

Primer

How Mammals Acquire and Distribute Iron Needed for Oxygen-Based Metabolism

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Virtually all cells and organisms require iron to perform basic cellular processes. In respiration, iron proteins capture energy released from oxidation of food by synthesizing high-energy compounds, such as NADH, that

are used to fuel cellular metabolism. Iron also enables hemoglobin in red blood cells to bind and transport oxygen to tissues throughout the body. Since iron is indispensable for respiration and oxygen transport, it is not surprising that cellular acquisition of iron has not been left to chance. It can be a challenge for organisms to obtain sufficient iron because much environmental iron is very insoluble. Thus, elaborate systems for uptake of

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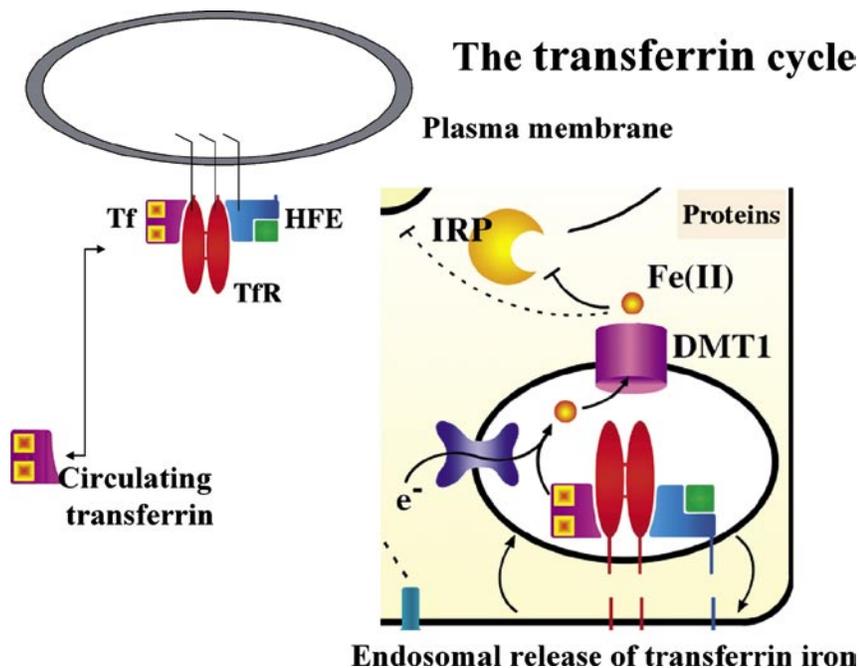
Abbreviation: IRP, iron regulatory protein

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DOI: 10.1371/journal/pbio.0000079

Primers provide a concise introduction into an important aspect of biology that is of broad and current interest.





DOI: 10.1371/journal.pbio.0000079.g001

Figure 1. Binding of Transferrin

Transferrin (purple rhombus) binds two ferric iron atoms (orange squares) and circulates in the bloodstream until it comes into contact with transferrin receptors, which pair together to form dimers (red torpedo shapes) on the plasma membrane surface of cells. A complex of HFE (blue square) and β 2-microglobulin (green square) binds to the transferrin receptor. Notably, the binding site for HFE overlaps the potential binding site of transferrin. Since it is likely that HFE associates with transferrin receptors shortly after synthesis, before the complex arrives at the plasma membrane, HFE can thus potentially prevent iron uptake through the transferrin receptor. Once transferrin binds the transferrin receptor (assuming that both potential binding sites are not blocked by HFE), the complex internalizes in an endosome, where acidification promotes release of ferric iron, and reduction by addition of an electron (e^-) to ferrous or diferric iron allows transport of free iron from the endosome, using the divalent metal transporter, DMT1, to the cytosol. Upon release into the cytosol, iron can be incorporated into proteins, and its presence can be sensed by iron regulatory proteins (IRPs).

iron and distribution within organisms are found throughout the kingdoms of life (reviewed in Lippard and Berg 1994).

Iron deficiency has been a common affliction throughout human history, and it is now estimated to affect 1 billion people worldwide (Baynes and Cook 1996). The characteristic syndrome of pallor, weakness, shortness of breath, and swelling was initially described by the ancient Egyptians, but it was not until the 20th century that doctors learned that these symptoms were caused by iron deficiency (Fairbanks and Beutler 1995). In mammals, dietary iron is absorbed from digestion of food in the duodenal portion of the gut. Specific iron transporters are present in epithelial cells that line the duodenum, and these transporters are much more abundant in iron-deficient animals. Mammals—including humans—have the ability to communicate their iron needs to the duodenum (Anderson et al. 2002; Andrews 2002).

In part because of iron's relevance to human disease, the molecular mechanisms by which mammals register their need for iron and activate responses that will restore iron balance are the subject of much ongoing study. Abnormal regulation of iron uptake is the cause of the common human disease hemochromatosis, in which excessive iron uptake eventually leads to iron overload and disease of the liver, heart, and other organs (Anderson and Powell 2002; Ajioka and Kushner 2003).

Once iron has crossed the barrier cells of the duodenum, it is released into blood and is circulated to tissues throughout the body. However, it is not practical to circulate free elemental iron because iron can bind indiscriminately to many proteins. Therefore, iron is transported in an unreactive form by a specific carrier protein, transferrin (Baker et al. 2003). Most of the iron that gains access to the circulating blood binds tightly to serum transferrin, an abundant protein that binds one (monoferric) or two ferric

iron atoms (diferric or holotransferrin) with high affinity. When ferric iron is bound to transferrin, it is nonreactive, meaning that it does not engage in single-electron transfers and it does not threaten other proteins and blood vessel walls with its reactivity. Also, because transferrin is a large protein, it remains in the circulation instead of being lost in urine when it passes through the kidney. Thus, cells and tissues that need to replenish their iron stores can do so by taking up transferrin that contains bound iron. Cells accomplish this task by synthesizing transferrin receptors, proteins that are present as pairs (dimers) on the cell surface. Membranes of iron-starved cells contain many more transferrin receptors than those of iron-replete cells, indicating that cells can appraise their own iron needs and increase transferrin receptor synthesis when they are iron-starved (Aisen 1994).

