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Iron Homeostasis in Insects

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Abstract

Iron is an essential micronutrient for all types of organisms; however, iron has chemical properties that can be harmful to cells. Because iron is both necessary and potentially damaging, insects have homeostatic processes that control the redox state, quantity, and location of iron in the body. These processes include uptake of iron from the diet, intracellular and extracellular iron transport, and iron storage. Early studies of iron-binding proteins in insects suggested that insects and mammals have surprisingly different mechanisms of iron homeostasis, including different primary mechanisms for exporting iron from cells and for transporting iron from one cell to another, and subsequent studies have continued to support this view. This review summarizes current knowledge about iron homeostasis in insects, compares insect and mammalian iron homeostasis mechanisms, and calls attention to key remaining knowledge gaps.

1. OVERVIEW OF IRON IN BIOLOGICAL SYSTEMS

Iron is an essential micronutrient for all types of organisms, mainly because iron functions as a cofactor for many indispensable enzymes (30). In insects, iron is a cofactor for enzymes involved in cellular respiration, DNA synthesis, detoxification of pesticides and plant defense compounds, neural function, and many other physiological processes (13, 81, 91, 102, 106). Some types of insects use iron for geomagnetic orientation (52, 104). A significant difference in the iron requirements between insects and mammals is the proportion of iron bound to hemoglobin. Most of the iron in mammals is bound to hemoglobin and functions in oxygen transport; in contrast, insects rely on a tracheal system for oxygen delivery and, thus, have much less hemoglobin-bound iron (14, 30).

Iron in biological systems is present in either the reduced ferrous (Fe^{2+}) form or the oxidized ferric (Fe^{3+}) form, and cycling between the two oxidation states is an important aspect of iron homeostasis (62). The need for redox cycling is related to the different properties of the two forms of iron: Ferrous iron is soluble under most physiological conditions, but it participates in toxic radical formation; ferric iron is less toxic but is insoluble at physiological pH (62). Iron in biological systems is typically in a protein-bound state that prevents iron precipitation and harmful chemical reactions (30, 63).

Iron homeostasis is better understood in mammals than in insects; thus, information about mammalian iron homeostasis informs iron-related studies in insects (81, 115). However, early studies of insect iron-binding proteins suggested that insects and mammals have quite different mechanisms of iron homeostasis, and subsequent studies have continued to support this view (81, 99, 115, 134, 144). This review highlights the similarities and differences (**Figure 1**).

2. BRIEF SUMMARY OF IRON HOMEOSTASIS IN MAMMALS

The first step in mammalian iron homeostasis is the uptake of heme (an iron-containing porphyrin) and nonheme iron from the diet (41) (**Figure 1**). Uptake of nonheme iron by intestinal absorptive cells (enterocytes) is better understood than heme uptake; it occurs in the acidic first section of the small intestine (duodenum) (18, 60). Nonheme iron in the diet is predominantly in the ferric form, and it needs to be reduced before it can be transported across the plasma membrane of an enterocyte (60). One ferric reductase that catalyzes this step is duodenal cytochrome b (DCytb), but at least one other ferric reductase or ferric reduction mechanism is likely to contribute to nonheme iron uptake (18, 30, 70). The resulting ferrous ions are transported across the duodenal cell membrane by the proton symporter divalent metal transporter 1 (DMT1), while the proton gradient needed for ferrous transport is likely to be provided by Na^+/H^+ exchanger-3 and a gastric H^+/K^+ ATPase that acidifies the stomach (67, 109, 142). How intestinal cells take up heme from the diet is still uncertain (18, 41). A proton-coupled folate transporter, also known as heme carrier protein 1 (HCP1), appears to be involved, but additional research is needed to fully understand the role of this protein in heme uptake (18, 41, 74).

Once iron is transported into a cell, it can be used, stored, or exported (**Figure 1**). Heme can remain intact or be degraded by heme oxygenase (HO) to release ferrous ions (23). Ferrous ions transported into the cell via DMT1 or released from heme may be bound by a member of the Poly(rC)-binding protein (PCBP) family; the PCBPs donate iron to enzymes that require iron as a cofactor, deliver iron to ferritin for storage, and traffic iron to the basal side of the cell for iron export (143). Iron is also delivered to mitochondria, where it is needed for enzyme metalation, heme synthesis, and iron–sulfur cluster biosynthesis (23, 60, 118). In mammals, iron is stored inside cytosolic ferritin (3). As ferrous ions enter ferritin, they become oxidized, and the resulting ferric ions (up to approximately 4,000) are stored within the ferritin cavity as ferrihydrite (3, 9).

