*Annu. Rev. Nutr. 1997. 17:501–26*


# COMPARATIVE NUTRITION OF IRON AND COPPER

*Joy J. Winzerling and John H. Law*
Departments of Biochemistry, Entomology, and Nutritional Sciences and the Center for Insect Science, University of Arizona, Tucson, Arizona 85721

KEY WORDS: yeast, invertebrate

ABSTRACT

The suggestion from nutritional studies with mammals of a link between iron and copper metabolism has been reinforced by recent investigations with yeast cells. Iron must be in the reduced ferrous (FeII) state for uptake by yeast cells, and reoxidation to ferric (FeIII) by a copper oxidase is part of the transport process. Thus, yeast cells deficient in copper are unable to absorb iron. In an analogous way, animals deficient in copper appear to be unable to move FeII out of cells, probably because it cannot be oxidized to FeIII. Invertebrate animals use copper and iron in ways very similar to vertebrates, with some notable exceptions. In the cases where vertebrates and invertebrates are similar, the latter may be useful models for vertebrate metabolism. In cases where they differ (e.g. predominance of serum ferritin in insects, oxygen transport by a copper protein in many arthropods, central importance of phenoloxidase, a copper enzyme in arthropods), the differences may represent processes that are exaggerated in invertebrates and thus more amenable to study in these organisms. On the other hand, they may represent processes unique to invertebrates, thus providing novel information on species diversity.

CONTENTS





0199-9885/97/0715-0501$08.00

## INTRODUCTION

In a recent, lively review, Kaplan & O'Halloran (80) remind us that ancient people associated iron with Mars and copper with Venus. The intricate and often intimate relationship of these two deities has its parallel in the biochemistry of the ionic forms of these elemental metals. The authors also note that copper and iron, selected for their abilities to bind oxygen reversibly and to transfer electrons in biological systems, have as dark sides their capacities to promote destructive free-radical reactions. It is thus important that these metal ions be confined within relatively innocuous complexes during their transport and incorporation into cellular components.

The past few years have seen an explosion in our understanding of how ionic iron and copper function in biological systems and of their complex interrelationships. Information has come not only from studies with vertebrate cells and organisms, but from studies on a variety of other eukaryotic cells, most notably yeast. This review, therefore, not only is timely, it also offers an opportunity to illustrate the value of a comparative approach to human nutrition.

We intend (*a*) to describe classical nutritional data that suggested an intimate relationship between iron and copper metabolism; (*b*) to offer an overview of recent exciting developments in our understanding of iron metabolism; (*c*) to show how a combination of biochemistry and genetics has illuminated the iron-copper connection in yeast cells; (*d*) to discuss how findings in yeasts may apply to vertebrates; and (*e*) to review recent investigations that suggest invertebrates could be useful models for understanding vertebrate micronutrition. We end with an assessment of the prospects for future use of a comparative approach to iron and copper metabolism.

## IRON AND COPPER—AN OLD STORY

In 1927, the Wisconsin nutrition group showed that iron salts failed to cure anemia in rats, while ashed foodstuffs, or acid extracts of the ashes, restored hemoglobin levels (154). The active constituent of the ashes was shown to be copper ion (67). Four decades later, Osaki et al (115) demonstrated that the

conversion of ferrous (FeII) to ferric (FeIII) in human serum could be correlated with the oxidation of *p*-phenylenediamine catalyzed by the blue copper protein, ceruloplasmin. These authors proposed that ceruloplasmin was responsible for the ferroxidase activity of serum that was necessary for oxidation of FeII to FeIII for transport by transferrin. At this same time, experiments by Lee et al (89) showed that copper-deficient pigs absorbed iron from the intestinal lumen into the mucosa at normal rates but failed to pass it on into the blood. When an iron supplement was injected intramuscularly into these pigs, increased amounts of iron were found in the reticuloendothelial system, hepatic parenchymal cells, and sideroblasts. Their conclusion was that copper deficiency impeded the release of iron from tissues. This brief historical review shows the close metabolic relationship between iron and copper (63, 80, 92).



## VERTEBRATE IRON AND COPPER METABOLISM

### *Overview of Vertebrate Iron Metabolism*

To understand the role of iron in other organisms, it is useful to summarize what is known about iron metabolism in vertebrate cells. Figure 1 depicts much of this information. The individual components and their roles are listed below. The reader is referred to several recent reviews (41, 71, 80, 84, 88), including two from this series (91, 100), for a more complete understanding.

IRON In aqueous solutions, both FeII and FeIII exist as ionic hexahydrates. Protons can be lost from these hexahydrates at characteristic pH values (hydrolysis) to yield insoluble hydrated oxides and oxide polymers. An important difference between the two ionic forms is that the $pK_a$ for loss of protons is much lower for FeIII hexahydrate (2–3) than for FeII hexahydrate ($\sim$7). This means that FeII ions are much more soluble at physiological pH than are FeIII ions (32). Both ionic forms occur in cells. However, since free ionic iron in combination with molecular oxygen has the capacity for wreaking havoc in cells through the generation of radicals, ionic iron in biological systems is complexed with a variety of small molecules and proteins. While FeIII coordinates with stronger affinity to "hard" bases containing oxygen, FeII has a greater affinity for "soft" ligands, such as those containing sulfur and nitrogen (32). Thus, reduction of a complexed form of FeIII to FeII can result in a less stable complex and a change of partner ligands. In the course of its journey through living organisms, iron undergoes many oxidation and reduction reactions.

HEME Heme, the FeII derivative of protoporphyrin IX, is the prothestic group of a large number of oxidative enzymes and oxygen carriers. In the presence of

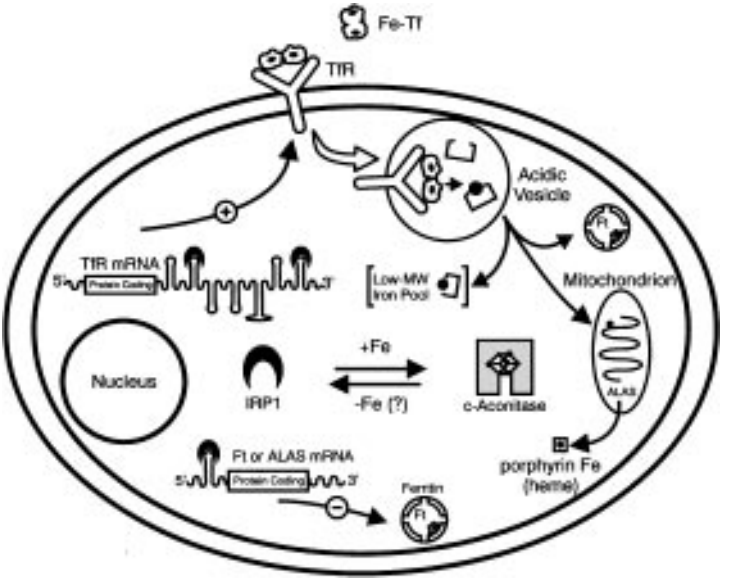


*Figure 1* Intracellular regulatory pathways of iron metabolism in vertebrates. Adapted from O'Halloran (113). Tf: Transferrin; TfR: transferrin receptor; ALAS: erythroid amino levulinate synthase; IRP1: iron regulatory protein 1; Ft: ferritin.

oxygen, heme also can generate destructive free radicals. This is prevented in biological systems by complexing heme to other molecules, or by destroying it through the action of the enzyme heme oxygenase (97). Heme can be transported safely in vertebrate blood on a carrier protein, hemopexin (105). The first enzyme in the biosynthetic pathway of heme, $\delta$-aminolevulinic acid synthase, is abundant in erythroid cells, where expression of the gene is influenced by iron availability (36).

TRANSFERRIN Transferrin (Tf) is an 80-kDa glycoprotein that circulates in vertebrate blood. Proteins of the same family are also found in milk (lactotransferrin) and avian eggs (ovotransferrin), where they are thought to play a bacteriostatic role by sequestering iron from bacterial invaders (32). Tfs are composed of two lobes—possibly the result of an early gene duplication event, since the lobes share similarity in amino acid sequence—each containing an iron-binding site. FeIII is tightly bound to Tf by six ligands, a histidine nitrogen, an aspartic acid carboxyl group, two tyrosine hydroxyl groups, and two oxygens of a carbonate or bicarbonate anion that is ion-paired to an arginine guanidinium group (1, 62).

TRANSFERRIN RECEPTOR Transferrin receptor (TfR) is a membrane glycoprotein composed of two identical 95-kDa subunits linked by disulfide bridges. TfRs have two extracellular binding sites with high affinity ($K_d \sim 10^{-9}$ M) for diferric Tf at physiological pH. The receptors are constantly taken up by endocytosis into clathrin-coated vesicles. After uncoating and fusion with endosomes, the receptors are sorted and returned to the cell surface (39). If diferric Tf is bound to TfR, FeIII is released within the acidified endosome. At the low pH of the endosome, apoTf remains bound to the TfR. Only when the apoTf-TfR complex returns to the more alkaline environment of the blood is the apoTf released to search for more FeIII. The process by which FeIII in an endosome is transferred to the cytoplasm and various cellular organelles is largely unknown.

FERRITIN Ferritins (Fts) appear to be distributed in virtually all living organisms. Vertebrate cytoplasmic Fts generally have variable proportions of two related subunits [heavy (H) and light (L), each about 20 kDa], the products of distinct genes. H subunits from different animals are more similar in amino acid sequence than are H and L subunits from the same animal. Of these highly helical Ft subunits, 24 combine to form a huge (10-nm outside diameter) hollow sphere, in which FeIII is stored as a mixed oxide/phosphate insoluble mineral (66, 146, 146). The interior of the sphere of some Fts can store as many as 4500 FeIII atoms. Iron enters the ferritin core through pores resulting from the symmetry of the molecule. Cellular FeII is oxidized by ferritin ferroxidase sites constituted from amino acid side chains, and the resulting FeIII is deposited on a growing mineral inside the sphere. Release of FeIII from Ft into the cell cytoplasm could involve the action of chelating agents or reduction to FeII, or both. Ft is also found in the serum of vertebrates and is thought to enter the blood from damaged cells. However, the facts that some subunits of serum Ft are glycosylated, while cytoplasmic Ft subunits are not, and that serum Ft has larger subunits than cytoplasmic Fts suggest that serum Ft is a distinct protein secreted from cells by exocytosis (94). Indeed, rat hepatoma cells in culture have been shown to secrete a serum-type Ft (130). Linder et al (94) also reported that serum Ft is a smaller molecule than is cytoplasmic Ft, and that it consists of hexameric, iron-poor molecules. However, the conditions used to isolate this preparation (70°C at pH 4.8), while they do not degrade cytoplasmic Ft, might be detrimental to this novel serum form. Of particular relevance to human nutrition is the report by Beard et al (15a) that ferritin is an effective dietary source of iron. These authors suggest that increasing plant ferritin content might enhance nutritional quality.

