Iron and copper metabolism in humans

Pathologies associated to iron and copper dysmetabolism

Pathology	Mutated gene	
Menkes syndrome	ATP7A	Copper deficiency (defective absorption of copper)
Wilson disease	ATP7B	Copper overload in liver and brain
Hemochromatosis type I-IV	HFE, TfR2, HJV, HAMP, Fpn	Iron overload in different organs
Friedreich ataxia	Frataxin	Neurodegeneration and iron overload in specific areas of the brain
Aceruloplasminemia	Ceruloplasmin	
Hallevorden-Spatz syndrome	PANK-2	
Neuroferritinopathy	L-ferritin	
Huntington chorea		
Alzheimer disease		
Parkinson disease		

Animal models for the study of human pathologies associated to iron dysmetabolism

Table 1. Animal Models Useful for Understanding Iron Biology and Related Diseases

Strain	Gene (Mutation)	Type of Mutation	Iron Phenotype	Analogous Human Disease
$Hfe^{-/-}$ and $Hfe^{C282Y/C282Y}$[a]	Hfe	TMM	Hepatocellular iron deposition, decreased macrophage iron, elevated transferrin saturation	HFE HC
$\beta2m^{-/-}$	Beta-2 microglobulin	TMM	Parenchymal iron deposition	ND
$Usf2^{-/-}$	Hepcidin[b]	TMM	Hepatocellular iron deposition, decreased macrophage iron, elevated transferrin saturation	Juvenile HC
Hamp—liver spec. transgene	Hepcidin	TgM	Iron deficiency and anemia	NA
$Hfe^{-/-}$ and Hamp transgene	Hepcidin/HFE	CMM	Amelioration of hepatic iron loading relative to $Hfe^{-/-}$ mice	NA
$TfR^{+/-}$	Transferrin receptor-1[c]	TMM	Microcytic hypochromic erythrocytes[f], decreased iron stores	ND
$TfRr2^{245x/245x}$	Transferrin receptor-2	TMM	Hepatocellular iron deposition, decreased macrophage iron, elevated transferrin-saturation	TfR2 HC
hpx	Transferrin	SMM	Microcytic hypochromic anemia, tissue iron deposition	Atransferrinemia
mk	DMT1 (G185R)	SMM	Systemic iron deficiency; impaired iron uptake in the duodenum and in erythroid precursors	ND
b (Belgrade rat)	DMT1 (G185R)	SRM	Systemic iron deficiency; impaired iron uptake in duodenum and in erythroid precursors	ND
cdy (Chardonnay)	DMT1 (missense, nonsense)	IZM	Hypochromic anemia	ND
weh (Weissherbst)	Ferroportin	IZM	Hypochromic anemia, impaired iron transfer from yolk sac to embryo	ND
$Cp^{-/-}$	Ceruloplasmin	TMM	Iron accumulation in hepatocytes and macrophages	Aceruloplasminemia
sla	Hephaestin (deletion)	SMM	Microcytic hypochromic anemia, impaired intestinal iron transfer	ND
$Fth^{+/-}$	H-Ferritin[d]	TMM	Elevated tissue and serum L-ferritin	ND
$Ireb2^{-/-}$	IRE binding protein 2	TMM	Iron deposition in enterocytes, neurons and oligodendrocytes	ND
f (flexed tail)	Sideroflexin 1 (frameshift)	SMM	Transient fetal and neonatal anemia with intracellular iron deposits	ND
Frda - tissue specific k.o. neuron/heart[e]	Frataxin	TMM	Mitochondrial iron deposits, neurodegeneration and cardiomyopathy	Friedreich ataxia
Frda - tissue specific k.o. muscle[e]	Frataxin	TMM	Mitochondrial iron deposits; cardiomyopathy	Friedreich ataxia
$Hmox1^{-/-}$	Heme oxygenase 1	TMM	Anemia, low serum iron levels, tissue iron deposition	Hmox 1 deficiency

ND: not described; SMM: spontaneous mouse mutant; TMM: targeted mouse mutant; NA: not applicable; SRM: spontaneous rat mutant; TgM: transgenic mouse;
HC: hemochromatosis; IZM: induced zebrafish mutant; CMM: compound mutant mouse
[a] Mutation is in mouse codon 294.
[b] Includes deletion of gene for upstream stimulatory factor-2.
[c] $Trfr^{-/-}$ mice: embryonic lethal by E 12.5.
[d] $Fth^{-/-}$ mice: early embryonic lethality.
[e] $Frda^{-/-}$ mice: early embryonic lethality; beta-cell ko: loss of beta cells, diabetes mellitus.
[f] Reduction in red cell size and hemoglobin content.

