Mitochondrial heme: an exit strategy at last

Mark D. Fleming¹ and Iqbal Hamza²

¹Department of Pathology, Boston Children’s Hospital, Harvard Medical School, Boston, Massachusetts, USA. ²Department of Animal and Avian Sciences, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland, USA.

The transport of heme across membranes is critical for iron absorption, the formation of hemoglobin and other hemoproteins, and iron recycling in macrophages. However, the identity of heme transport proteins has been elusive. In this issue of the JCI, Chiabrando et al. reveal that an isoform of the feline leukemia virus subgroup C receptor (FLVCR1) exports heme from the mitochondria and is critical for erythroid differentiation.

Nearly two-thirds of the body’s iron endowment is in the form of hemoglobin in erythrocytes, and each erythrocyte contains more than a billion iron atoms in the form of heme (1). Consequently, it is not surprising that inherited or acquired defects in hemoglobin synthesis, including the thalassemias, hemoglobinopathies, and iron deficiency, are among the most prevalent human diseases. Importantly, the approximately 360 billion erythrocyte produced daily require over 250 mg of heme to assemble into hemoglobin. Heme is synthesized in the mitochondria, but globin is translated in the cytosol, and it is unclear how newly synthesized heme is transported out of the mitochondria for incorporation into hemoglobin (2). Heme transport across membranes is important for dietary iron absorption and crucial for erythrocyte heme iron recycling in the reticuloendothelial system (RES) macrophage. Despite the physiologic importance of these processes, the molecular pathways of transmembrane heme transport have, for the most part, remained obscure, in large part due to technical difficulties in identifying heme-specific transporters in mammalian cells and the inability to translate these findings to whole organisms. In this issue of the JCI, using a combination of siRNA studies and targeted mutations in mice, Chiabrando et al. provide compelling evidence that an isoform of the feline leukemia virus subgroup C receptor (FLVCR1) exports heme from the mitochondria (3).

The requirement for heme transport

For more than 50 years, it has been known that nutritional heme-iron is absorbed in the intestine by a active, energy-dependent, and inducible process that requires a heme transporter in enterocytes. This is because elemental iron has limited bioavailability in the intestine due to the presence of natural iron chelators, such as phytates and tannins, as well as its tendency to oxidize (i.e., to rust) and precipitate. In contrast, even though heme-iron constitutes only one-third of total dietary iron, it is more easily absorbed and is the source for two-thirds of body iron in meat-eating individuals (4). This is a consequence of heme’s solubility at intestinal pH and because its uptake is not known to be influenced by other dietary factors.

Heme must also be transported across mitochondrial membranes because the final steps of heme synthesis occur in the mitochondria, but some hemoproteins such as hemoglobin are cytosolic (2). Likewise, heme transport out of phagolysosomes is an essential component of iron recycling by macrophages, as heme oxygenase (Hmox), the enzyme that catalyzes the oxidation of heme to biliverdin, carbon monoxide, and ferric iron, is found largely tethered to the cytosolic surface of the endoplasmic reticulum (5). Iron recycling out of the macrophage is mediated by the ferrous iron exporter ferroportin (FPN1) (6), whose cell-surface expression is tightly controlled by the systemic iron regulatory hormone hepcidin (7). In this manner, iron catabolized by RES macrophages from heme can be released immediately to the plasma to replenish the Fe₂⁺-transferrin pool necessary for erythropoiesis or stored as ferritin for subsequent use.

Mammalian heme transporters

The identification and characterization of heme and other porphyrin transporters in mammals has proven to be difficult (2), in part due to a lack of genetic and molecular tools, but also as a consequence of the promiscuity of proteins capable of transporting heme with low affinity. Furthermore, in vivo investigation of transporter proteins identified in vitro or on the basis of their expression pattern has been misleading or has provided ambiguous results. For example, Hep1, a putative apical intestinal heme importer, was eventually proven to be essential for folate transport in the intestine when it was found to be mutated in patients with congenital folate deficiency (8, 9). Whether Hep1 also contributes to the absorption of heme in the intestine is uncertain at this time. Some evidence suggests that the breast cancer...
BCRP resistance protein (11). Furthermore, null can mediate the efflux of a large number of heme-responsive genes (HRGs) and protects cells from heme toxicity (12). Using the heme auxotroph Caenorhabditis elegans, Hamza and colleagues identified a mitochondrial isoform, FLVCR1b, transports heme from the mitochondria to the cytosol and thus is critical for the synthesis of hemoglobin and differentiation of erythroid cells.

