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Heme transport and erythropoiesis

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In humans, systemic heme homeostasis is achieved via coordinated regulation of heme synthesis, transport and degradation. Although the heme biosynthesis and degradation pathways have been well characterized, the pathways for heme trafficking and incorporation into hemoproteins remain poorly understood. In the past few years, researchers have exploited genetic, cellular and biochemical tools, to identify heme transporters and, in the process, reveal unexpected functions for this elusive group of proteins. However, given the complexity of heme trafficking pathways, current knowledge of heme transporters is fragmented and sometimes contradictory. This review seeks to focus on recent studies on heme transporters with specific emphasis on their functions during erythropoiesis.

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Introduction

Heme homeostasis is a highly coordinated process during erythropoiesis, marked by a dramatic increase of heme synthesis which is essential for proper hemoglobinization of red blood cells (RBCs) [1,2]. Heme is also involved in transcriptional and translational regulation of erythroid specific gene expression, which is critical for coupling heme synthesis with protein production for erythroid cell differentiation [3,4]. In addition, a large amount of heme-iron is recycled for re-packing into hemoglobins by erythrophagocytosis (EP) in macrophages of the reticuloendothelial system (RES) [1,5••,6•]. Although heme biosynthesis and its regulation have been well characterized, the mechanisms for heme transport in eukaryotes remain poorly understood. Comprehensive reviews for generic heme trafficking and interorganellar transfer pathways have been covered elsewhere [5••,6•,7,8]. In this review we will seek to cover the following. How does newly synthesized heme exit the mitochondria for incorporation into hemoglobins and other hemoproteins? How does heme released from lysed RBCs cross the phagolysosomal membrane to be delivered to downstream effectors such as heme oxygenase-1 (HO-1) for degradation? Can heme be redistributed between different tissues through heme transporters and chaperones? Extensive efforts to identify heme trafficking pathways have been underway for over a decade and a number of heme transporters have been identified recently.

Heme import

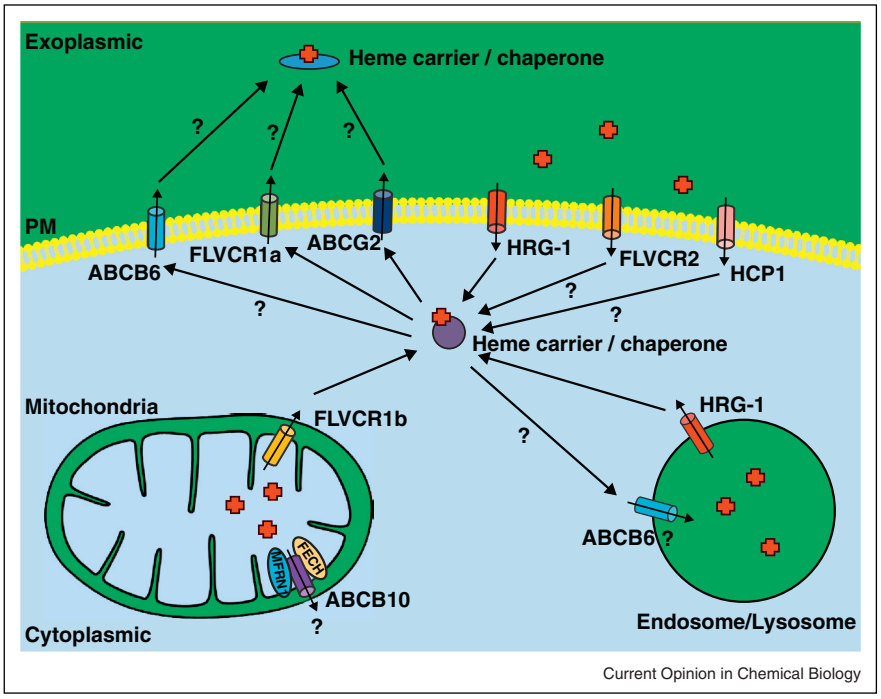
Heme is a more readily bioavailable iron source and contributes to two-third of body iron, even though heme constitutes only a third of total dietary iron [9,10]. In mammals, dietary heme is apparently taken up intact by enterocytes in the intestine. However, heme is a large amphipathic porphyrin and free heme can be cytotoxic. Thus, specific molecules and pathways are required for heme uptake and trafficking (Figure 1).