IRON RESPONSIVE ELEMENT The iron responsive element (IRE) is a stem loop structure formed from a stretch of ribonucleotide sequence (26a, 84a, 146a).

Untranslated regions (UTRs) of the mRNAs of several proteins involved in iron metabolism, as well as some other messages (71), have IREs. Binding of the iron regulatory protein 1 (IRP1) to these IREs can influence the translation of the message or it can protect the message against degradation.

IRON REGULATORY PROTEIN There are two related IRPs in mammalian cells, IRP1 and IRP2 (71, 91). IRP1, the best studied of the two, is a cytoplasmic protein that shows about 30% amino acid sequence identity to pig heart mitochondrial aconitase, an enzyme of the tricarboxylic acid cycle (16). IRP1 has two distinct functions. As a holoprotein, IRP1 contains a 4Fe4S cluster and is identical with cytoplasmic aconitase (83). When iron levels in the cell are low, the iron-sulfur cluster is destroyed; the protein loses aconitase activity and gains a high affinity for IREs (16, 59). Ft mRNA contains an IRE in the 5′ UTR, and binding of IRP1 or IRP2 prevents translation. TfR mRNA has several IREs in the 3′ UTR, and binding of IRP1 to these IREs stabilizes the message and prolongs its half-life. Thus, IRP1 could serve as a cellular iron sensor that exerts reciprocal control on the synthesis of Ft and TfR. IRP2 shows similar binding to IREs but lacks aconitase activity and is degraded rapidly in the presence of iron (58, 10, 117, 128). IRP1 may also be activated by phosphorylation by protein kinase C (131), by nitric oxide, and by hydrogen peroxide (71). Regulation of ferritin synthesis in plants is quite different (83a) and is not discussed further.

## *The Iron Pathway*

Based on current knowledge, the following general pathway can be postulated for iron metabolism in vertebrates. Iron is absorbed by vertebrates more readily in the heme form than in the non-heme form (133). In the blood, FeIII circulates in blood tightly complexed with Tf. Diferric Tf binds to TfRs found on cell surfaces and enters the cells by receptor-mediated endocytosis (Figure 1). FeIII is subsequently released from the Tf-TfR complex in the acidified endosome, probably reduced to FeII, and transferred to the cell cytoplasm. Intracellular iron is stored in the Ft sphere as FeIII; mobilization of FeIII from Ft stores likely involves reduction or chelation of ionic iron to small molecules that can diffuse through the Ft pores. Although much is known about iron entry into cells via the TfR, neither the mechanisms of intracellular transport nor the reduction and oxidation steps required for iron movement inside the cell and incorporation into proteins are well defined. In addition to cellular iron uptake via TfRs, mammalian cells have less well-understood mechanisms for the entry of non-Tf bound iron (see below).

## *Overview of Vertebrate Copper Metabolism*

Mechanisms of vertebrate copper metabolism are less well understood (and more controversial) than those of vertebrate iron metabolism. However, some

of the key components have been defined. A reasonable picture of the current state of this field is found in recent reviews (65, 92a, 93, 153).

CUII TRANSPORTERS IN BLOOD CuII is transported in blood by reversible carrier proteins that can deliver this ion to tissues. Transcuprein, a large (270-kDa) serum protein with a high affinity for CuII ($K_d \sim 10^{-17}$ M), appears to be the first acceptor of CuII to enter the blood (93). Little is known of the molecular nature of this protein (92). Serum albumin, an abundant 67-kDa protein, also has a high affinity for CuII and can exchange CuII with transcuprein (93). Some blood CuII also appears to be associated with histidine (153). How copper bound to these carriers is taken up by cells is not clear.



CERULOPLASMIN The main copper-containing protein of human blood is the multi-copper (blue) ferroxidase, ceruloplasmin (Cp). Cp is synthesized primarily by the liver, and the six copper ions incorporated during synthesis (64, 129) are mandatory for ferroxidase activity of the holoprotein (64). The primary structure of Cp has been known for several years (142); more recently, the cDNA sequence was completed (46, 86, 161). Yet, the function of Cp as a vehicle for copper transport from liver to peripheral tissues remains a subject of debate (for contrary views, see 63, 153). The resolution to this debate could be that Cp has at least two essential functions (ferroxidase activity and copper transport) and possibly others as well.

Several studies have documented the uptake of copper from Cp by tissues and cells (43a, 62a, 118a), and the presence of putative Cp receptors on cell surfaces (37a) suggests that Cp transports copper. On the other hand, early work in mice (53) showed that only a small amount of copper from the Cp pool was exchanged per day. Later work established that copper does not affect the rate of synthesis or secretion of Cp, only the oxidase activity (54, 64). The recently completed X-ray crystal structure of Cp (165) accords well with function as an oxidase, rather than as a transport protein. (Structurally, Cp belongs to a family of copper oxidases that includes ascorbate oxidase and nitrite reductase.) Finally, patients with aceruloplasminemia, a genetic disorder resulting in absent Cp (64, 104), have relatively normal copper tissue levels (102), which suggests that Cp is not mandatory for copper distribution.

Cp also plays an important role in iron mobilization. The current hypothesis is that FeII is transported to the cell surface, oxidized to FeIII in a reaction catalyzed by Cp, and immediately bound to plasma Tf (115). Although this view has been challenged in the past (see, for example, 155), and the form of iron presented at the cell surface is virtually unknown, evidence strongly favors this hypothesis. Biochemical and physiological studies have shown that (*a*) copper deficiency in animals impedes iron release from tissues (89), (*b*) FeII

is oxidized to FeIII in the presence of molecular oxygen in plasma (125), and (*c*) conversion of FeII to FeIII in human serum correlates with the oxidation of *p*-phenylenediamine catalyzed by Cp (115). In addition, recent clinical findings in patients with aceruloplasminemia indicate that Cp is involved in iron transport. Symptoms of aceruloplasminemia include low serum iron, elevated serum Ft, and iron deposition in parenchymal and reticuloendothelial tissues (104, 163). Cp administered to a patient with aceruloplasminemia resulted in enhanced iron mobilization with increased serum iron (96). Taken together, these findings suggest that Cp has an important role in the movement of iron out of tissues. However, Tf iron transport may not be entirely dependent upon Cp.

CELLULAR COPPER METABOLISM Within cells, CuII may be carried by small chelator molecules or by metallothioneins, small metal ion-binding proteins, although the role of the latter is uncertain (153).



## THE IRON-COPPER CONNECTION

### *Iron and Copper Transport in Yeast Cells*

The transport of copper and iron in bacterial organisms has been reviewed (153) and is not discussed further here. The many excellent recent reviews of copper and iron metabolism in yeasts attest to the rapid expansion of knowledge in this area (5, 8, 41, 80, 113, 153). *Saccharomyces cerevisiae* has provided a model for copper and iron transport into eukaryotic cells (Figure 2). These studies show that high-affinity iron uptake in this organism is dependent upon reduction of extracellular FeIII to FeII by two membrane reductases, Fre1p (34) and Fre2p (52). Reduced iron is then bound to Ftrp, a membrane transport protein (137), reoxidized by a second membrane-anchored, multi-copper oxidase (Fet3p), and transported into the cell (9, 40). Fet3p is necessary for placement of Ftrp in the plasma membrane (137), and Ftrp is required for Fet3p maturation and copper acquisition. The copper for Fet3p is provided by two copper transport proteins, Ctr1p (33, 35) and Ccc2p (not shown in Figure 2; 164). Ctr1p transports CuI across the plasma membrane, while Ccc2p is involved in intracellular membrane copper transport (164).

Neither the iron-binding sites nor the oxidation state of the iron transported by Ftrp have been clearly defined. Nevertheless, Ftrp, Fet3p, and Fre1p are all independently required for high-affinity iron uptake by these organisms (40, 137). Why reoxidation of iron is necessary for high-affinity iron uptake is not immediately apparent. Stearman et al (137) suggested it could be necessary for transport through the membrane by Ftrp (40, 80, 137).

*S. cerevisiae* also has a mechanism for low-affinity iron transport; Fet4p is involved in this process and uses FeII as a substrate (43). When overexpressed,

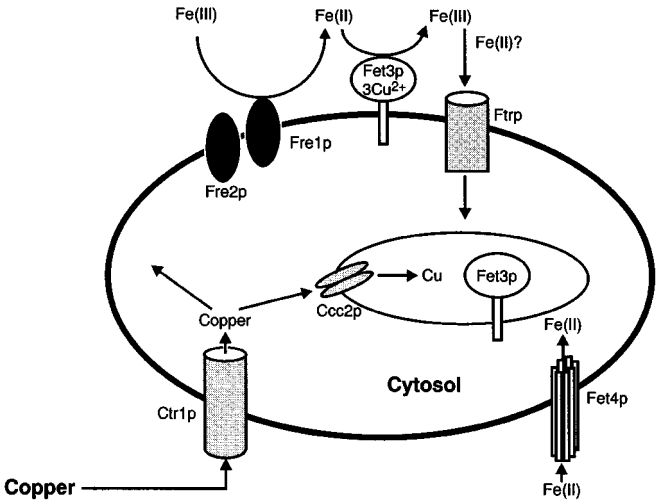


*Figure 2* A model linking copper and iron uptake in *Saccharomyces cerevisiae*. Adapted from De Silva et al (41). Fre1p and Fre2p are ferrireductases, Ctr1p is a copper transporter, Fet4p is a low-affinity iron transporter, Ccc2p is a copper transporter P-type ATPase, Fet3p is an extracellular multicopper oxidase a, and Ftrp is an iron transporter membrane protein.

the gene product of FET4 compliments that of FET3. This system also will transport other metal ions.

