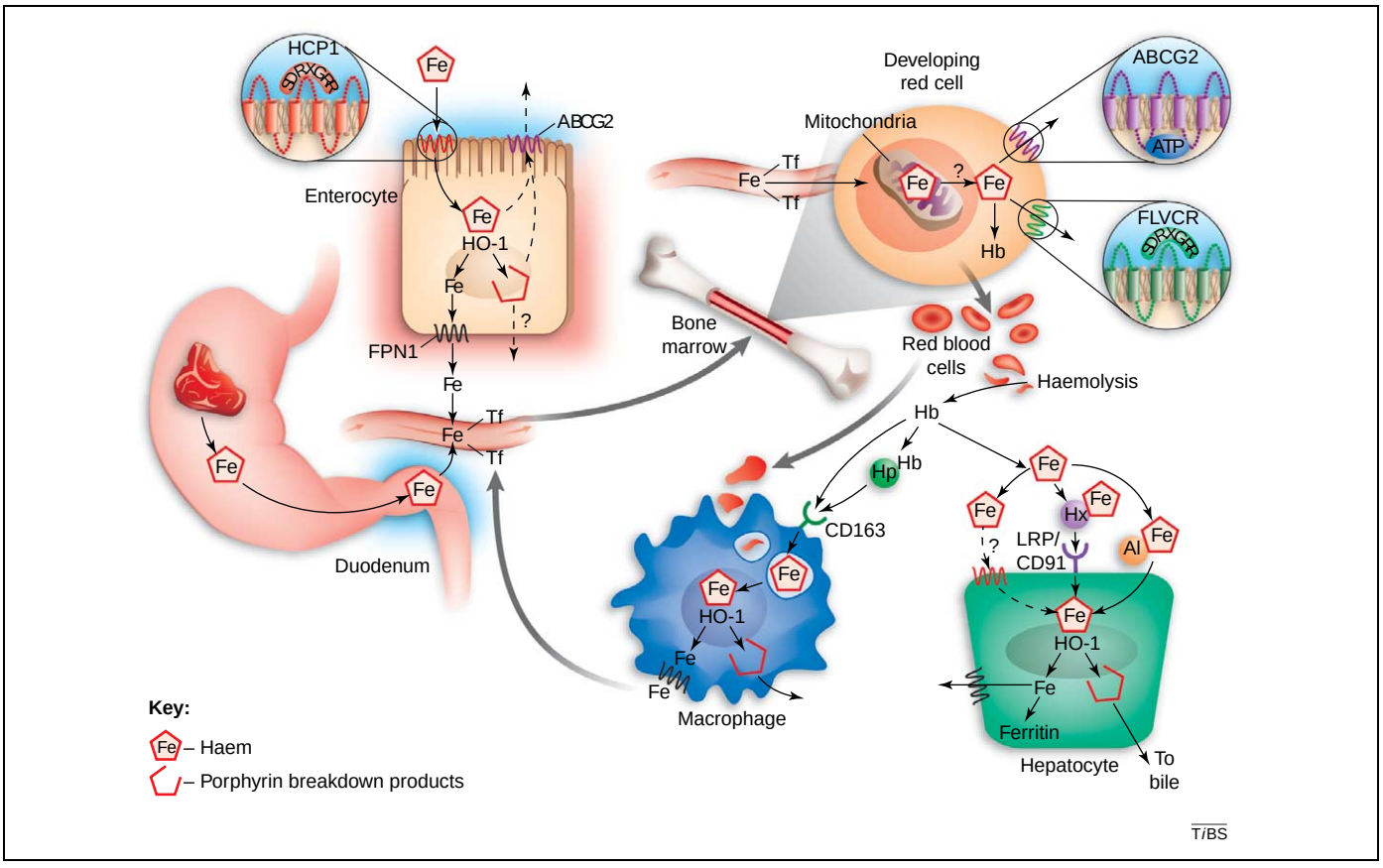

Figure 1. Overview of transmembrane haem transport in mammalians showing the sites of haem transport and the role of recently identified proteins HCP1, ABCG2 and FLVCR. HCP1 is responsible for absorption of haem iron from the diet. Haem is transported by HCP1 as an intact metalloporphyrin. Once inside the cytosol, HO-1 degrades haem and releases iron, which then exits the enterocyte by ferroportin. ABCG2 expressed on the apical membrane of enterocytes might function to rid these cells of excess haem and/or other haem breakdown products. The site of FLVCR expression in the enterocyte is not known but might also be involved in efflux of haem and/or haem breakdown products from either apical or basolateral membranes. ABCG2 and FLVCR have roles in maturation of red cells and seem to have overlapping functions that prevent developing cells from apoptosis by controlling intracellular haem by regulating efflux. Free haem resulting from haemolysis is bound to albumin and haemopexin (Hx). These complexes are rapidly cleared by the liver via specific a low-density lipoprotein receptor-related protein (LRP; also known as CD91) in the case of haemopexin and non-specific uptake mechanisms for haem bound to albumin. Macrophages have a major role in scavenging haem by phagocytosis of effete red cells or by uptake of Hb–haptoglobin (Hp) complexes via surface CD163 receptors. The haem is broken down within the cell and the iron is recycled to the plasma. Only a representative number of TM domains for HCP1, ABCG2 and FLVCR are shown. Likewise, the positions of the motifs and ATP molecules in the transporters are not accurate. Broken arrows indicate hypothetical pathways. Abbreviations: FPN1, ferroportin 1; Tf, transferrin.

stores is not as potent as seen for non-haem Fe absorption, at least in humans [20]. Studies on regulation of haem absorption by stored iron in rats have not been consistent: some report no effect [20] but others find increased haem absorption [21] in iron-deficiency. The post-translational regulation of HCP1 by redistribution and translocation between apical and cytosolic membrane compartments is particularly noteworthy [18]. HCP1 was seen to be distributed between the apical membrane and cytosolic compartments. The protein was mainly at the apical membrane in iron deficiency, whereas the protein was confined to the cytoplasmic region in iron-loaded mice. Recycling and trafficking of iron-transport proteins between the plasma membrane and internal organelles in response to iron has been noted previously and is likely to be important in regulation, however, the mechanisms involved are completely unknown at present [22]. Independent lines of evidence strongly indicate that HCP1 functions as an intestinal haem transporter. First, functional studies conducted in *Xenopus* oocytes and cultured cells have shown that, when HCP1 is expressed, there is a 2–3-fold increase in