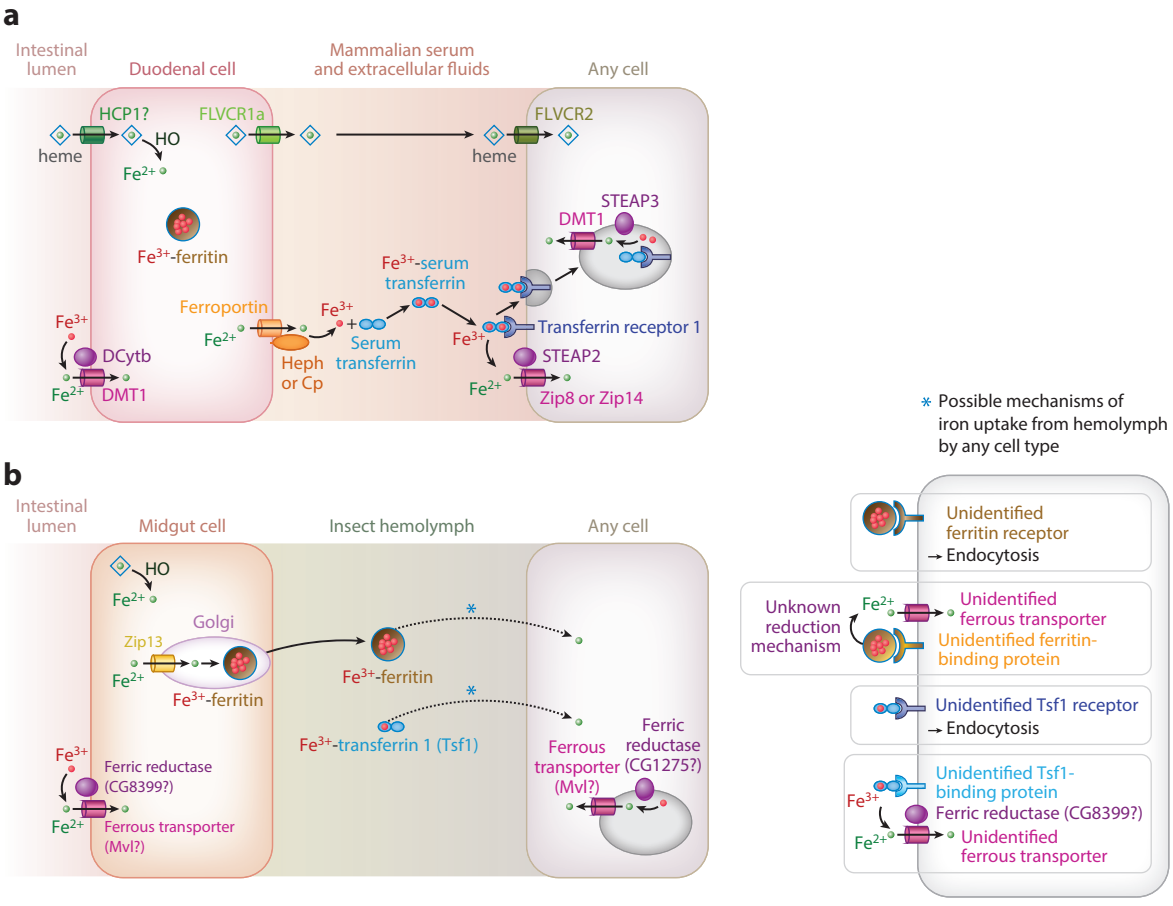

Figure 1

Iron transport in mammals and insects. Some key aspects of iron transport in (*a*) mammals and (*b*) insects are shown. Abbreviations: Cp, ceruloplasmin; DCytb, duodenal cytochrome b; DMT1, divalent metal transporter 1; FLVCR, feline leukemia virus subgroup C receptor; HCP1, heme carrier protein 1; HO, Heme Oxygenase; Heph, hephaestin; Mvl, Malvolio; STEAP, six-transmembrane epithelial antigen of the prostate; Tsf1, Tranferrin 1; Zip, Zinc-regulated, iron-regulated transporter-like protein.

The best-understood iron export mechanism involves efflux of ferrous ions (**Figure 1**), although iron can also be exported as heme or as ferritin-bound iron. Ferrous ions are exported from enterocytes and other mammalian cells by the ferrous permease ferroportin (24). Once outside the cell, the ferrous ions are oxidized by an extracellular multicopper ferroxidase (e.g., membrane-bound hephaestin or soluble ceruloplasmin) (49, 98). The resulting ferric ions are bound by serum transferrin, an extracellular protein that binds two ferric ions with high affinity (37). Serum transferrin then traffics iron to other cells (37). Export mechanisms for heme and ferritin are not well characterized. Only one heme exporter, feline leukemia virus subgroup C receptor 1a (FLVCR1a), has been studied in detail (16, 23). Mammalian ferritin, although primarily a cytosolic protein, is also released from cells, after which it is referred to as serum ferritin (127).