The pathways in yeast reveal many interrelationships between iron and copper metabolism. First, the copper transport proteins are required to provide CuII for Fet3p. Second, although Fre1p is required for high-affinity iron uptake, its reductive activity is not limited to iron. Fre1p also reduces CuII to CuI (68) and thereby likely contributes CuI for transport by Ctr1p (35). Third, the presence of either copper or iron will repress transcription of FRE1 (78, 160). Metal-dependent regulation of gene expression of FRE1 is mediated by at least two separate gene products. Iron-dependent gene expression of FRE1, FRE2, and FET3 is mediated by the AFT1 gene product (160). Both basal (78) transcription and copper-dependent (55, 68) control of expression of FRE1 appear mediated by the MAC1 gene product. Thus, these two transcriptional regulators seem to allow metal-dependent control of gene expression of separate, but overlapping, subsets of genes involved in both copper and iron metabolism (160).

## *Comparative Mechanisms in Yeast and Vertebrates*

Mechanisms of mammalian mineral metabolism with similarities to those of yeast copper and iron transport occur in at least three areas: (*a*) intracellular

transport and efflux of copper; (*b*) role of copper oxidases in iron metabolism; and (*c*) cellular uptake of non-Tf bound iron.

INTRACELLULAR COPPER TRANSPORT AND EFFLUX A remarkable similarity between the yeast pathway and the pathway of copper metabolism in humans was found when Yuan et al (164) reported that the gene encoding Ccc2p is a homologue of the Menkes and Wilson disease genes. Both of these inherited disorders are characterized by defective copper efflux from cells caused by mutations of two separate genes with expression in different tissues (24, 25, 153). Like yeast CCC2, the genes for Menkes (MNK) (152) and Wilson (WND) (25, 143, 159) disease encode copper-transporting P-type ATPases. Recent work indicates that similar to the yeast protein, the human copper-transporting P-type ATPases are intracellular copper transport proteins (65). The predicted amino acid sequences of the proteins encoded by MNK, WND, and CCC2 also have several conserved regions characteristic of P-type ATPases. Two of these regions are of particular interest: N-terminal CXXC motifs (X denoting any other aa) that are predicted heavy metal association sites; and a 30–amino acid predicted transmembrane region that contains a CXC motif thought responsible for the movement of metal ions through the membrane (24, 49, 65). These regions are of interest not only for their predicted functions, but also because they are highly conserved among yeast, human, and bacterial proteins (for review see 24) that participate in known pathways of mineral metabolism in these various organisms.

ROLE OF COPPER OXIDASES IN IRON METABOLISM As noted in the discussion of Cp, this blue multi-copper oxidase is thought responsible for the conversion of FeII to FeIII in plasma. Multi-copper oxidases couple oxidation of a substrate to reduction of molecular oxygen (80). The copper binding domains of yeast Fet3p show a high degree of similarity with those of Cp (9). Yeast Fet3p is involved in the oxidation of iron for entry into cells, while Cp appears involved in the oxidation of iron for efflux from cells. As noted above, all cells also must have mechanisms for intracellular iron oxidation, reduction, and transport in order to maintain iron solubility, to prevent oxidative damage, and to provide iron to the appropriate intracellular organelles. It seems probable that other copper oxidases similar to Fet3p, as well as reductases similar to Fre1p/Fre2p, would be involved in these vertebrate intracellular pathways.

UPTAKE OF NON-TRANSFERRIN BOUND IRON IN VERTEBRATES The intestinal endothelial cell, which is responsible for absorption of dietary non-heme iron, is the most obvious tissue where mechanisms similar to those found in yeast iron transport could apply. Two components of iron absorption could have similarity to the iron uptake mechanisms of yeasts: surface reductases, which

reduce FeIII to the more soluble FeII form; and apical membrane proteins, which transport iron into the cell. Studies of the FeIII reductase activity of mucosal tissues are far from complete. Several reports have documented binding of both FeII and FeIII to intestinal brush border membrane vesicles (BBMV) from different mammals (99, 134, 135). Raja et al (121) demonstrated reduction of FeIII by mouse duodenal tissues, as well as transport of FeII into both mucosal tissues and BBMV. Nichols et al (109) have shown that the IEC-6 rat intestinal cells have two different binding sites for FeII and at least four iron-binding surface proteins. Adenocarcinoma colonic cells (Caco-2) transport radiolabeled iron across the cell membrane as low-molecular-weight complexes, but results vary regarding the presence and requirement for apical surface ferrireductase(s) (3, 4, 61, 110). Whether or not oxidation or reduction are required for iron uptake by the gut remains unknown.

Several proteins that could serve as transporters during iron absorption have been isolated, but their roles in vivo remain to be defined. Teichmann & Stremmel (144) purified a glycoprotein from human brush border membranes that bound both FeII and FeIII. Conrad & Umbreit (29) isolated two proteins from rat duodenal mucosal homogenates, a higher molecular mass protein (ca 230 kDa) that appeared to be a surface integrin, and mobilferrin, a cytosolic protein of 56 kDa (30). Finally, O'Donnell & Cox (112) obtained an FeII binding glycoprotein of 100 kDa from BBMV from rabbit duodenum. These studies confirm that intestinal tissues have surface iron-binding proteins that could function as reductases or iron transporters. (For more details on intestinal iron absorption, see 18, 41, 45).

Certain clinical disorders, as well as studies in animal models of these disorders, substantiate that mechanisms for the uptake of non-transferrin bound iron (NTBI) exist in vivo for tissues other than the intestine. In both hypotransferrinemia (69) and genetic hemachromatosis (11, 108, 151), complicating liver disease occurs as a result of secondary iron overload due to increased intestinal absorption. In hypotransferrinemia, absorbed iron is transported in portal blood in the relative absence of Tf. In genetic hemachromatosis, blood Tf is saturated, and excess circulating iron, probably a citrate complex (57), deposits in the liver. Studies of animals (31) indicate that NTBI has a half-life of $<0.5$ min in plasma, and that iron from low-molecular-weight complexes is deposited in hepatic tissues (22, 31). From these observations, one can conclude that there is transport of NTBI from the intestine to the liver, that this iron is cleared rapidly from the circulation, and that uptake of NTBI will allow iron deposition in hepatic tissues in the presence of high hepatic intracellular iron (79, 138).

A candidate for a membrane surface protein that could serve as a transporter of NTBI is melanotransferrin (MTf), the human melanoma tumor-associated antigen, p97. MTf, a sialoglycoprotein that belongs to the Tf family (12, 126),

has one binding site for iron (12). MTf is attached to cell membranes by a glycosyl-phosphatidylinositol anchor (2, 47) and is present on numerous cell types (23, 123), including the apical surface of both fetal porcine intestinal endothelial cells (37) and human epithelial cells derived from colorectal cancer cells (2). Recent work by Kennard et al (82) demonstrated that mutant Chinese hamster ovary (CHO) cells transfected with MTf had increased uptake of NTBI.

Several other mammalian cultured cells and tissues show NTBI uptake. These cell types and tissues include: HeLa cells (79, 138), rat hepatocytes (13, 132, 148), rat liver (22, 158), HepG2 cells (118, 122), melanoma cells (124), CHO cells (28), K562 cells (75, 76), cultured human fibroblasts (116), leukemic cells (L1210) (15), and reticulocytes (120). The characteristics of NTBI uptake vary greatly with the type of cells and tissues studied and are dependent upon how the studies are conducted. For further details of these studies, the reader is referred to De Silva et al (41). In summary, many cells are capable of NTBI uptake, and these mechanisms are operative in certain clinical conditions. NTBI uptake is currently considered a cryptic transport mechanism that is induced in vertebrate cells upon exposure to NTBI (41). However, such mechanisms in other organisms probably represent key survival pathways.

In addition to NTBI uptake, several studies have indicated that certain types of cells have mechanisms for the removal and uptake of iron from Tf at the surface membrane apart from the well-described TfR pathway (21, 111, 141, 147, 148). Such mechanisms appear to have similarity to the yeast pathway in that release of iron from Tf involves surface reductases.

## IRON AND COPPER IN INVERTEBRATE ANIMALS

### *Why Invertebrates?*

Invertebrate animals encompass the greatest diversity in the animal kingdom. Adaptation to a wide variety of life styles has necessitated resolution of a number of problems not encountered by vertebrates. Thus, the metabolism of invertebrate animals sometimes emphasizes processes that are not well represented in vertebrates. Examples are the abundance of serum Ft in insects, as opposed to its scarcity in vertebrates, and the predominance of copper proteins in some invertebrate sera, as opposed to a minor presence in vertebrates. These examples illustrate that one can find materials and processes in invertebrates that magnify and help to elucidate analogous, but uncommon, materials and processes in vertebrates. Furthermore, invertebrate animals are more economical to maintain, are easier to manipulate, and have faster growth and developmental rates than do vertebrates. Some invertebrates, e.g. *Drosophila melanogaster*, are amenable to genetic studies; indeed, study of this organism has often led to new understanding of vertebrate nutrition, biochemistry, and molecular biology.