When holotransferrin binds to the transferrin receptor, the complex of transferrin bound to the receptor is internalized by a process known as clathrin-mediated endocytosis, a mechanism for moving the surface receptor complexes into discrete membrane-bound physical environments within cells known as endosomal vesicles (Mousavi et al. 2003). These endosomes are then acidified by vesicular membrane proteins that pump protons, and iron is released from transferrin upon vesicular acidification. The transferrin that now lacks iron (apotransferrin) remains bound to the transferrin receptor, and this complex recycles to the cell surface, where apotransferrin dissociates from the transferrin receptor upon exposure to normal pH. The iron that was released within the acidified endosome is then transported across the vesicular membrane into the cytoplasm, where it is used to meet the nutritional needs of the cell (reviewed in Klausner et al. 1993).

In the inherited human disease hemochromatosis, researchers spent many years trying to identify the genetic abnormality responsible for iron overload. In 1996, after several decades of searching, the main gene that causes genetic hemochromatosis was identified (Feder et al. 1996). Much to the surprise of investigators, this gene, known as HFE, proved to be related to a group of proteins important in immunology, known as major histocompatibility complex antigens. More surprising still was the



discovery that HFE binds to transferrin receptor dimers (Feder et al. 1998). When proteins bind to one another in cells, it is usually because they are collaborating to accomplish an important task. However, in the case of the transferrin receptor–HFE complex, it remains unclear how HFE contributes to transferrin receptor function and iron metabolism.

Often in biology, information about the shape of protein complexes and their location in cells can facilitate insights into mechanisms of protein function. Previously, the structure of the transferrin receptor dimer bound to HFE and a smaller molecule known as β 2-microglobulin has been described in great detail (Lebrón et al. 1998; Bennett et al. 2000). However, though the structures of various transferrins alone have been obtained, attempts to obtain structural information about the transferrin–transferrin receptor complex have been unsuccessful. When the individual structures of proteins that bind to one another are known, as is the case for transferrin and the transferrin receptor, educated guesses about amino acid contact points between proteins can be evaluated by substituting various amino acids in each protein to see whether binding is affected.

In an article in this issue of *PLoS Biology* by Giannetti et al. (2003), the authors have substituted specific amino acids in the transferrin receptor with different amino acids to determine whether they are important in binding either HFE or diferric transferrin. They have reached an interesting conclusion: their data indicate that diferric transferrin and HFE bind to physically and functionally overlapping sites on the transferrin receptor (West et al. 2001). A logical extension of this conclusion is that if each monomer in the transferrin receptor dimer were bound to HFE, the transferrin receptor would not be able to bind and internalize diferric transferrin. Thus, it would be very interesting to know how many transferrin receptor complexes contain HFE and whether there are situations when there are two HFE molecules bound to each transferrin receptor dimer. If HFE has the potential to prevent binding of transferrin, which is the reason that the transferrin receptor exists in the first place, why is HFE present at all? Is it possible that HFE plays a role in vesicular trafficking? Does the HFE

protein contain information that allows the iron-loaded vesicle to interact with specific proteins or compartments that will efficiently monitor iron influx and appropriately distribute the iron within the cell? Is HFE synthesized to prevent iron uptake through the transferrin receptor under special circumstances? Many additional studies using imaging and biochemical techniques will be needed to address the role of HFE.

Structural information can also help to model how iron is released from the transferrin–transferrin receptor complex within acidified endosomes. Giannetti et al. (2003) suggest that several of the amino acids to which holotransferrin is bound at neutral pH cease to act as tight binding sites once they undergo protonation at acidic pH. The holotransferrin then is free to move about and adopt different binding conformations, some of which favor the release of iron from transferrin within the endosome. This arrangement ensures that iron is released only when it is in an enclosed vesicle that is designed to handle the free iron.

Once the iron is exported from the endosome, little is known about how it is transported and made available to proteins in the cell that require iron incorporation to function. Several cytosolic proteins, known as iron regulatory proteins (IRPs), are responsible for monitoring cytosolic iron levels and regulating iron availability. When iron supplies are low, IRPs increase transferrin receptor expression and thereby help the cell to restore iron balance (reviewed in Rouault and Klausner 1997; Schneider and Leibold 2000).

Thus, transferrin and the transferrin receptor are proteins that play central roles in mammalian iron metabolism. Transferrin solves the problem of how to move iron through the body safely and efficiently. Similarly, the transferrin receptor protects cells from indiscriminate and potentially harmful iron uptake. The transferrin receptor is present on the surface of only those cells that need iron, because cells regulate how much transferrin receptor they make according to their needs. Clearly, HFE binding will be important in transferrin receptor function, but we do not yet understand how it functions. However, all future models will need to account for the fact that the binding site of HFE on the transferrin receptor overlaps with the binding site of

transferrin. The fact that we cannot yet offer a good reason for this design reveals that there is much more work to do on the relationship between transferrin receptors and HFE. ■

Acknowledgments

I thank Caroline Philpott for allowing me to incorporate her original artwork into the figure.

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