Uptake of iron by cells other than enterocytes can occur via multiple pathways (**Figure 1**). Iron uptake from Fe^{3+}-transferrin mainly occurs via two pathways, both involving transferrin receptor 1 (2, 65). The most widely known mechanism is receptor-mediated endocytic uptake of

Fe^{3+}-transferrin. In the acidified endosome, iron is released from serum transferrin, reduced by a ferric reductase [e.g., Six-transmembrane epithelial antigen of the prostate 3 (STEAP3)], and transported across the endosome membrane through a ferrous transporter (e.g., DMT1) (30). A less recognized mechanism for the uptake of transferrin-bound iron involves a conformational change of the receptor-bound transferrin that allows reduction of ferric ions (e.g., by STEAP2) and transport into the cell through a ferrous transporter [e.g., Zinc-regulated, iron-regulated transporter-like protein 8 or 14 (Zip8 or Zip14)] (65). Uptake of heme by nonenterocytes is not well understood but is thought to involve the transporters HCP1 and FLVCR2 (16, 23). Endocytic uptake of serum ferritin can be mediated by one of several multifunctional receptors (127).

In mammals, the amount of iron in the body is regulated primarily by controlling the export of iron from enterocytes through a process regulated by the hormone hepcidin (18). Hepcidin expression increases under iron-replete conditions, which results in the ubiquitination and degradation of ferroportin, leading to decreased iron efflux (18, 30). The iron content of individual cells is controlled by many mechanisms that affect iron uptake and export (30).

3. UPTAKE OF IRON FROM THE DIET IN INSECTS

Insect diets vary in the quantity and form of iron that they contain; for example, herbivores that feed on the leaves of woody plants have a diet containing approximately 220–2,100 μmol L^{-1} iron, presumably in the form of nonheme iron, whereas hematophagous insects that feed on mammalian blood have a diet containing approximately 10 mmol L^{-1} iron, mainly in the form of heme bound to hemoglobin (6, 50, 148). Given the diversity of insect diets, it seems likely that significant differences in iron homeostasis exist among types of insects. In fact, multiple mechanisms for handling the large concentration of heme in a blood meal have evolved in hematophagous insects (113, 134). In addition, among various types of insects, several differences in other aspects of iron homeostasis have been identified (described below).

The nonheme iron in insect diets is expected to be predominantly in the ferric form, but all known iron transporters in animals are specific for ferrous ions (61, 64); therefore, ferric ions in the midgut lumen must be reduced by some mechanism prior to uptake. How this process occurs in insects is still unknown. A member of the Cytochrome b561 (Cytb561) family of proteins, DCytb, catalyzes ferric reduction in the apical membrane of mammalian enterocytes (70) (**Figure 1**). Three conserved Cytb561s in insects have been considered as candidate ferric reductases: Two, named CG1275 and No extended memory (Nemy) in *Drosophila melanogaster*, are similar to DCytb, and a third, CG8399 in *D. melanogaster*, is more distantly related (73, 81, 101, 115, 121, 122, 135). Of the three, only CG8399 is known to have ferric reductase activity (101, 121). CG8399 is located in the plasma membrane and is expressed in the midgut, making it a promising candidate for a role in iron uptake from the midgut lumen (17, 58). CG1275 is expressed in the midgut but may be present in endosomal and lysosomal membranes rather than in the plasma membrane; therefore, CG1275 is more likely to function in the endocytic pathway than in the apical membrane of midgut cells (73). Nemy does not appear to have midgut expression (17).

Ferrous ions that have been generated in the midgut lumen could be transported into midgut cells through a ferrous transporter. Because different ferrous transporters function at either acidic pH or neutral pH (65), it is likely that the pH of an insect's digestive system specifies what type of iron transporter is functional in the midgut. Midgut lumenal pH differs among insects and is correlated with insect phylogeny (116). Cyclorrhaphous dipterans, including *D. melanogaster*, have a specialized, acidic midgut region that is involved in uptake of iron from the diet and is thus referred to as the iron region of the midgut (86, 88, 130, 131). The digestive systems of some

orthopteran and hemipteran insects may also have restricted regions of iron uptake, although these regions are not necessarily restricted to acidic areas (131).