The differences in vertebrate and invertebrate animals goes beyond the presence or absence of a calcified central nerve cord. Invertebrate circulatory systems can vary from fully closed (annelid worms), as in vertebrates, to the open system found in arthropods, in which the blood, usually called hemolymph, is pumped from posterior to anterior by a tubular heart but then diffuses through the body cavity (hemocoel) to the tissues. Hemolymph of invertebrates usually lacks specialized oxygen-carrying cells, and hemoglobin is a rarity in invertebrates, restricted to annelid worms and other animals that live in an oxygen-poor environment. Thus, invertebrates have oxygenation strategies that differ sharply from those of vertebrates. The largest group of invertebrates, the insects, do not carry oxygen in the hemolymph; instead, they use a system of tracheal tubes that conduct oxygen down to the tissue level, where it reaches the cells by passive diffusion. Another group of invertebrates, including sipunculid worms and brachiopods, employ a nonheme binuclear iron oxygen-transporting protein, hemerythrin, which is carried in erythrocytes in the hemolymph. Most other invertebrates transport oxygen by means of copper proteins, the hemocyanins (Hc). Thus, because of the difference in oxygenation mechanisms, the iron requirement of some invertebrates (insects) is much less than that of vertebrates and, in some cases, is supplanted by an increased demand for copper (most other arthropods and mollusks). Nonetheless, all invertebrate animals have the universal iron and copper cellular proteins: cytochromes, iron-sulfur proteins, superoxide dismutase, cytochrome oxidase, etc. Finally, in many invertebrates another copper enzyme, phenoloxidase, assumes important roles in the immune response, in pigment formation, and in sclerotization, a process for cross-linking proteins to form a hard exoskeleton.

## *Invertebrate Transferrins*

At the time the sequences of vertebrate Tfs became known, it was postulated that the two lobes represent a duplication of a gene encoding a precursor iron-binding protein of approximately half the size of Tf. It has been suggested that the gene duplication was a fairly recent event, paralleling the development of the vertebrate kidney. This suggestion was based on two lines of evidence. First, proteolytic fragments of vertebrate Tf—approximately representing the two lobes of the intact molecule—injected into the bloodstream of rats were readily cleared through the kidney, whereas intact Tf was not (156). Furthermore, a 41-kDa iron-binding protein was identified in the hemolymph of a tunicate, *Pyura* (98), the DNA of which hybridized with human Tf cDNA on Southern blots (20). These results suggested that this protochordate, which lacks a kidney, contained putative progenitor of vertebrate Tf. However, no sequence data are available to show that the isolated *Pyura* protein is related to Tf, or that the *Pyura* DNA detected on Southern blots encodes a member of the Tf family.

On the other hand, a Tf with a molecular weight of 78 kDa was isolated from an insect, *Manduca sexta* (14, 74). The amino acid sequence of this protein showed sufficient similarity to that of vertebrate Tfs to suggest a common origin. However, the *M. sexta* Tf bound a single FeIII, and examination of the deduced sequence showed that amino acid side chain ligands known to be responsible for iron binding in vertebrate Tfs were present only in the N-terminal lobe of this insect protein.

Later, a Tf was isolated from a cockroach (77) that bound two FeIII and had appropriate ligands for FeIII binding in both lobes. It was subsequently demonstrated that the cockroach Tf closely resembled human serum Tf in its requirement for bicarbonate anion, in its pH dependence for iron binding, and in the circular dichroism and FeIII and CuII protein electron paramagnetic resonance spectra (51). These studies demonstrated that an insect Tf is an authentic member of the Tf superfamily, and that the gene duplication event had to predate the separation of vertebrate and invertebrate lines. Two other insect Tfs, from a fly (87) and a mosquito (*Aedes aegypti*, T Yoshiga, JH Law, unpublished data), also contain functional N-terminal iron-binding lobes. However, the C-terminal lobes of these Tfs not only lack appropriate ligands for iron binding, they also have large deletions in the polypeptide chains. Whether the C-terminal lobe has taken on some other function cannot be answered at present. In this regard, it is interesting to note that the bullfrog, *Rana catesbeiana*, produces a member of the Tf family that lacks iron-binding ligands but binds a neurotoxin, saxitoxin (103).

Tf-like proteins have been isolated from other invertebrate organisms (73, 90), but they are less well characterized than those of insects. It is assumed that insect Tfs transport iron in insect hemolymph, a role analogous to Tfs in vertebrates, but this remains to be proven. Injection of $^{59}$FeIII-labeled *M. sexta* Tf into hemolymph of the same animal showed that the label was quickly cleared and transferred to tissues (74). It remains to be seen if insect and other invertebrate tissues have TfR. It is also possible that serum Tf in invertebrates could serve a protective, antibiotic role by sequestering iron and making it unavailable to invading organisms. The fly Tf appears to be taken up by the developing oocyte, where it presumably functions to store iron for the developing embryo (87).

## *Invertebrate Ferritins*

Fts have been isolated and characterized from insects, crustacea, mollusks, and trematode worms, and Ft-like particles have been observed microscopically in other invertebrate animals. Locke & Nichol (95, 107) reviewed the distribution of Fts in insects and noted that in most insects, Fts, though present in the cytoplasm, are predominantly found in the lumen of the endoplasmic reticulum and

in the hemolymph. Insect Fts and their subunits also have larger apparent sizes than those of mammals (26, 44, 74, 106, 157). Some Ft subunits are glycosylated, and it seems likely that insect Fts are heteropolymers composed of both glycosylated and nonglycosylated subunits (44, 106, 157). Two insect Ft subunits from two species (mosquito and moth) have been cloned and sequenced. Each deduced amino acid sequence contains a putative signal sequence, indicating that the mature protein will be exported from the cell (44, 119). It is clear that at least one additional subunit, the product of a separate gene, exists in both of these insects, but the genes for these subunits have not yet been isolated.

When *M. sexta* Ft labeled with $^{59}$FeIII was injected into larvae, 20% of the label was rapidly incorporated into tissues, while the rest remained in the hemolymph. When pupae were similarly injected, Ft iron was largely sequestered in the fat body within 24 h (74). In contrast, housefly Ft leaves the fat body just prior to the pupal stage (26). These results indicate that insect Fts can move in and out of insect cells. (The functions of the insect fat body tissues are comparable to the combined roles of the liver, adipose, and endocrine tissues of vertebrates. Fat body is the site of synthesis of most hemolymph proteins, and a site of storage of hemolymph proteins during the pupal stage.) In many of their properties—i.e. glycosylation, mass, and location—the insect Ft subunits more closely resemble those of mammalian serum Fts than those of mammalian cytoplasmic Fts. It would be of great interest to determine the sequence of vertebrate serum Ft and to see whether it resembles insect Ft more closely than it resembles vertebrate cytoplasmic Fts.

As we examine other invertebrates, the situation is very different. In the crayfish, Ft is strictly cytoplasmic—none was found in the hemolymph—and the subunits are of similar size to those of vertebrates. The deduced amino acid sequence of one subunit showed no signal sequence and no potential glycosylation sites. The sequence was much more similar to vertebrate H chains than to insect Ft chains (72). Another crustacean protein, artemin, has sequence similarity to Ft, but this protein is not involved in iron metabolism; it seems instead to be a storage protein produced in the cyst form of the brine shrimp (38). The blood fluke *Schistosoma mansoni* also has Ft subunits of similar size to those of vertebrate cytoplasmic Fts, and the deduced amino acid sequences have no signal peptides and are much more similar to vertebrate H chains than to insect Fts. While worms of each sex have both subunits, one is preferentially expressed in males and the other predominantly expressed in females (42).

The snail *Lymnaea stagnalis* produces two distinct Ft subunits. One subunit is cytoplasmic (soma Ft) and similar in sequence to vertebrate H chains, while the other (yolk Ft) is a vitellogenic protein, secreted into the hemolymph and taken up by developing oocytes. Like the insect Fts, yolk Ft has a larger subunit (25 kDa) and a signal sequence (150). Yet, the yolk Ft sequence has

less similarity to the mosquito Ft sequence than does the soma Ft sequence (44). For that matter, the yolk Ft sequence shows only modest similarity to either the soma or the vertebrate Fts (150).

## *Invertebrate Iron Responsive Elements and Iron Regulatory Proteins*

Rothenberger et al (127) presented evidence for IRPs in cell extracts from chicken, frog, fish, fly, and annelid worms by means of gel-shift assays performed with labeled RNA transcripts that contained a human IRE. Sequences encoding putative IREs have been reported in the Ft cDNAs from snails (in the soma Ft cDNA but not in the yolk Ft cDNA) (150), two insects (44, 119), and the crayfish (72). A functional IRE has been identified in *D. melanogaster* succinate dehydrogenase subunit b cDNA (56, 85, 101). Thus, it seems probable that the other putative IREs identified in invertebrate Ft cDNAs are functional.

We have recently begun to purify insect proteins that can bind IREs. Extracts of *M. sexta* fat body contain such a protein, which has now been purified to near homogeneity (D Zhang, JJ Winzerling, unpublished data). Furthermore, a polymerase chain reaction product representing about 30% of the coding sequence of a cDNA for an *M. sexta* IRP has been sequenced that has 69% identity and 79% similarity to vertebrate IRP1. These preliminary findings indicate that IRP1 has been a very well-conserved component of the iron regulatory system.

## *Invertebrate Heme Metabolism*

Since hemoglobin is uncommon in invertebrates, disposal of excess heme is rarely a problem, unless the animal chooses a diet rich in this substance. Animals that feed on vertebrate blood can encounter this difficulty. Ticks digest the blood proteins not in the gut lumen but in the cells lining the gut. The female tick incorporates heme from the hemoglobin into vitellogenin, the precursor protein of the major yolk protein, which is then incorporated into the developing oocyte (19). Another insect, *Rhodnius prolixus*, exploits heme released from the blood meal to aid in blood feeding. This hemophagous Hemipteran, a vector of trypanosome parasites (Chagas disease), absorbs heme from the midgut, transports it on a hemopexin-like protein, and deposits it in the salivary glands (114). There it is incorporated into heme proteins that reversibly bind nitric oxide (nitrophorins) and are secreted in the saliva. When the insect feeds on a vertebrate host, it injects the nitric oxide-loaded nitrophorins. Dissociation of the nitric oxide causes vasodilation, which facilitates feeding (27). Finally, there is the classical case of the malarial parasite, *Plasmodium falciparum*. In the vertebrate host, these animals live within erythrocytes and ingest hemoglobin. Digestion releases heme that could be harmful to cell membranes.

These organisms deal with the problem by converting heme molecules to an insoluble polymer, hemozoin (136).