**FLVCR1 and human disease**

The association of pure red cell aplasia and skeletal abnormalities has suggested that mutations in FLVCR1 might be found in human patients with the inherited form of pure red cell aplasia termed Diamond-Blackfan anemia (DBA), which is also associated with skeletal abnormalities. FLVCR1 mutations in DBA patients have not been found as of yet (19); however, if they were to exist, one would predict that they would occur in the FLVCR1b isoform. Interestingly, nonsense mutations in FLVCR1 have recently been described in patients with the rare autosomal recessive disease posterior column ataxia and retinitis pigmentosa (PCARP) (20, 21). Three of the four reported disease-associated FLVCR1 variants, all of which are present in the homozygous state in PCARP patients, occur on exon 1 of the gene — the part of the gene that is omitted in the FLVCR1b transcript and protein. Based on this finding, one might speculate that heme export by the cell-surface FLVCR1a may have a uniquely important function in neuronal cells. It is equally possible, as has been observed with many other proteins capable of transporting heme, that FLVCR1a binds other substrates with higher affinity than heme. It is, however, more problematic to reconcile the fourth patient mutation, which occurs in a transmembrane domain common to the FLVCR1a and FLVCR1b isoforms. Individuals harboring this mutation are not anemic, as the murine model would predict (20), raising the possibility that this genetic lesion has a unique effect on transport of an alternative substrate. If FLVCR1b localizes to the inner membrane of the mitochondria, does heme exit the mitochondrial outer membrane through unconventional means? Furthermore, how do eukaryotes that lack obvious FLVCR homologs, such as yeast, export heme from the mitochondria? Pertinent biochemical questions also remain: FLVCR1a is proposed to transport heme out of the cytoplasm, while FLVCR1b transports heme into the cytoplasm. Because FLVCR1 is an MFS family member of secondary transporters, are the sources of energy to translocate heme and the kinetic mechanisms (uniporter, symporter or antiporter) different between the two isoforms? Only time will reveal the truth in the myriad of theoretical possibilities.

Address correspondence to: Mark D. Fleming, Department of Pathology, Boston Children’s Hospital, S. Burt Wollbach

---

**Figure 1**

Two isoforms of FLVCR1 may regulate heme levels. FLVCR1a isomoph had previously been postulated to transport cytosolic heme across the plasma membrane. Chiabrando et al. now show that a mitochondrial isoform, FLVCR1b, transports heme from the mitochondrion into the cytosol and thus is critical for the synthesis of hemoglobin and differentiation of erythroid cells.

![Diagram of FLVCR1 isoforms](https://example.com/diagram.png)
Taking a bite: endocytosis in the maintenance of the slit diaphragm

Rosemary V. Sampogna and Qais Al-Awqati

Department of Medicine, College of Physicians and Surgeons of Columbia University, New York, New York, USA.

In the kidney, the slit diaphragm joins adjacent podocytes, forming an epithelial barrier that filters plasma into the urinary space, yet retains blood cells and proteins within the circulation. In this issue of the JCI, Soda et al. have identified clathrin-mediated endocytosis as a central mechanism by which the function and structural integrity of the slit diaphragm are maintained.

The glomeruli of the human kidney filter almost 200 liters of fluid every day, but must retain the albumin and other large proteins within the circulation. The fluid has to cross almost 200 liters of fluid every day, but must be filtered through a membrane that filters plasma into the urinary space, yet retains blood cells and proteins. The GBM (1) and the slit diaphragm (2), a structure that joins adjacent podocytes, are two barriers to protein filtration, but the endothelial cell also likely plays a role. Disruption of these barriers results in proteinuria, a consequence of many kidney diseases. Podocytes begin life as tall epithelial cells that have typical tight junctions near the apex separating the apical surface from the lateral cell membranes. As development progresses, these junctions migrate downward toward the basal surface of the mature podocytes, becoming the slit diaphragm (3). Unlike other polarized epithelia, the lateral surface is above these specialized tight junctions and is continuous with and identical to the apical surface. The basal surface differentiates into thin interdigitating structures called foot processes that are joined together by the cell-cell junctions of the slit diaphragm. During proteinuric disease, the foot processes lose their fine structure and collapse into a mass of cytoplasm on top of the basement membrane (4). The observation that a great abundance of actin and actin-binding proteins accumulates in these diseased foot processes suggested that actin dysregulation might play a key role in the pathogenic progression (5). The discovery that α-actinin4 mutations were also associated with heavy proteinuria also helped bring attention to the role of actin (6).

Since then, mutations in humans or animals in other actin-binding proteins—including Nck, CD2AP, and dynamin—were also found to mediate proteinuria (7). Further, the cytoplasmic domains of many slit diaphragm components can bind actin or signal to actin, further suggesting a key role for this cytoskeletal protein (8). But what was the actual cell biological process in which actin was so central? Was the primary function of actin to maintain the slit diaphragm, or was the actin rearrangement merely a consequence of the permeability change? Here, one might turn to basic cell biology and physiology to find the definitive answers. Unfortunately, the podocyte, when grown in culture, loses its complex shape; it has neither foot processes nor a real slit diaphragm. Given that actin is directly involved in cell shape determination and maintenance, this has placed the field in a difficult position. However, an article in this issue by Soda et al. has provided the first insight into the process of maintenance of the slit diaphragm (9). The authors show that clathrin-mediated endocytosis is required for podocyte function and is the likely effector of the changes in actin that are so often observed.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: J Clin Invest. 2012; 122(12):4330–4333. doi:10.1172/JCI66785.

In this kidney, the slit diaphragm joins adjacent podocytes, forming an epithelial barrier that filters plasma into the urinary space, yet retains blood cells and proteins within the circulation. In this issue of the JCI, Soda et al. have identified clathrin-mediated endocytosis as a central mechanism by which the function and structural integrity of the slit diaphragm are maintained.