Ferrous iron uptake by mammalian enterocytes occurs via DMT1; therefore, an insect homolog of DMT1, Malvolio (Mvl), is predicted to play a similar role in insects (81, 115) (**Figure 1**). Because these types of transporters are proton symporters, Mvl is predicted to function in low-pH environments such as acidic regions of the midgut and in late endosomes and lysosomes (65). Most insect species have one or two Mvl homologs, although Mvl is lacking in culicine mosquitoes (84, 120, 125). Several lines of evidence indicate that Mvl is involved in iron uptake. *Anopheles albimanus* Mvl was shown to enhance iron uptake by *Xenopus laevis* oocytes (83). *D. melanogaster* Mvl is expressed in the acidic region of the midgut where iron uptake occurs, although its presence in the plasma membrane has not yet been verified (12). Recombinant *D. melanogaster* Mvl was targeted to the plasma membrane and to intracellular sites in cultured cells, but endogenous Mvl was detected only in intracellular locations in the anterior and posterior midgut (29, 111). *D. melanogaster* Mvl loss-of-function mutants had less iron in the iron region of the midgut, and this phenotype was not rescued by dietary iron supplementation, indicating that Mvl is important for iron uptake in the iron region (8). Mvl mutants also had phenotypes (low whole body iron content and an abnormal taste preference) that were rescued by iron supplementation, suggesting that iron uptake in the iron region of the midgut is not the only role for Mvl in iron homeostasis (8, 96).

Although Mvl seems likely to transport ferrous ions from the diet in cyclorrhaphous dipterans, a different transporter must function in insects that lack an acidic midgut section. In addition, the well-documented uptake of iron by the anterior midgut of *D. melanogaster* is expected to involve a transporter that functions at neutral pH (8, 88, 108). The two well-characterized mammalian ferrous importers that function at neutral pH are Zip8 and Zip14 (61). Although Zip family members are present in insects, orthologs of Zip8 and Zip14 have not been identified (103, 120, 125). RNA sequencing–based and RNA interference (RNAi)-based screens for iron transporters in *Aedes aegypti* identified candidate iron importers, including Zinc transporter 7 (ZnT7) and Dyspepsia (119, 120). ZnT7, which transports zinc into the Golgi in mammalian and *D. melanogaster* cells, is expressed in the *A. aegypti* midgut, and knockdown in cultured cells resulted in decreased ferritin-reporter expression, suggesting that it plays a role in iron uptake (46, 78, 103, 120). Dyspepsia is a member of the SLC16 family of transporters, which are not known to be iron transporters (43); however, knockdown of Dyspepsia in *A. aegypti* cultured cells resulted in decreased ferritin-reporter expression, suggesting that it too plays a role in iron uptake (119). Additional studies of ZnT7, Dyspepsia, and other candidate iron transporters will clarify their roles in iron homeostasis.

Heme uptake by the insect midgut has been studied mostly in blood-feeding insects, which consume large quantities of heme. In *A. aegypti*, greater than 95% of the iron transported from a blood meal to various tissues originates from hemoglobin (148). An insect heme transporter has not been definitively identified. The insect ortholog of mammalian HCP1 (CG30345 in *D. melanogaster*) is expressed in the midgut, but whether it plays a role in iron homeostasis has not been established (17, 18, 81). An RNAi-based screen, informed by gene expression analyses, identified four candidate heme importers in *A. aegypti* (AAEL000417, AAEL003318, AAEL004513, and AAEL012440); future studies will resolve their possible role in heme uptake (27). A midgut-associated heme transporter has been identified in ticks, although this protein transports heme not through the apical membrane but instead through the membrane surrounding digestion vesicles (72). The insect ortholog of this protein, ABCB10, is predicted to be a mitochondrial membrane protein and has not been implicated in heme transport (27, 72, 73, 134). Insects also have an uncharacterized homolog of the mammalian heme importer FLVCR2 (CG1358 in *D. melanogaster*) (73).

4. INTRACELLULAR IRON IN INSECTS

Some of the heme that is transported into cells is degraded by HO, a conserved, ubiquitously expressed enzyme that is localized to the endomembrane system and positioned to cleave heme in the cytosol (39) (**Figure 1**). *D. melanogaster* and *Anopheles gambiae* HOs have been biochemically characterized (112, 146). Like mammalian HOs, they cleave heme, leading to the release of a ferrous ion (112, 146). The affinities of *A. gambiae* HO and human HO for heme are similar (112). The affinity of *D. melanogaster* HO for heme is somewhat less, and the active site is thought to have structural differences compared with mammalian and other insect HOs (146). Ubiquitous knockdown of *D. melanogaster* HO resulted in larval and pupal lethality, demonstrating that it is an essential gene, and knockdown in eye discs resulted in iron accumulation and apoptosis in the adult eye (19, 56). Chemical inhibition or RNAi-mediated knockdown of HO in *A. gambiae* and *Rhodnius prolixus* females caused a decrease in fecundity, likely due to a disruption of iron homeostasis or possibly heme toxicity in the ovaries (112, 125).

In mammalian cells, some of the ferrous ions that are transported directly into cells or released from heme are bound by chaperones (e.g., PCBPs) and shuttled to various subcellular destinations (100). No iron chaperones have been identified in insects, but a PCBP family member has been identified in *D. melanogaster* and other insect species (73, 136). The *D. melanogaster* protein, named Mushroom-body expressed (Mub), influences circadian rhythm in flies, as does PCBP1 in cultured mammalian cells (136). It is not known if the circadian rhythm phenotypes are related to changes in iron homeostasis; disruptions in iron homeostasis can affect circadian rhythms in *D. melanogaster*, but PCBP proteins have non-iron-related functions (73, 80, 100, 136).