### *Invertebrate Copper Metabolism*

Hc is a dominant form of copper in many invertebrates; it may constitute more than 80% of the hemolymph proteins (149). Two quite different forms of Hc exist. The arthropod form, found in the Crustacea and other arthropods, excluding Insecta, is based on a hexameric protein (a trigonal antiprism) consisting of one or more types of subunits of about 75 kDa. The hexamers may associate into higher order structures, including 12-mers, 24-mers, 36-mers, and 48-mers. Each subunit contains a binuclear copper site that can bind one oxygen molecule. CuI is oxidized to CuII when oxygen is bound reversibly (149). Molluscan Hcs are composed of much larger subunits, about 450 kDa, each containing a single binuclear copper oxygen-binding site. The subunits are organized into decamers or dodecamers that form immense cylinders, 30 nm in diameter and 15 nm high (149). The large size of these proteins has impeded their complete characterization.

Although insects generally do not utilize oxygen carriers in the hemolymph, and thus lack Hc, all insects produce a set of Hc-related proteins, hexamerins (17, 145). These hexamerins have a variety of binding sites for other ligands, as well as a variety of functions, not all well defined (145).

Both arthropod and molluscan Hcs are similar to two other groups of important proteins, the phenoloxidases (PO) of invertebrates and the tyrosinases of vertebrates (50, 81). The POs play extremely important roles in arthropods. They are produced as inactive proenzymes in hemocytes and secreted into the hemolymph, where the zymogen form may be activated by a proteolytic cascade, reminiscent of the blood coagulation cascade of vertebrates (7, 140). Arthropod POs are polypeptides of about 80 kDa with two copper-binding sites (162). Since they can catalyze oxidation of aromatic amino acids and compounds derived from aromatic amino acids, they participate both in the production of melanin, which gives the dark color to the exoskeleton of many arthropods, and in the protein cross-linking reactions that harden the exoskeleton (139). For some time, it has been believed that the hemolymph and cuticular POs were separate enzymes. However, it has been shown in the case of the silkworm that cuticular proPO is not synthesized in the epidermis but is transported from the hemolymph into the cuticle, where it can be activated by a cascade (6).

Five proPO cDNAs have been cloned and sequenced, one for a crustacean (10) and four for insects (50, 60, 81). All show similarity to each other and to the arthropod Hcs and hexamerins, but to plant or animal tyrosinases they show similarity only in the copper-binding sites. PO also plays an important role in the immune response of arthropods by catalyzing reactions that lead

to the encapsulation of invading organisms. Essentially, a cuticular capsule is constructed around the invader, cutting it off from the nutritive resources of the host. It may be of interest that some proPOs have a short stretch of sequence similar to regions in complement C3 and C4 of mammals responsible for the formation of a reactive thiol ester that can anchor those proteins to a substrate (60). If the insect proPO contains such a reactive thiol ester, it could allow the active PO molecule to bind covalently to the exterior of an invading organism, thus facilitating the encapsulation reactions.

## FUTURE PROSPECTS

Much remains to be done in order to determine if the copper-iron connection, established in yeast cells, has direct counterparts in animals. The uptake of iron in yeast cells seems most closely to resemble the efflux of iron from mammalian cells. If vertebrate proteins corresponding to the yeast proteins can be identified, then their functions can be defined in the context of vertebrate metabolism, and the power of the comparative approach will be amply verified.

Once a connection is established between the yeast processes and correlates in mammals, invertebrates could prove useful as models because of their specialized systems. For example, absorption of iron and copper in the invertebrate alimentary tract could be a useful model of the vertebrate system. Insects are very tolerant of an overload of these ions. Yet their responses are similar to those of vertebrates. Insect ferritin may prove a useful model for vertebrate serum ferritin. If, indeed, Cp plays a key role in oxidation of FeII, is there an invertebrate analogue? Could Hc or PO serve this role in invertebrates? Comparative nutrition using this varied group of organisms holds promise for unraveling these and other complex metabolic issues.

As we were putting the finishing touches on this review, an exciting group of papers appeared (reviewed in 48). It was shown that raising the concentration of iron to nanomolar ranges in the open tropical Pacific Ocean leads to phytoplankton proliferation to 20 times normal levels. Concomitantly, $CO_2$ levels dropped in the sea water, leading to absorption of more $CO_2$ from the atmosphere. The effect was short-lived, probably because the FeIII hydrolyzed to insoluble oxides in sea water. These findings open an exciting new frontier for comparative nutritional studies of phytoplankton, tracing iron through a food chain, and seeking ways to prolong the half-life of soluble and bioavailable iron in sea water.

### ACKNOWLEDGMENTS

We thank Ms. Susan Anderson for manuscript preparation and Mr. William Manlove for assistance in literature search. Work in the authors' laboratories was supported by grants from the US Public Health Service, Nos. GM 29238

and AI 32595, and by the John D. and Catherine T. MacArthur Foundation. We wish to thank Matthias W. Hentze, Maria C. Linder, and Elizabeth C. Theil for reading the manuscript and making valuable suggestions for improvements.





*Literature Cited*


1. Aisen P. 1994. Iron metabolism: an evolutionary perspective. In *Iron Metabolism in Health and Disease*, ed. JH Brock, JW Halliday, MJ Pippard, RJ Ward, pp. 1–30. London: Saunders
2. Alemany R, Vilá MR, Francí C, Egea G, Real FX, Thomson TM. 1993. Glycosyl phosphatidylinositol membrane anchoring of melanotransferrin (p97): apical compartmentalization in intestinal epithelial cells. *J. Cell Sci.* 104:1155–62
3. Alvarez-Hernandez X, Nichols GM, Glass J. 1991. Caco-2 cell line: a system for studying intestinal iron transport across epithelial cell monolayers. *Biochim. Biophys. Acta* 1070:205–8
4. Alvarez-Hernandez X, Smith M, Glass J. 1994. Regulation of iron uptake by transferrin in Caco-2 cells, and an intestinal cell line. *Biochim. Biophys. Acta* 1192:215–22
5. Anderson GJ, Dancis A, Roman DG, Klausner RD. 1994. Ferric iron reduction and iron uptake in eukaryotes: studies with the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe. Adv. Exp. Med. Biol.* 356:81–89
6. Ashida M, Brey PT. 1995. Role of the integument in insect defense: prophenoloxidase cascade in the cuticular matrix. *Proc. Natl. Acad. Sci. USA* 92:10698–702
7. Ashida M, Yamazaki HI. 1990. Biochemistry of the phenoloxidase system in insects with special reference to its activation. In *Molting and Metamorphosis*, ed. E Ohnishi, H Ishizaki, pp. 239–65. Berlin: Springer
8. Askwith CC, De Silva D, Kaplan J. 1996. Molecular biology of iron acquisition in *Saccharomyces cerevisiae. Mol. Microbiol.* 20:27–34
9. Askwith CC, Eide D, Van Ho A, Bernard PS, Li L, et al. 1994. The *FET3* gene of *S. cerevisiae* encodes a multicopper oxidase required for ferrous iron uptake. *Cell* 76:403–10
10. Aspán A, Huang T, Cerenius L, Söderhäll K. 1995. cDNA cloning of prophenoloxidase from the freshwater crayfish *Pacifastacus leniusculus* and its activation. *Proc. Natl. Acad. Sci. USA* 92:939–43
11. Bacon BR, Britton RS. 1990. The pathology of hepatic iron overload: a free radical-mediated process? *Hepatology* 11:127–37
12. Baker EN, Baker HM, Smith CA, Stebbins MR, Kahn M, et al. 1992. Human melanotransferrin (p97) has only one functional iron-binding site. *FEBS Lett.* 298:215–18
13. Barisani D, Berg CL, Wessling-Resnick M, Gollan JL. 1995. Evidence for a low $K_m$ transporter for non-transferrin-bound iron in isolated rat hepatocytes. *Am. Physiol. Soc.* 269:G570–76
14. Bartfeld NS, Law JH. 1990. Biochemical and molecular characterization of transferrin from *Manduca sexta. J. Biol. Chem.* 265:21684–91
15. Basset P, Quesneau Y, Zwiller J. 1986. Iron-induced L1210 cell growth: evidence of a transferrin-independent iron transport. *Cancer Res.* 46:1644–47

15a. Beard JL, Burton JW, Theil E. 1996. Purified ferritin and soybean meal can be sources of iron for treating iron deficiency in rats. *J. Nutr.* 126:154–60

16. Beinert H, Kennedy MC. 1993. Aconitase, a two-faced protein: enzyme and iron regulatory factor. *FASEB J.* 7:1442–49
17. Beintema JJ, Stam WT, Hazes B, Smidt MP. 1994. Evolution of arthropod hemocyanins and insect storage proteins (hexamerins). *Mol. Biol. Evol.* 11:493–504
18. Bezkorovainy A. 1989. Biochemistry of nonheme iron in man. *Clin. Physiol. Biochem.* 7:53–69
19. Boctor FN, Kamel MY. 1976. Purification and characterization of two lipovitellins from eggs of the tick *Dermacentor andersoni*. *Insect Biochem.* 6:233–40