HRG-1

Rao *et al.* have demonstrated that the roundworm *Caenorhabditis elegans* is a unique model for heme trafficking studies because even though it is a heme auxotroph it acquires dietary heme via the intestine and subsequently disseminates heme throughout the organism for viability [11]. Genomic screens in *C. elegans* identified CeHRG-1 and CeHRG-4 as the first *bona fide* eukaryotic heme importers [12••]. CeHRG-1 has orthologs in vertebrates, while CeHRG-4 is worm-specific. Transient knockdown of *hrg-1* in zebrafish resulted in hydrocephalus, yolk tube malformations and severe anemia. Worm HRG-1 fully rescued all phenotypes observed due to knockdown of *hrg-1* in zebrafish [12••]. The phenotypes resulting from knockdown of zebrafish *hrg-1* were restricted specifically to the erythroid lineage and did not impact other hematopoietic cell lineages. Additionally, significant heme-induced inward currents were observed in *Xenopus* oocytes injected with cRNA for CeHRG-1, CeHRG-4, and the human homolog, hHRG-1, indicating heme-dependent transport across cell membranes [12••].

Human *HRG-1* (*SLC48A1*) mRNA was abundant in the brain, kidney, heart and skeletal muscle and in cell lines derived from duodenum, kidney, bone marrow and brain [12••]. hHRG-1 localized to acidic endosomal and lysosomal organelles in HEK293 cells, and its affinity for heme decreased with increasing pH. Additionally, tyrosine (YxxxØ) and acidic-dileucine (DxxIL) based sorting motifs were found in the C-terminus of both *C. elegans* and

Figure 1



A schematic description of known heme transporters. HRG-1 is a heme importer that localizes to endosomal/lysosomal compartments, but can traffic to the plasma membrane. HCP1 and FLVCR2 are two putative heme importers. The cell surface FLVCR1a and the ABC transporter ABCG2 have been implicated in heme export in erythroid cells, whereas the mitochondrial isoform FLVCR1b transports heme into the cytosol. ABCB6 was previously proposed to be a mitochondrial porphyrin/heme importer, but has recently been shown to localize to the plasma membrane and endosomal/lysosomal vesicles. ABCB10 forms a complex with MFRN1 and FECH, and stabilizes MFRN1. It is not clear whether ABCB10 transport heme. Heme carrier/chaperone that is responsible for intracellular and intercellular heme trafficking remains unknown. Question marks represent the presumptive heme trafficking pathways. PM, plasma membrane.

human HRG-1 [12••]. Yanatori and colleagues recently reported hHRG-1 localized to the plasma membrane and lysosomes in non-polarized HEp2 cells. In polarized MDCK cells, hHRG-1 was located to the basolateral membrane and a cytosolic organelle just under the apical membrane [13]. A recent study showed that hHRG-1 interacted with the c subunit of the vacuolar proton ATPase (V-ATPase) pump and enhanced endosomal acidification [14]. Together these studies suggest hHRG-1 plays a role in the transport of heme from the exoplasmic space or lumen of acidic endosome–lysosome compartments into the cytoplasm.

Interestingly, in addition to lysosomal localization in HEK293 cells, hHRG-1 is also recruited and colocalizes with Nramp1 at the erythrophagosomal membrane, surrounding ingested RBCs in bone marrow derived macrophages (BMDMs) [15]. However, the absence of HO-1 at this location indicates that during EP, at least a portion of heme released from degraded hemoglobin is mobilized by hHRG-1 to the cytoplasm [15]. The cytosolic heme can then undergo intracellular redistribution including degradation by HO-1 for iron recycling, or be exported by heme effluxers. Indeed, a recent study shows that HRG1 is essential for macrophage iron homeostasis and transports heme from the phagolysosome to the cytoplasm during EP [16••]. HRG1 is strongly expressed in macrophages of the reticuloendothelial system and specifically localizes to the phagolysosomal membranes during EP. Depletion of Hrg1 in mouse macrophages causes attenuation of heme transport from the phagolysosomal compartment suggesting that HRG1 is the heme transporter for heme-iron recycling in macrophages. The study proposes that genetic variation in *HRG1* may be an important genetic determinant in inherited iron disorders in humans [16••]. HRG-1, as observed for HO-1, was recently identified as a target of the heme-regulated transcription factor BACH1 in microarray expression analysis and ChIP-Seq experiments, further suggesting that HRG-1 may be an important player in erythropoiesis and the phagolysosomal heme transporter [17].

HCP1

Heme carrier protein 1 (HCP1/SLC46A1) is a membrane protein expressed by enterocytes in the duodenum implicated in the absorption of heme in the intestine [18]. Ectopically overexpressing *HCP1* in *Xenopus* oocytes revealed a 2–3-fold increase in heme uptake. Subsequent