haem uptake. The HCP1-dependent uptake component is both temperature-dependent and saturable, indicating a carrier-mediated process. HCP1 also facilitates the uptake of Zn-protoporphyrin, a structural homologue of haem, suggesting the requirement for the porphyrin ring in the uptake process. Second, it was found that incubation of duodenal tissue with anti-HCP1 antibodies produced statistically significant inhibition of radiolabelled haem uptake. HCP1 joins the array of proteins exerting effects on body iron status. It remains to be seen if the gene encoding HCP1 is responsible for modifying phenotypic penetrance of iron-overload diseases such as haemochromatosis and other disorders of iron metabolism.

It has been suggested that haem degradation by HO-1 is the rate-liming step of haem absorption in the gut because the activity of HO-1 is increased during Fe deficiency [14]. It, therefore, seems possible that the post-translational regulation of HCP1 is coordinately regulated with HO-1 levels and activity. HO-1 degrades haem to inorganic iron in the cytosolic milieu of the enterocyte, which is then transferred through the basolateral side of the gut cells via ferroportin. Thus, although entry of haem into the enterocyte is specifically regulated by the haem transporter HCP1, exit of iron resulting from

haem ingestion is controlled by ferroportin and, therefore, presumably directly influenced by levels of the circulating iron regulatory peptide hepcidin [23].

Haem transport in peripheral tissues and organelles

Despite the fact that a large proportion of Fe from the reticuloendothelial system is recycled daily for erythropoiesis, little information exists on the molecular details of this process. Senescent erythrocytes are selectively phagocytosed by macrophages in the liver, spleen and bone marrow. Phagosome maturation and involvement of endoplasmic reticulum will lead to the degradation of red cell components. Haem is degraded by HO-1 and the resultant ferrous iron is presumably exported out of the cell by the iron-efflux protein Ferroportin [24,25,26]. Consequently, ferroportin expression tends to increase with increased HO-1 expression after erythrophagocytosis [27]. Furthermore, the importance of ferroportin in iron recycling in macrophages was recently demonstrated in genetic knock-out mice [28]. Reticuloendothelial iron recirculation from phagocytosed effete (i.e. aged or damaged) erythrocytes was inhibited when ferroportin was globally inactivated in these mice. Subsequently, this resulted in iron retention in both hepatic and splenic macrophages of the mice. Similarly, the importance of HO-1 is shown by knockout mice that show anaemia, depressed serum iron and macrophage iron overload [30]. In humans, a rare phenotype that is characteristic of a variety of missense mutations in ferroportin collectively referred to as autosomal dominant haemochromatosis or ferroportin disease has been reported [29].