20. Bowman BH, Yang F, Adrian GS. 1988. Transferrin: evolution and genetic regulation of expression. *Adv. Genet.* 25:1–38
21. Brieland JK, Vissers MCM, Phan SH, Fantone JC. 1989. Human platelets mediate iron release from transferrin by adenine nucleotide-dependent and -independent mechanisms. *Biochim. Biophys. Acta* 978:191–96
22. Brissot P, Wright TL, Ma WL, Weisiger RA. 1985. Efficient clearance of non-transferrin-bound iron by rat liver. *J. Clin. Invest.* 76:1463–70
23. Brown JP, Nishiyama K, Hellström I, Hellström KE. 1981. Structural characterization of human melanoma-associated antigen p97 with monoclonal antibodies. *J. Immunol.* 127:539–46
24. Bull PC, Cox DW. 1994. Wilson disease and Menkes disease: new handles on heavy-metal transport. *Trends Genet.* 10:246–52
25. Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. 1993. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nature Genet.* 5:327–37
26. Capurro MD, Iughetti P, Ribolla PEM, DeBianchi AG. 1996. *Musca domestica* hemolymph ferritin. *Arch. Insect Biochem. Physiol.* 32:197–207
26a. Casey JL, Hentze MW, Koeller DM, Caughman SW, Rouault TA, et al. 1988. Iron-responsive elements: regulatory RNA sequences that control mRNA levels and translation. *Science* 240:924–28
27. Champagne DE, Nussenzveig RH, Ribeiro JMC. 1995. Purification, partial characterization, and cloning of nitric oxide-carrying heme proteins (nitrophorins) from salivary glands of the blood-sucking insect *Rhodnius prolixus*. *J. Biol. Chem.* 270:8691–95
28. Chan RYY, Ponka P, Schulman HM. 1992. Transferrin-receptor-independent but iron-dependent proliferation of variant Chinese hamster ovary cells. *Exp. Cell Res.* 202:326–36
29. Conrad ME, Umbreit JN. 1993. A concise review: iron absorption—the mucin-mobilferrin-integrin pathway. A competitive pathway for metal absorption. *Am. J. Hematol.* 42:67–73
30. Conrad ME, Umbreit JN, Moore EG, Uzel C, Berry MR. 1994. Alternate iron transport pathway. Mobilferrin and integrin in K562 cells. *J. Biol. Chem.* 269:7169–73
31. Craven CM, Alexander J, Eldridge M, Kushner JP, Bernstein S, Kaplan J. 1987. Tissue distribution and clearance kinetics of non-transferrin-bound iron in the hypotransferrinemic mouse: a rodent model for hemochromatosis. *Proc. Natl. Acad. Sci. USA* 84:3457–61
32. Crichton RR. 1991. *Inorganic Biochemistry of Iron Metabolism.* Luvain le Neuve, Belgium: Ellis Horwood. 263 pp.
33. Dancis A, Haile D, Yuan DS, Klausner RD. 1994. The *Saccharomyces cerevisiae* copper transport protein (Ctrlp). *J. Biol. Chem.* 269:25660–67
34. Dancis A, Roman DG, Anderson GJ, Hinnebusch AG, Klausner RD. 1992. Ferric reductase of *Saccharomyces cerevisiae*: molecular characterization, role in iron uptake, and transcriptional control by iron. *Proc. Natl. Acad. Sci. USA* 89:3869–73
35. Dancis A, Yuan DS, Haile D, Askwith C, Eide D, et al. 1994. Molecular characterization of a copper transport protein in *S. cerevisiae*: an unexpected role for copper in iron transport. *Cell* 76:393–402
36. Dandekar T, Stripecke R, Gray NK, Goossen B, Constable A, et al. 1991. Identification of a novel iron-responsive element in murine and human erythroid $\delta$-aminolevulinic acid synthase mRNA. *EMBO J.* 10:1903–9
37. Danielsen EM, van Deurs B. 1995. A transferrin-like GPI-linked iron-binding protein in detergent-insoluble noncaveolar microdomains at the apical surface of fetal intestinal epithelial cells. *J. Cell Biol.* 131:939–50
37a. Davidson LA, McOrmond SL, Harris ED. 1994. Characterization of a particulate pathway for copper in K562 cells. *Biochim. Biophys. Acta* 1221:1–6
38. DeGraff J, Amons R, Möller W. 1990. The primary structure of artemin from *Artemia* cysts. *Eur. J. Biochem.* 193:737–50
39. deJong G, van Dijk JP, van Eijk HG. 1990. The biology of transferrin. *Clin. Chim. Acta* 190:1–46
40. De Silva DM, Askwith CC, Eide D, Kaplan J. 1995. The *FET3* gene product required for high affinity iron transport in yeast is a cell surface ferroxidase. *J. Biol. Chem.* 270:1098–101
41. De Silva DM, Askwith CC, Kaplan J. 1996. Molecular mechanisms of iron uptake in eukaryotes. *Physiol. Rev.* 76:31–47
42. Dietzel J, Hirzmann J, Preis D, Symmons P, Kunz W. 1992. Ferritins of

*Schistosoma mansonii*: sequence comparison and expression in female and male worms. *Mol. Biochem. Parasitol.* 50:245–54

43. Dix DR, Bridgham JT, Broderius MA, Byersdorfer CA, Eide DJ. 1994. The *FET4* gene encodes the low affinity Fe(II) transport protein of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 269:26092–99
44. Dunkov BC, Zhang D, Choumarov K, Winzerling JJ, Law JH. 1995. Isolation and characterization of a mosquito ferritin and cloning of a cDNA that encodes one subunit. *Arch. Insect Biochem. Physiol.* 29:293–307
45. Flanagan PR. 1990. Intestinal iron metabolism and absorption. In *Iron Transport and Storage*, ed. P Ponka, HM Schulman, RC Woodworth, 15:247–61. Boca Raton, FL: CRC. 336 pp.
46. Fleming RE, Gitlin JD. 1990. Primary structure of rat ceruloplasmin and analysis of tissue-specific gene expression during development. *J. Biol. Chem.* 265:7701–7
47. Food MR, Rothenberger S, Gabathuler R, Haidl ID, Reid G, Jefferies WA. 1994. Transport and expression in human melanomas of a transferrin-like glycosylphosphatidylinositol-anchored protein. *J. Biol. Chem.* 269:3034–40
48. Frost BW. 1996. Phytoplankton bloom on iron rations. *Nature* 383:475–76
49. Fu D, Beeler TJ, Dunn TM. 1995. Sequence, mapping and disruption of *CCC2*, a gene that cross-complements the $Ca^{2+}$-sensitive phenotype of *csg1* mutants and encodes a P-type ATPase belonging to the $Cu^{2+}$-ATPase subfamily. *Yeast* 11:283–92
50. Fujimoto K, Okino N, Kawabata S-I, Iwanaga S, Ohnishi E. 1995. Nucleotide sequence of the cDNA encoding the proenzyme of phenol oxidase $A_1$ of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 92:7769–73
51. Gasdaska JR, Law JH, Bender CJ, Aisen P. 1996. Cockroach transferrin closely resembles vertebrate transferrins in its metal ion-binding properties. A spectroscopic study. *J. Inorg. Biochem.* 64:247–58
52. Georgatsou E, Alexandraki D. 1994. Two distinctly regulated genes are required for ferric reduction, the first step of iron uptake in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 14:3065–73
53. Gitlin D, Janeway CA. 1960. Turnover of the copper and protein moieties of ceruloplasmin. *Nature* 185:693
54. Gitlin JD, Schroeder JJ, Lee-Ambrose LM, Cousins RJ. 1992. Mechanisms of caeruloplasmin biosynthesis in normal and copper-deficient rats. *Biochem. J.* 282:835–39
55. Graden JA, Winge DR. 1996. MAC1: Novel metallo-regulation of gene expression by copper-repression of transactivation in yeast. *Inorg. Biochem. Summer Workshop Cent. Metalloenzyme Studies, Univ. GA, Athens*, p. 21 (Abstr.)
56. Gray NK, Pantopoulos K, Dandekar T, Ackrell BAC, Hentze MW. 1996. Translational regulation of mammalian and *Drosophila* citric acid cycle enzymes via iron-responsive elements. *Proc. Natl. Acad. Sci. USA* 93:4925–30
57. Grootveld M, Bell JD, Halliwell B, Aruoma OI, Bomford A, Sadler PJ. 1989. Non-transferrin-bound iron in plasma or serum from patients with idopathic hemochromatosis. *J. Biol. Chem.* 264:4417–22
58. Guo B, Yang Y, Leibold EA. 1994. Iron regulates cytoplasmic levels of a novel iron-responsive element-binding protein without aconitase activity. *J. Biol. Chem.* 269:24252–60
59. Haile DJ, Rouault TA, Harford JB, Kennedy MC, Blondin GA, et al. 1992. Cellular regulation of the iron-responsive element binding protein: Disassembly of the cubane iron-sulfur cluster results in high-affinity RNA binding. *Proc. Natl. Acad. Sci. USA* 89:11735–39
60. Hall M, Scott T, Sugumaran M, Söderhäll K, Law JH. 1995. Proenzyme of *Manduca sexta* phenol oxidase: purification, activation, substrate specificity of the active enzyme, and molecular cloning. *Proc. Natl. Acad. Sci. USA* 92:7764–68
61. Halleux C, Schneider YJ. 1994. Iron absorption by CaCo-2 cells cultivated in serum-free medium as in vitro model of the human intestinal epithelial barrier. *J. Cell. Phys.* 158:17–28
62. Harris DC, Aisen P. 1989. Physical biochemistry of the transferrins. In *Iron Carriers and Iron Proteins*, ed. TM Loehr, pp. 239–351. New York: VCH. 533 pp.

62a. Harris ED. 1991. Copper transport: an overview (43171B). *Proc. Soc. Exp. Biol. Med.* 196:130–40