In contrast to mammalian ferritin, which is mainly a cytosolic storage protein, insect ferritin is present in the secretory pathway (93, 99) (**Figure 1**). In mammals, PCBP-type iron chaperones deliver iron to ferritin in the cytosol, but these chaperones are not expected to cross the membranes of the secretory pathway (100); instead, delivery of iron to insect ferritin is mediated by a ferrous transporter, Zip13, located in the endoplasmic reticulum (ER) and Golgi (138). Phylogenetic analyses have shown that insect Zip13 proteins are orthologous to human Zip13, which functions as a zinc importer (although it may also export iron) (120, 125, 134, 138, 139). Biochemical and genetic evidence that Zip13 exports iron in *D. melanogaster* and *A. aegypti* is very convincing (120, 138–140, 147). Biochemical analyses of *D. melanogaster* Zip13 (Zip99C) demonstrated that it transports radioactive iron, has a DNXXH motif in the fourth transmembrane domain that is essential for iron specificity, and is stabilized by iron through interactions with the amino domain (138, 140, 147). Ubiquitous knockdown of Zip13 in *D. melanogaster* resulted in less iron in the ER and Golgi and in whole bodies, and midgut-specific knockdown resulted in increased activity of a cytosolic iron sensor, aconitase (indicating iron accumulation), and a decrease in the amount of ferritin-bound iron in the midgut cells; overexpression produced opposite outcomes (138). In *A. aegypti*, Zip13 is upregulated after a blood meal, particularly in the midgut (120). Knockdown in adult *A. aegypti* females resulted in accumulation of iron in the midgut and decreased iron in the ovaries, suggesting that iron export from midgut cells was hindered by the lack of Zip13; knockdown in cultured cells resulted in increased expression of an intracellular iron reporter (120).

Ferritin is the best-studied iron-binding protein in insects, and previous reviews have described the pioneering research on ferritin from many insect species (79, 92, 99, 115). Expression of insect ferritin is ubiquitous (99). Like mammalian ferritin, insect ferritin oxidizes ferrous ions, and the resulting ferric ions (up to approximately 3,000 ferric ions per molecule of ferritin) are stored as ferrihydrite within the large ferritin shell (3, 15, 123). The 24 ferritin subunits are encoded by two genes, *Fer1HCH* and *Fer2LCH*, and the two subunits are often, but not always, present in a 1:1 ratio (3, 42, 44, 94, 99). The Fer1HCH subunit oxidizes ferrous ions, while Fer2LCH is

thought to facilitate the formation of ferrihydrite (3, 44, 88). *A. aegypti* is unusual in having two *Fer2LCH* genes, each with a different expression pattern but with unknown functional differences (33, 34). Unlike mammalian ferritin subunits, insect Fer1HCH and Fer2LCH are synthesized with a signal peptide that targets the subunits to the secretory pathway (26, 115). The process of ferritin assembly within the secretory pathway is still not well understood, but it is likely that assembly starts with the formation of heterodimers in the ER, and it is known that temporal regulation of expression is important (44, 105).

Some iron-loaded (holo-)ferritin in the secretory pathway is exported into the hemolymph, but some is stored in the Golgi and other membrane-bound compartments (92, 93, 99). Evidence for storage of holo-ferritin in midgut cells is particularly convincing (88, 92). The addition of iron to cultured *A. gambiae* 4a3b cells resulted in an increase in the amount of ferritin in the Golgi and no detectable ferritin secretion, indicating that the 4a3b cells were storing holo-ferritin in the Golgi (31). In addition, cultured 4a3b and *A. aegypti* CCL-125 cells were found to have ferritin in punctate vesicles that lacked organelle markers, suggesting that they contain a storage form of ferritin (31). Intracellular iron storage is most obvious in xylem-feeding homopterans that have cytosolic and nuclear ferritin (92). The mechanism by which iron is released from ferritin, such that it becomes available to the cell, is not known, but degradation in the lysosome seems likely, since insect ferritin has been observed in lysosomes, and a lysosome-based mechanism of iron release occurs in mammalian cells (31, 76).

A key destination for intracellular iron is the mitochondria, where iron is needed as an enzyme cofactor and for heme and iron–sulfur cluster synthesis (21). In contrast to many aspects of iron homeostasis, the transport and storage of iron in mitochondria appear to be similar between mammals and insects (82, 115). For example, insect mitoferrin and its mammalian homologs transport ferrous ions to the mitochondrial matrix (85, 126). Likewise, insect mitochondrial ferritin (Fer3HCH in *D. melanogaster*) and mammalian mitochondrial ferritin are both expressed primarily in the testis and appear to have similar iron storage functions (25, 87, 126).