Considerable interest has been generated over the years regarding the import and export of intact haem as a metabolite in cellular processes (Figure 1), particularly the export of haem from the site of its synthesis in the mitochondria to the endoplasmic reticulum for incorporation into haemoproteins. Furthermore, as it is involved in transcriptional regulation of some genes, transport of haem into the nucleus must also occur. Several putative haem-carrier proteins such as isoforms of glutathione S-transferase – ligandin [31], Yb_2Yb_2 and Z-type-fatty-acid-binding protein [32] – have been reported. Other proteins including haem-binding protein 23 (HBP23), p22HBP and SOUL have been identified in mammals as soluble cytosolic haem-binding proteins [33–35]. However, homologues of HBP23 – peroxiredoxins – in yeast and mouse do not bind to haem but, instead, exhibit peroxidase and antioxidant properties [36]. It has also been suggested that members of ATP-binding cassette (ABC) transporters ABC7, ABC3 and ABCme [37], export haem in addition to iron and FeS clusters from mitochondrial matrix into the cytosol [3] (Table 1). Further work is required to delineate the transport, binding and anti-oxidant properties of these haem-associated proteins.

Identification of mammalian haem-efflux proteins

Receptor for feline leukaemic virus, subgroup C

Recently, two haem exporters from maturing erythroid cells were reported. Feline leukaemic virus receptor (FLVCR) was originally identified and cloned [38,39] as a cell-surface protein receptor for feline leukaemic virus, subgroup C (FeLV-C). Other variants of the non-oncogenic retrovirus group, FeLV-A and FeLV-B, differed in host range, receptors and interference pathogenesis [40]. FeLV-C infection in cats causes pure red-blood-cell aplasia (also termed erythroid aplasia and aplastic anaemia) [41]. This is a type of anaemia that involves an erythroid lineage in which the erythroid progenitor, burst-forming units-erythroid (BFU-E), fails to mature and differentiate to the colony-forming units-erythroid (CFU-E). Profound anaemia develops because erythropoiesis is arrested specifically by FeLVC infection at this stage [42]. The main determinant of this FeLVC-induced anaemia has been mapped to a small region of the virus surface envelope glycoprotein region 1, which specifically binds to the host protein FLVCR [42].

FLVCR was cloned from a human T-lymphocyte cDNA library in a retroviral vector [38,39] and, like HCP1, is a member of the major facilitator superfamily. The predicted cDNA encodes a protein of 560 amino acids with a molecular weight of 60 kDa. It has significant amino acid sequence homology (45% identity) to a *Caenorhabditis elegans* protein of unknown function and weak homologies (21% identity) to bacterial glycerol-3-phosphate and glucarate transporters [39]. The hydrophobicity plot of FLVCR indicates the presence of 12 hydrophobic membrane-spanning domains, a large hydrophilic loop between TM6 and TM7 and a highly conserved signature sequence GxxSDRxGRR in the hydrophilic region between TM2 and TM3, which is similar to HCP1. FLVCR was shown to be expressed in different haematopoietic cells including peripheral blood lymphocytes and T cells and weakly expressed in tissues such as fetal liver, pancreas and kidney [38].

Until recently, the physiological role of FLVCR was unknown; however, recent work has revealed it to be a human haem exporter that is essential for erythropoiesis [43]. FLVCR was shown to mediate zinc mesoporphyrin efflux in cultured rat renal epithelia cells and the haematopoietic K562 cells. Haem efflux by these cells overexpressing the protein was temperature dependent. Furthermore, the haem efflux via FLVCR was shown to be essential for erythroid differentiation in K562 cells and blocked by incubation with the ligand feline leukaemia virus. FLVCR, therefore, functions as a haem exporter that is likely to protect the cells from excess haem build up during the CFU-E stage of erythropoiesis. FLVCR expression is up-regulated in CFU-E when haem synthesis increases, presumably to protect the cells from haem toxicity [43]. FLVCR expression is down-regulated when globin synthesis increases and haemoglobinization commences. Therefore, FLVCR acts as an overflow valve for the efflux of excess free haem from the CFU-E cells and this is particularly crucial during this stage of erythroid differentiation. FLVCR, thus, prevents haem cytotoxicity and maintains haem at levels that regulate globin transcription and haem biosynthesis.