63. Harris ED. 1995. The iron-copper connection—the link to ceruloplasmin grows stronger. *Nutr. Rev.* 53:170–73

64. Harris L, Takahashi Y, Miyajima H, Serizawa M, MacGillivray TA, Gitlin JD. 1995. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. *Proc. Natl. Acad. Sci. USA* 92:2539–43
65. Harris ZL, Gitlin JD. 1996. Genetic and molecular basis for copper toxicity. *Am. J. Clin. Nutr.* 63:836–41S
66. Harrison PM, Lilley TH. 1989. Ferritin. In *Iron Carriers and Iron Proteins,* ed. TM Loehr, 2:123–32. New York: VCH. 533 pp.
67. Hart EB, Steenbock H, Waddell J, Elvehjem CA. 1927. Iron in nutrition VI. Copper as a supplement to iron for hemoglobin building in anemic rats. *J. Biol. Chem.* 77:797–812
68. Hasset R, Kosman DJ. 1995. Evidence for Cu(II) reduction as a component of copper uptake by *Saccharomyces cerevisiae. J. Biol. Chem.* 270:128–34
69. Hayashi A, Wada Y, Suzuki T, Shimizu A. 1993. Studies on familial hypotransferrinemia: unique clinical course and molecular pathology. *Am. J. Hum. Genet.* 53:201–13
70. Henderson BR, Seiser C, Kühn LC. 1993. Characterization of a second RNA-binding protein in rodents with specificity for iron-responsive elements. *J. Biol. Chem.* 268:27327–34
71. Hentze MW, Kühn LC. 1996. Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress. *Proc. Natl. Acad. Sci. USA* 93:8175–82
72. Huang TS, Law JH, Söderhäll K. 1996. Purification and cDNA cloning of ferritin from the freshwater crayfish *Pacifastacus leniusculus. Euro. J. Biochem.* 236:450–56
73. Huebers HA, Huebers E, Finch CA, Martin AW. 1982. Characterization of an invertebrate transferrin from the crab *Cancer magister* (Arthropoda). *J. Comp. Physiol.* 148:101–9
74. Huebers HA, Huebers E, Finch CA, Webb BA, Truman JW, et al. 1988. Iron binding proteins and their roles in the tobacco hornworm *Manduca sexta* (L). *J. Comp. Physiol. B* 158:291–300
75. Inman RS, Coughlan MM, Wessling-Resnick M. 1994. Extracellular ferrireductase activity of K562 cells is coupled to transferrin-independent iron transport. *J. Biochem.* 33:11850–57
76. Inman RS, Wessling-Resnick M. 1993. Characterization of transferrin-independent iron transport in K562 cells. *J. Biol. Chem.* 268:8521–28
77. Jamroz RC, Gasdaska JR, Bradfield JY, Law JH. 1993. Transferrin in a cockroach: molecular cloning, characterization, and suppression by juvenile hormone. *Proc. Natl. Acad. Sci. USA* 90:1320–24
78. Jungmann J, Reins HA, Lee J, Romeo A, Hassett R, et al. 1993. MAC1, a nuclear regulatory protein related to Cu-dependent transcription factors is involved in Cu/Fe utilization and stress resistance in yeast. *EMBO J.* 12:5051–56
79. Kaplan J, Jordan I, Sturrock A. 1991. Regulation of the transferrin-independent iron transport system in cultured cells. *J. Biol. Chem.* 266:2997–3004
80. Kaplan J, O'Halloran TV. 1996. Iron metabolism in eukaryotes: Mars and Venus at it again. *Science* 271:1510–12
81. Kawabata T, Yasuhara Y, Ochiai M, Matsuura S, Ashida M. 1995. Molecular cloning of insect pro-phenol oxidase: a copper containing protein homologous to arthropod hemocyanin. *Proc. Natl. Acad. Sci. USA* 92:7774–78
82. Kennard ML, Richardson DR, Gabathuler R, Ponka P, Jefferies WA. 1995. A novel iron uptake mechanism mediated by GPI-anchored human p97. *EMBO J.* 14:4178–86
83. Kennedy MC, Mende-Mueller L, Blondin GA, Beinert H. 1992. Purification and characterization of cytosolic aconitase from beef liver and its relationship to the iron-responsive element binding protein. *Proc. Natl. Acad. Sci. USA* 89:11730–34

83a. Kimata Y, Theil EC. 1994. Posttranscriptional regulation of ferritin during nodule development in soybean. *Plant Physiol.* 104:263–70

84. Klausner RD, Dancis A. 1994. A genetic approach to elucidating eukaryotic iron metabolism. *FEBS Lett.* 355:109–13

84a. Koeller DM, Casey JL, Hentz MW, Gerhardt EM, Chan LN, et al. 1989. A cytosolic protein binds to structural elements within the iron regulatory region of the transferrin receptor mRNA. *Proc. Natl. Acad. Sci. USA* 86:3574–78

85. Kohler SA, Henderson BR, Kühn LC. 1995. Succinate dehydrogenase b mRNA of *Drosophila melanogaster* has a functional iron-responsive element in its 5′-untranslated region. *J. Biol. Chem.* 270:30781–86
86. Koschinsky ML, Funk WD, van Oost BA, MacGillivray RTA. 1986. Complete

cDNA sequence of human preceruloplasmin. *Proc. Natl. Acad. Sci. USA* 83:5086–90
87. Kurama T, Kurata S, Natori S. 1995. Molecular characterization of an insect transferrin and its selective incorporation into eggs during oogenesis. *Eur. J. Biochem.* 228:229–35
88. Lash A, Saleem A. 1994. Iron metabolism and its regulation. *Ann. Clin. Lab. Sci.* 25:20–30
89. Lee GR, Nacht S, Lukens JN, Cartwright GE. 1968. Copper-deficient swine. *J. Clin. Invest.* 47:2058–69
90. Lee MY, Huebers E, Martin AW, Finch CA. 1978. Iron metabolism in a spider, *Dugesiella hentzi*. *J. Comp. Physiol.* 127:349–54
91. Leibold EA, Guo B. 1992. Iron dependent regulation of ferritin and transferrin receptor expression by the iron-responsive element binding protein. *Annu. Rev. Nutr.* 12:345–68
92. Linder MC. 1991. Copper and metabolic regulation. In *Biochemistry of Copper.* New York: Plenum. 525 pp.
92a. Linder MC. 1997. Copper transport. *Am. J. Clin. Nutr.* In press
93. Linder MC, Hazegh-Azam M. 1996. Copper biochemistry and molecular biology. *Am. J. Clin. Nutr.* 63:797–811
94. Linder MC, Schaffer KJ, Hazegh-Azam M, Zhou CYJ, Tran TN, Nagel GM. 1996. Serum ferritin: Does it differ from tissue ferritin? *J. Gastroenterol. Hepatol.* 11:1033–36
95. Locke M, Nichol H. 1992. Iron economy in insects: transport, metabolism, and storage. *Annu. Rev. Entomol.* 37:195–215
96. Logan JI, Harveyson KB, Wisdom GB, Hughes AE, Archbold GPR. 1994. Hereditary caeruloplasmin deficiency, dementia and diabetes mellitus. *Q. J. Med.* 87:663–70
97. Maines MD. 1988. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J.* 2:2557–68
98. Martin AW, Huebers E, Huebers H, Webb J, Finch CA. 1984. A monosited transferrin from a representative deuterostome: the ascidian *Pyura stolonifera* (subphylum Urochordata). *Blood* 64:1047–52
99. Marx JJM, Aisen P. 1981. Iron uptake by rabbit intestinal mucosal membrane vesicles. *Biochim. Biophys. Acta* 649:297–304
100. Mascotti DP, Rup D, Thach RE. 1995. Regulation of iron metabolism—translational effects mediated by iron, heme, and cytokines. *Annu. Rev. Nutr.* 15:239–61
101. Melefors O. 1996. Translational regulation in vivo of the *Drosophila melanogaster* mRNA encoding succinate dehydrogenase iron protein via iron responsive elements. *Biochem. Biophys. Res. Commun.* 221:437–41
102. Miyajima H, Nishimura Y, Mizoguchi K, Sakamoto M, Shimizu T, Honda N. 1987. Familial apoceruloplasmin deficiency associated with blepharospasm and retinal degeneration. *Neurology* 37:761–67
103. Morabito MA, Moczydlowski E. 1994. Molecular cloning of bullfrog saxiphilin: a unique relative of the transferrin family that binds saxitoxin. *Proc. Natl. Acad. Sci. USA* 91:2478–82
104. Morita H, Ikeda S, Yamamoto K, Morita S, Yoshida K, et al. 1995. Hereditary ceruloplasmin deficiency with hemosiderosis: a clinicopathological study of a Japanese family. *Ann. Neurol.* 37:646–56
105. Muller-Eberhard U. 1988. Hemopexin. *Methods Enzymol.* 163:536–65
106. Nichol H, Locke M. 1989. The characterization of ferritin in an insect. *Insect Biochem.* 19:587–602
107. Nichol H, Locke M. 1990. The localization of ferritin in insects. *Tissue Cell* 22:767–77
108. Nichols GM, Bacon BR. 1989. Hereditary hemochromatosis: pathogenesis and clinical features of a common disease. *Am. J. Gastroenterol.* 84:851–62
109. Nichols GM, Pearce AR, Alverez X, Bibb NK, Nichols KY, et al. 1992. The mechanisms of nonheme iron uptake determined in IEC-6 rat intestinal cells. *J. Nutr.* 122:945–52
110. Nunez MT, Alvarez X, Smith M, Tapia V, Glass J. 1994. Role of redox systems on $Fe3^{+}$ uptake by transformed human intestinal epithelial (Caco-2) cells. *Am. J. Physiol.* 267:C1582–88
111. Nunez MT, Cole ES, Glass J. 1983. The reticulocyte plasma membrane pathway of iron uptake as determined by the mechanism of $\alpha,\alpha'$-dipyridyl inhibition. *J. Biol. Chem.* 258:1146–51
112. O'Donnell MW, Cox TM. 1982. Microvillar iron-binding glycoproteins isolated from the rabbit small intestine. *J. Biochem.* 202:107–15
113. O'Halloran TV. 1993. Transition metals in control of gene expression. *Science* 261:715–25