5. IRON EXPORT IN INSECTS

Whereas the primary iron export mechanism for mammalian cells involves the ferrous transporter ferroportin (**Figure 1**), insects lack an identifiable ferroportin homolog (2, 81, 115). An RNAi-based screen of predicted metal transporters from *A. aegypti* identified two possible iron exporters: AAEL014762 (Zip13, described in Section 4), which exports iron to the secretory pathway, and AAEL013490, which is orthologous to mammalian Zip11 and insect Zip48C (73, 120, 145). Mammalian Zip11 and *D. melanogaster* Zip48C influence zinc homeostasis, but their possible roles in iron homeostasis have not yet been investigated (20, 145). Additional research is needed to determine whether AAEL013490 functions as a ferrous exporter in vivo.

In mammals, ferrous ions that are exported through ferroportin are oxidized by one of two ferroxidases in the multicopper oxidase family, hephaestin and ceruloplasmin (2) (**Figure 1**). Whether insects have a ferroxidase that functions similarly to hephaestin and ceruloplasmin has not been fully resolved. Insects have two conserved multicopper oxidases: MCO1 and MCO2 (22). MCO2 functions as a laccase (22, 71, 97). MCO1 was thought to be a ferroxidase because MCO1 from *D. melanogaster* has detectable ferroxidase activity, and knockdown interfered with iron homeostasis (71). Likewise, MCO1 knock down in *A. gambiae* and *Helicoverpa armigera* affected the iron content of whole bodies and Malpighian tubules, respectively (77, 97). Like hephaestin, the dipteran MCO1s were detected on the basal surface of tissues (71, 97). However, an analysis of the kinetic properties of MCO1 from *D. melanogaster*, *A. gambiae*, *Manduca sexta*, and *Tribolium castaneum*, using various substrates, demonstrated that MCO1 is unlikely to function as a

ferroxidase in vivo (97). When assayed for ferroxidase activity, the enzymes had a high K_m relative to the expected concentration of ferrous ions in hemolymph; therefore, the affinity of MCO1 for iron is apparently inadequate for MCO1 to function as a ferroxidase in vivo (97). The catalytic efficiency of the MCO1s as ascorbate oxidases was much higher than their catalytic efficiency as ferroxidases, and their K_m values were similar to the concentration of ascorbate in hemolymph (66, 97). Taken together, studies of MCO1 suggest that it functions as an ascorbate oxidase rather than a ferroxidase, and that MCO1 influences iron homeostasis through an unknown mechanism involving ascorbate (97). Cyclorrhaphous dipterans have an additional multicopper oxidase, Mco3 (not orthologous to mosquito MCO3) (8, 22, 73, 128). *D. melanogaster* Mco3 has significant ferroxidase activity, and, like ceruloplasmin, it is a secreted, soluble protein (128). Expression is mainly restricted to pupae (40, 128). Mco3 null phenotypes include increased iron content of the whole body, head, and gut (8, 128). If the affinity of Mco3 for ferrous ions is found to be physiologically pertinent, then Mco3 most likely oxidizes ferrous ions in pupal hemolymph.

Iron export from insect cells is primarily accomplished through the secretion of holo-ferritin (81, 99, 115) (**Figure 1**). The concentration of ferritin in *M. sexta* and *Musca domestica* hemolymph is approximately 100–500 nmol L^{-1}, which is hundreds of times higher than a typical concentration of serum ferritin in humans (15, 53). Iron export via holo-ferritin secretion has been carefully documented for *A. aegypti* CCL-125 cells (31, 32, 36). In *D. melanogaster*, cells with insufficient ferritin accumulate an abnormal amount of iron, resulting in cellular damage consistent with an excess of iron (89, 115). Studies of ferritin mutants and RNAi-mediated knockdown phenotypes in diverse insect species have shown that ferritin is an essential protein; both subunits were shown to be essential in *D. melanogaster* and the planthopper *Nilaparvata lugens* (38, 75, 88, 110, 125).

Some types of mammalian cells export heme through FLVCR1a (23) (**Figure 1**). Insects have a protein in the FLVCR family, but whether it functions in heme export, like FLVCR1a; heme import, like FLVCR2; or no heme transport at all is not known (113, 134). A homolog in *R. prolixus* is upregulated after a blood meal, and knockdown results in a reduction in lifespan and in fecundity (125). A combination of gene expression analyses and RNAi-based screens in *A. aegypti* cultured cells and midgut cells identified one candidate heme exporter (AAEL00864), although the *D. melanogaster* ortholog of this protein, Pummelig, is a phospholipase with functions related to lipid metabolism; therefore, future studies are needed to establish whether this protein has a role in heme export (27, 48).