ABC transporter ABCG2

In another recent study, a second haem-efflux protein ABCG2 [also known as BCRP (breast cancer resistance protein), MXR (for mitoxantrone resistance) and ABCP

(ATP-binding cassette placenta); hereafter referred to as ABCG2] was identified [44]. Unlike HCP1 or FLVCR, ABCG2 is a member of the ABC transporter family that was originally found to confer drug resistance in breast cancer cells (hence the name BCRP). ABCG2 and others in the group mediate resistance of cancer cells by the active efflux of chemotherapeutic drugs [45]. ABC transporters are varied in their substrate specificities and consequent mutational phenotypes (reviewed in Ref. [46]). Briefly, ABC transporters are characterized by a conserved cytosolic domain, two hydrophilic cytosolic nucleotide-binding domains and a minimum of two membrane-spanning domains. Specifically, ABCG2 belongs to the G family of ABC transporter proteins in which the nucleotide-binding domains are localized towards the N terminus of the polypeptide chain [46]. ABCG2 was first isolated [47] from a breast cancer cell line and is located on human chromosome 4q22. The gene consists of 16 exons and 15 introns [48]. ABCG2 has only one ABC cassette in a single peptide of 70 kDa that consists of six putative transmembrane domains. It is referred to as a half transporter because only the homodimer is functional [49]. ABCG2 has several *cis*-acting regulating elements in its promoter region, of which a hypoxia response element has been shown to be functional (discussed later). ABCG2 transcription is therefore regulated by hypoxia-inducible transcription factor 1 (HIF1), and Akt1 – an upstream regulator of HIF1 [50] – signals ABCG2 localization to the plasma membrane. ABCG2 is expressed in several tissues in a pattern that varies in different species. It is expressed in hepatic canalicular membranes, renal proximal tubules, intestinal epithelium and the placenta [51], where it performs the role of detoxifying diverse drugs, toxins and metabolites from these tissues [48]. This function is favoured by its localization to the plasma membrane rather than in organelles like most other ABC transporters [52]. ABCG2 is also expressed in haematopoietic stem cells and particularly in a sub-population called 'side population'. It confers on haematopoietic cells the ability to transport Hoechst dye, prevents cytotoxicity of chemotherapeutic agents and maintains survival under hypoxic conditions. ABCG2 has wide substrate specificity and it seems, therefore, that inducible expression is important for detoxification of xenobiotics and food metabolites particularly via hepatobiliary circulation [53].

The importance of ABCG2 in haem transport was serendipitously discovered when ABCG2-null mice fed a modified diet developed skin phototoxicity [54]. This was caused by the accumulation of a compound structurally similar to protoporphyrin IX, pheophorbide a, which is a degradation product of chlorophyll present in the diet. *Abcg2*$^{-/-}$ mice accumulate protoporphyrin IX and other porphyrin-like compounds in erythrocytes and other cells including skin cells, which led to the photosensitivity, implying that ABCG2 has a role in cellular efflux of these compounds [54]. The regulation of ABCG2 expression by HIF1 and its ability to transport porphyrin under hypoxic conditions were shown in an elegant series of studies [55]. Analysis of *Abcg2*$^{+/+}$ and *Abcg2*$^{-/-}$ progenitor cells showed diminished survival of the mutant under hypoxic conditions. Inhibition of ABCG2 with fumitremorgin C or reserpine in wild-type mice also impaired the survival of the progenitor cells under hypoxia. By contrast, however, the inhibition of haem biosynthesis with succinyl acetone ensured the survival *Abcg2*$^{-/-}$ progenitor cells. This averted the accumulation of porphyrins in these cells. An enhanced level of protoporphyrin IX in erythroid progenitors of *Abcg2*$^{-/-}$ confirms porphyrins as endogenous substrates of ABCG2 [44]. Moreover, erythroid cells overexpressing ABCG2 were shown to have reduced levels of protoporphyrin [56]. Therefore, ABCG2 confers cytoprotection on various stem cells. Its expression is upregulated in hypoxia via HIF1, and it confers a strong survival advantage to stem cells under hypoxic stress by reducing intracellular haem or porphyrin levels. Thus, ABCG2 enhances tolerance to hypoxic stress by regulating intracellular porphyrin levels in haematopoietic cells, which probably reduces the generation of reactive oxygen species by haem and also prevents mitochondrial damage leading to apoptosis [57]. Although the *Abcg2*$^{-/-}$ phenotype does not exhibit the characteristic photosensitivity of erythropoietic protoporphyria, it is speculated that human polymorphisms of the gene encoding ABCG2 could account for the variable penetrance of the disease [58].