114. Oliveira PL, Kawooya JK, Ribeiro JMC, Meyer T, Poorman R, et al. 1995. A heme-binding protein from hemolymph and oocytes of the blood-sucking insect, *Rhodnius prolixus*—isolation and characterization. *J. Biol. Chem.* 270:10897–901
115. Osaki S, Johnson DA, Frieden E. 1966. The possible significance of ferrous oxidase activity of ceruloplasmin in normal human serum. *J. Biol. Chem.* 241:2746–51
116. Oshiro S, Nakajima H, Markello T, Krasnewich D, Bernardini I, Gahl WA. 1993. Redox, transferrin-independent, and receptor-mediated endocytosis iron uptake systems in cultured human fibroblasts. *J. Biol. Chem.* 268:21586–91
117. Pantopoulos K, Gray NK, Hentze MW. 1995. Differential regulation of two related RNA-binding proteins, iron regulatory protein (IRP) and $IRP_{B.}$ *RNA* 1:155–63
118. Parkes JG, Randell EW, Olivieri NF, Templeton DM. 1995. Modulation by iron loading and chelation of the uptake of non-transferrin-bound iron by human liver cells. *Biochim. Biophys. Acta* 1243:373–80
118a. Percival SS, Harris ED. 1990. Copper transport from ceruloplasmin: characterization of the cellular uptake mechanism. *Am. J. Physiol.* 258:C140–46
119. Pham DQ-D, Zhang D, Hufnagel DH, Winzerling JJ. 1996. *Manduca sexta* hemolymph ferritin: cDNA sequence and mRNA expression. *Gene* 172:255–59
120. Quail EA, Morgan EH. 1994. Role of membrane surface potential and other factors in the uptake of non-transferrin-bound iron by reticulocytes. *J. Cell. Physiol.* 159:238–44
121. Raja KB, Simpson RJ, Peters TJ. 1992. Investigation of a role for reduction in ferric iron uptake by mouse duodenum. *Biochim. Biophys. Acta* 1135:141–46
122. Randell EW, Parkes JG, Olivieri NF, Templeton DM. 1994. Uptake of non-transferrin-bound iron by both reductive and nonreductive processes is modulated by intracellular iron. *J. Biol. Chem.* 269:16046–53
123. Real FX, Furukawa KS, Mattes MJ, Gusik SA, Cordon-Cardo C, et al. 1988. Class 1 (unique) tumor antigens of human melanoma: identification of unique and common epitopes on a 90-kDa glycoprotein. *Proc. Natl. Acad. Sci. USA* 85:3965–69
124. Richardson DR, Baker E. 1992. The effect of desferrioxamine and ferric ammonium citrate on the uptake of iron by the membrane iron-binding component of human melanoma cells. *Biochim. Biophys. Acta* 1103:275–80
125. Roeser HP, Lee GR, Nacht S, Cartwright GE. 1970. The role of ceruloplasmin in iron metabolism. *J. Clin. Invest.* 49:2408–17
126. Rose TM, Plowman GD, Teplow DB, Dreyer WJ, Hellström KE, Brown JP. 1986. Primary structure of the human melanoma-associated antigen p97 (melanotransferrin) deduced from the mRNA sequence. *Proc. Natl. Acad. Sci. USA* 83:1261–65
127. Rothenberger S, Mullner EW, Kühn LC. 1990. The mRNA-binding protein which controls ferritin and transferrin receptor expression is conserved during evolution. *Nucleic Acids Res.* 18:1175–79
128. Samaniego F, Chin J, Iwai K, Rouault TA, Klausner RD. 1994. Molecular characterization of a second iron responsive element binding protein, iron regulatory protein 2. *J. Biol. Chem.* 269:30904–10
129. Sato M, Gitlin JD. 1991. Mechanisms of copper incorporation during the biosynthesis of human ceruloplasmin. *J. Biol. Chem.* 266:5128–34
130. Schaffer KJ, Tran TN, Hazegh-Azam M, Linder MC. 1996. Expression and regulation of the secretion of serum ferritin. In *Trace Elements in Man and Animal*, ed. M L'Abbe, vol. 9. New York: Plenum. In press
131. Schalinske KL, Eisenstein RS. 1996. Phosphorylation and activation of both iron regulatory proteins 1 and 2 in HL-60 cells. *J. Biol. Chem.* 271:7168–76
132. Scheiber B, Goldenberg H. 1993. NAD(P)H: ferric iron reductase in endosomal membranes from rat liver. *Arch. Biochem. Biophys.* 305:225–30
133. Scrimshaw NS. 1991. Iron deficiency. *Sci. Am.* 267:46–52
134. Simpson RJ, Peters TJ. 1986. Mouse intestinal $Fe^{+3}$ uptake kinetics in vivo. The significance of brush-border membrane vesicle transport in the mechanism of mucosal $Fe^{3+}$ uptake. *Biochim. Biophys. Acta* 856:115–22
135. Simpson RJ, Raja KB, Peters TJ. 1986. $Fe^{2+}$ uptake by mouse intestinal mucosa in vivo and by isolated intestinal brush-border membrane vesicles. *Biochim. Biophys. Acta* 860:229–35
136. Slater AFG, Swiggard WJ, Orton BR, Flitter WD, Goldberg DE, et al. 1991. An iron-carboxylate bond links the heme

units of malaria pigment. *Proc. Natl. Acad. Sci. USA* 88:325–29
137. Stearman R, Yuan DS, Yamaguchi-Iwai Y, Klausner RD, Dancis A. 1996. A permease-oxidase complex involved in high-affinity iron uptake in yeast. *Science* 271:1552–57
138. Sturrock A, Alexander J, Lamb J, Craven CM, Kaplan J. 1990. Characterization of a transferrin-independent uptake system for iron in HeLa cells. *J. Biol. Chem.* 265:3139–45
139. Sugumaran M. 1996. Roles of the insect cuticle in host defense reactions. In *New Directions in Invertebrate Immunology*, ed. K Söderhäll, G Vasta, S Dwanaga, 18:1–20. Fair Haven, NJ: SOS
140. Sugumaran M, Kanost MR. 1993. Regulation of insect hemolymph phenoloxidases. In *Parasites and Pathogens of Insects*, ed. NE Beckage, SN Thompson, BA Federici, 1:317–42. San Diego: Academic
141. Sun IL, Navas P, Crane FL, Morré, Löw H. 1987. NADH diferric transferrin reductase in liver plasma membrane. *J. Biol. Chem.* 33:15915–21
142. Takahashi N, Ortel TL, Putnam FW. 1984. Single-chain structure of human ceruloplasmin: the complete amino acid sequence of the whole molecule. *Proc. Natl. Acad. Sci. USA* 81:390–94
143. Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, et al. 1993. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nature Genet.* 5:344–50
144. Teichmann R, Stremmel W. 1990. Iron uptake by human upper small intestine microvillous membrane vesicles. *J. Clin. Invest.* 86:2145–53
145. Telfer WH, Kunkel JG. 1991. The function and evolution of insect storage hexamers. *Annu. Rev. Entomol.* 36:205–28
146a. Theil EC. 1994. Iron regulatory elements (IREs): a family of mRNA non-coding sequences. *Biochem. J.* 304:1–11
146. Theil EC. 1990. The ferritin family of iron storage proteins. *Adv. Enzymol. Mol. Biol.* 63:421–29
147. Thorstensen K. 1988. Hepatocytes and reticulocytes have different mechanisms for the uptake of iron from transferrin. *J. Biol. Chem.* 263:16837–41
148. Thorstensen K, Trinder D, Zak O, Aisen P. 1995. Uptake of iron from N-terminal half-transferrin by isolated rat hepatocytes. Evidence of transferrin-receptor-independent iron uptake. *Eur. J. Biochem.* 232:129–33
149. VanHolde KE, Miller KI. 1995. Hemocyanins. *Adv. Protein Chem.* 47:1–81
150. Von Darl M, Harrison PM, Bottke W. 1994. cDNA cloning and deduced amino acid sequence of two ferritins: soma ferritin and yolk ferritin, from the snail *Lymnaea stagnalis* L. *Eur. J. Biochem.* 222:353–66
151. von Herbay A, de Groot H, Hegi U, Stremmel W, Strohmeyer G, Sies H. 1995. Low vitamin E content in plasma of patients with alcoholic liver disease, hemochromatosis and Wilson's disease. *J. Hepatol.* 20:41–46
152. Vulpe C, Levinson B, Whitney S, Packman S, Gitschier J. 1993. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nature Genet.* 3:7–13
153. Vulpe CD, Packman S. 1995. Cellular copper transport. *Annu. Rev. Nutr.* 15:293–322
154. Waddell J, Steenbock H, Elvehjem CA, Hart EB. 1927. Iron in nutrition V. Iron salts and iron-containing ash extracts in the correction of anemia. *J. Biol. Chem.* 77:777–95
154a. Waldo GS, Theil EC. 1995. Ferritin and iron biomineralization. In *Comprehensive Supramolecular Chemistry*, ed. JL Atwood, JED Davies, DD MacNicol, F Vögtle, JM Lehn, 5:65–89. New York: Plenum
155. Weisenberg E, Halbreich A, Moger J. 1980. Biochemical lesions in copper-deficient rats caused by secondary iron deficiency. Derangement of protein synthesis and impairment of energy metabolism. *Biochem. J.* 188:633–41
156. Williams J, Grace SA, Williams JM. 1982. Evolutionary significance of renal excretion of transferrin half-molecule fragments. *Biochem. J.* 201:417–19
157. Winzerling JJ, Nez P, Porath J, Law JH. 1995. Rapid and efficient isolation of transferrin and ferritin from *Manduca sexta. Insect Biochem. Mol. Biol.* 25: 217–224
158. Wright TL, Brissot P, Ma WL, Weisiger RA. 1986. Characterization of non-transferrin-bound iron clearance by rat liver. *J. Biol. Chem.* 261:10909–14
159. Yamaguchi Y, Heiny M, Gitlin JD. 1993. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson's disease. *J. Biol. Chem.* 263:1842–47
160. Yamaguchi-Iwai Y, Dancis A, Klausner RD. 1995. AFT1: a mediator of

iron regulated transcriptional control in *Saccharomyces cerevisiae. EMBO J.* 14:1231–39

161. Yang F, Naylor SL, Lum JB, Cutshaw S, McCombs JL, et al. 1986. Characterization, mapping, and expression of the human ceruloplasmin gene. *Proc. Natl. Acad. Sci. USA* 83:3257–61
162. Yasuhara Y, Koizumi Y, Katagiri C, Ashida M. 1995. Reexamination of properties of prophenoloxidase isolated from larval hemolymph of the silkworm *Bombyx mori*. *Arch. Biochem. Biophys.* 320:14–23
163. Yoshida K, Furihata K, Takeda S, Nakamura A, Yamamoto K, et al. 1995. A mutation in the ceruloplasmin gene is associated with systemic hemosiderosis in humans. *Nature Genet.* 9:267–72
164. Yuan DS, Stearman R, Dancis A, Dunn T, Beeler T, Klausner RD. 1995. The Menkes/Wilson disease gene homologue in yeast provides copper to a ceruloplasmin-like oxidase required for iron uptake. *Proc. Natl. Acad. Sci. USA* 92:2632–36
165. Zaitseva I, Zaitsev V, Card G, Moshkov K, Bax B, et al. 1996. The X-ray structure of human serum ceruloplasmin at 3.1 Å: nature of the copper centers. *J. Biol. Inorg. Chem.* 1:15–23