Mammalian serum transferrin, which is secreted in its apo form, is not thought to export iron from cells. Like serum transferrin, insect transferrin 1 (Tsf1; described in Section 6), is assumed to be secreted as an apo-protein, and evidence of apo-Tsf1 in *M. sexta* hemolymph supports this assumption (53). However, knockdown of Tsf1 in the midgut of *D. melanogaster* resulted in increased iron content of the midgut, and knockdown in muscles resulted in iron accumulation in muscle cell mitochondria (137, 141). One explanation for these results is that Tsf1 can be loaded with ferric ions in the ER or Golgi before being secreted as holo-Tsf1.

6. IRON UPTAKE FROM INSECT HEMOLYMPH

The mechanisms of iron uptake from insect hemolymph are still unknown (**Figure 1**). In mammalian plasma, almost all of the iron is bound to serum transferrin; therefore, most iron uptake involves serum transferrin and transferrin receptor 1 (2). In contrast, based on studies of iron in the plasma of *M. sexta* larvae, most of the iron in insect plasma appears to be bound to ferritin. This assumption is based on the following calculations: Ferritin is present at approximately 0.2 μmol L^{-1}, and each ferritin molecule contains approximately 2,000 ferric ions; thus, the concentration of ferritin-bound iron should be approximately 400 μmol L^{-1}. In contrast, Tsf1 is

present at approximately 1 μmol L^{-1} and can bind no more than one ferric ion; thus, the concentration of Tsf1-bound iron should be no higher than approximately 1 μmol L^{-1} (11, 53, 132). Non-protein-bound iron was undetectable in *M. sexta* plasma (1). The concentration of heme in *M. sexta* plasma is not known. Taken together, these results suggest that iron uptake from hemolymph must involve iron bound to ferritin and/or transferrin.

There is strong evidence that ferritin participates in cellular uptake of iron from hemolymph. Radioactive iron bound to ferritin is transferred to insect tissues, and iron-loaded ferritin is a mitogen for cultured insect cells (75, 114, 148). In addition, knockdown of ferritin in midgut cells results in iron deficiency in other cell types; this result indicates that ferritin secreted from midgut cells delivers iron to other cells in the body (114). The mechanism by which ferritin delivers iron to cells is not yet known (**Figure 1**). Mammalian serum ferritin can be taken up via endocytosis after binding to various receptors [e.g., Transmembrane immunoglobulin and mucin domain 2 (TIM-2) and Scavenger Receptor Class A Member 5 (Scara5)] (65, 127). Orthologs of serum ferritin receptors have not been identified in insects, but endocytic uptake via an unidentified receptor seems likely (115). Alternatively, the ferric ions in ferritin could be reduced and removed from ferritin prior to uptake through a ferrous transporter. This type of mechanism has not been observed in insects or mammals, but it is similar to one utilized by the bacterium *Bacillus cereus* to remove iron from host ferritin (107).

Insect Tsf1, which arose early in insect evolution, is homologous but not orthologous to serum transferrin (4, 90). Serum transferrin evolved with the placental mammal lineage and arose from a duplication of an ancestral gene encoding lactoferrin, which has immune rather than iron transport functions (28, 54, 129). The amino acid sequence of Tsf1 from *D. melanogaster* is equally similar to human serum transferrin and human lactoferrin sequences, consistent with studies demonstrating a role for Tsf1 in both iron transport and immunity (35, 51, 55, 137). Tsf1 and serum transferrin have biochemical and structural differences, including in the way in which they coordinate iron; however, like serum transferrin, Tsf1 has a very high affinity for ferric ions (5, 132, 133). In addition, like serum transferrin, Tsf1 releases iron under moderately acidic conditions, suggesting that Tsf1 could release iron in acidified endosomes after endocytic uptake via an unidentified receptor (5, 132).

Several lines of evidence indicate that Tsf1 is involved in iron uptake. Radioactive iron bound to Tsf1 is taken up by insect cells, and ^{125}I-labeled Tsf1 is transported into developing insect eggs (53, 68). In addition, RNAi-mediated knockdown of Tsf1 in *D. melanogaster* results in changes in iron distribution in the insect body, and a Tsf1 null mutation interferes with the immune-induced transfer of iron from hemolymph to fat body (55, 137). However, Tsf1 null mutant flies have the same amount of iron in fat body, hemolymph, ovaries, and thoraxes as control flies, and they are viable and fertile (55). Additional studies are needed to reconcile the differences in phenotypes resulting from RNAi-mediated knockdown versus a mutant genotype. Notably, the knockdown and null phenotypes are different from those of mice and humans with a severe deficiency of serum transferrin; affected mice and humans have iron overload of various tissues, and affected mice die shortly after birth (7, 45, 47). Despite most types of insects having a Tsf1 ortholog, the louse, aphid, and thrips lineages have lost the *Tsf1* gene and, thus, must have evolved to manage iron without it (90). [Note that insects have three additional orthologous groups of transferrins; however, these transferrins are not likely to have a role in iron homeostasis (90, 117).]