Functions of FLVCR and ABCG2

As stress proteins, FLCVR and ABCG2 seem to function similarly by getting rid of excess toxic haem or porphyrins during early and later stages of haematopoiesis. This might act as a supplement for HO-1 in bone marrow, where the requirement for oxygen by HO-1 might partially limit haem degradation in the physiological hypoxic conditions of the marrow [59]. Whereas FLVCR mRNA was shown to be expressed in intestinal and hepatic cell lines by reverse-transcription PCR, no data has been presented on protein or mRNA levels of expression in either liver or intestine and, hence, its expression in these tissues remains unknown. By contrast, ABCG2 has been clearly localized on the apical membrane of duodenal enterocytes and other regions of the gastrointestinal tract [53]. Further studies are necessary to define the exact histological localization of FLVCR in the gut and whether both ABCG2 and FLVCR are regulated by iron. Presumably, these proteins, if functional in the gut, could transport excess haem from the enterocyte into the lumen or plasma. Indeed, recent evidence from Caco-2, an intestinal cell line, indicates the existence of a secretory or efflux pathway for haem from the basolateral to the apical side of the fully differentiated cells [60]; an earlier report suggested haem trafficking into the intestine from the basolateral side [61]. Uc *et al.* [60] showed that the treatment of cells with trypsin on the apical side enhanced haem uptake, suggesting the presence of a protein that functions as a negative regulator of haem uptake at this histological site. Perhaps FLVCR and ABCG2 could, in this regard, be involved in regulating the endogenous levels of haem in the enterocyte.

Haem-detoxification systems: regulation of systemic transport and storage

The liver plays a pivotal part in systemic metabolism of both haem and non-haem Fe. It serves as both a depot and a buffer compartment that enhances iron supply

in situations of high demand, in addition to detoxifying when excesses occur. The liver is particularly important in haem biosynthesis (for cytochrome P450s), utilization, recycling, (Kupffer cells of the reticuloendothelial system) and catabolism [62]. A large proportion of haem transport and utilization is by phagocytosis of effete erythrocytes by macrophages. Haemoglobin (Hb) can be released into plasma as a result of intravascular lysis of red cells. Free Hb in plasma binds to haptoglobin (Hp), an α_2 acid glycoprotein acute-phase protein, produced by the liver. The Hb–Hp complex is endocytosed by the Hb scavenger receptor CD163 [2,63]. (Plasma Hp binds 150 mg/dl of Hb.) The Hb–Hp complex is then broken down to release free haem, globin and unconjugated bilirubin, which are released into the blood stream. When intravascular haemolysis exceeds the binding capacity of Hp, free Hb dissociates into dimers, which are filterable by the glomerulus in the kidneys. Excess Hb is also oxidized to methaemoglobin, which dissociates to free haem and forms complexes with a β-globulin haemopexin. The circulating plasma is rid of potentially free toxic haem by processes that involve uptake of haem-bound complexes in the liver. Haem forms complexes with albumin [64], with a dissociation constant (K_d) of 10^{-8} M and haemopexin [65] with high affinity (K_d of $\sim 10^{-12}$M). Haem–haemopexin is endocytosed by a receptor-mediated mechanism in the liver [65] akin to diferric transferrin receptor uptake of inorganic iron. Contrary to the latter, however, the cloning, expression and characterization of haemopexin receptor await completion, although a recent report has identified the human haemopexin receptor as the low-density lipoprotein receptor-related protein CD91 (LRP/CD91) [66]. Moreover, some other studies have indicated that haemopexin, as an exogenous anti-oxidant, binds to haem extracellularly, inhibits influx of free haem into cells and thereby confers cytoprotection against intracellular toxicity [67]. These pathways normally preclude haemoglobinuria. However, during chronic intravascular haemolysis, megalin and cubilin complexes of the kidney reabsorb free Hb. Megalin and cubilin mediate Hb endocytosis and both proteins, which have broad substrate specificities, are involved in the reabsorption and intracellular trafficking of free Hb during chronic haemolysis [68,69] The production of erythropoietin and the recent findings of the expression of iron-transport genes in the kidney highlight the importance of this organ in the maintenance of iron homeostasis [70] (Table 1).

Concluding remarks

Recent advances in haem transport have led to the identification of both haem-import and haem-efflux proteins. HCP1, which is strongly expressed in the duodenum, is thought to mediate haem import into enterocytes. HCP1 is iron regulated and might have an important role in regulating iron stores in mammals. By contrast, FLCVR and ABCG2 have been identified as haem-efflux proteins. Because haem is both essential and toxic, cells require mechanisms to tightly control intracellular haem levels; this is probably achieved through regulation of the transport proteins described here in addition to that of several cytosolic and extracellular haem chaperone proteins and HO-1. The identification of HCP1, FLVCR and ABCG2 has important implications for the mechanism of haem ingress and egress and will no doubt stimulate further research in the area of transcellular haem transport. Future work will focus on the role and the regulation of these proteins in disorders of haem and iron metabolism.

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