Serum transferrin–mediated iron uptake requires transferrin receptor 1 (65) (**Figure 1**). Insects lack a mammalian transferrin receptor homolog; therefore, hypothetical endocytic uptake of Tsf1 requires an unidentified receptor (35, 69). In mammalian cells, once serum transferrin is endocytosed, iron is released in the acidic environment of the endosome, reduced by a ferric

reductase such as STEAP3, and transported into the cytoplasm through a ferrous transporter such as DMT1 (30) (**Figure 1**). Assuming that endocytic uptake of Tsf1 occurs, iron would probably be reduced by a ferric reductase prior to export to the cytosol through the ferrous transporter Mvl (described in Section 3) (81, 115). Insects are not known to have homologs of the mammalian STEAP proteins; however, candidate ferric reductases include CG1275 and Nemy proteins (described in Section 3) (81, 115). Both enzymes are thought to be present in intracellular membranes (57, 73). The physiological functions of CG1275 have not been studied; Nemy is known to participate in peptide amidation in neuroendocrine neurons and influence olfactory memory, but a possible role in iron homeostasis has not been investigated (57, 59).

Serum transferrin can also deliver iron to cells through a nonendocytic mechanism that involves binding to transferrin receptor 1, ferric reduction by a ferric reductase such as STEAP2, and transport through a ferrous transporter such as Zip8 or Zip14 (65) (**Figure 1**). As described above, insects lack homologs of transferrin receptor 1 and STEAP proteins and do not have orthologs of Zip8 or Zip14; however, the insect ortholog of mammalian ferric chelate reductase 1 (CG8399 in *D. melanogaster*) may function as a plasma membrane ferric reductase (101, 103, 121).

Possible conserved heme uptake mechanisms are described in Section 3. A novel type of heme transport occurs in the hematophagous insect *R. prolixus*. *Rhodnius* heme-binding protein binds heme that is exported from midgut cells and delivers it to developing oocytes via receptor-mediated endocytosis (10, 95, 124).

7. REGULATION OF IRON HOMEOSTASIS IN INSECTS

Many pathways that regulate systemic and cellular iron homeostasis in mammals are known, but regulation of iron homeostasis in insects is still poorly understood. In mammals, systemic iron homeostasis is primarily controlled through the action of the hormone hepcidin through its effect on the iron efflux transporter ferroportin (18, 30). Insects do not synthesize hepcidin and do not have a ferroportin homolog; therefore, insects and mammals must regulate systemic iron homeostasis differently (18, 81, 115). At least one aspect of cellular iron regulation is similar: a mechanism that involves the binding of an RNA-binding iron regulatory protein to mRNA iron-response elements when cellular iron levels are low (18, 30). Some of the earliest research on iron homeostasis in insects was centered on this subject, and this work has been reviewed previously (81, 92, 115). At least in some types of insects, excess iron that has been taken up by midgut cells is loaded onto ferritin, which is then secreted into the gut lumen and excreted, thus avoiding iron overload (81, 99, 115). Some blood feeding insects have evolved mechanisms to handle the high concentration of heme in blood, including excretion of heme aggregates and synthesis of a heme-binding protein in hemolymph that protects against heme toxicity (39, 113, 134). An interesting hypothesis about the possible role of manganese superoxide dismutase 2 as a mitochondrial iron sensor has been proposed (82). Control of iron homeostasis in the context of immunity has been recently reviewed (51).

8. CONCLUSIONS AND REMAINING QUESTIONS

Although there are obvious similarities between mammals and insects in the way in which iron is transported, stored, and regulated, there are also important differences. For example, mitochondrial iron homeostasis appears to be similar in mammals and insects, but many of the key players in mammalian iron homeostasis are lacking in insects, including hepcidin, ferroportin, conserved ferroxidases, and homologous receptors for transferrin and ferritin. In addition, among various types of insects, significant differences in iron homeostasis have been identified; for example, culicine mosquitoes lack Mvl, and lice, aphids, and thrips lack Tsf1. Future studies of iron

homeostasis in more diverse insect groups will provide a more complete picture of similarities and differences among types of insects, and findings that are currently restricted to dipterans may not be applicable to all insect taxa. Future progress in understanding iron homeostasis in insects will require the identification of ferrous and heme transporters, putative Tsf1 and ferritin receptors, iron chaperones, and ferric reductases. Important remaining questions include how heme is imported, exported, and transported; how ferritin and Tsf1 deliver iron to cells; and how systemic iron homeostasis is regulated. In addition, not covered in this review are interesting studies on the relationship between iron homeostasis and insect–microbe interactions. Related remaining questions include how mechanisms of iron sequestration inhibit growth of pathogenic but not commensal or symbiotic microbes and how bacterial symbionts influence insect iron homeostasis.

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