Systemic iron homeostasis

Iron is absorbed by **enterocytes** in duodenum, released in the bloodstream and transported to different organs by transferrin. Reticulo-endothelial **macrophages** represent the major site of iron recycling from senescent erythrocytes.

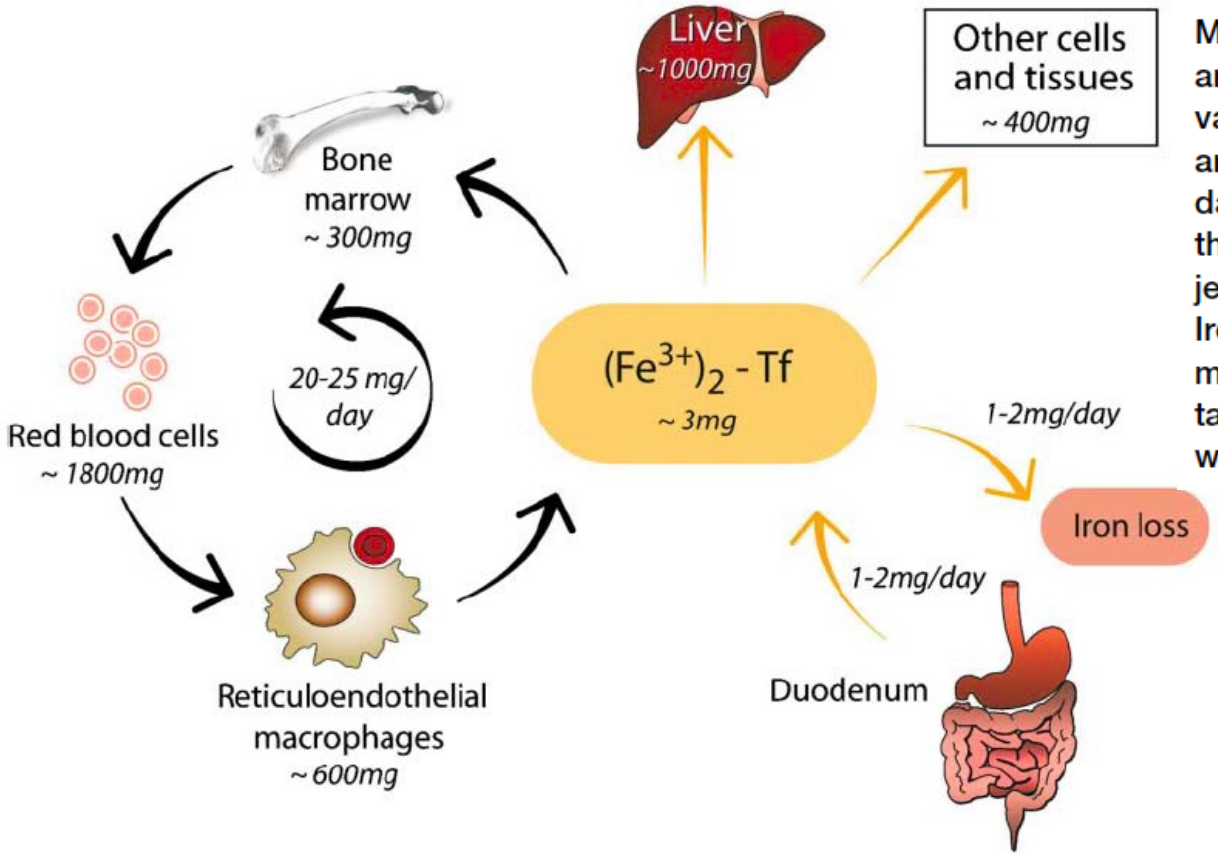


Figure 3. Systemic Iron Homeostasis

Major pathways of iron traffic between cells and tissues are depicted. Normal (human) values for the iron content of different organs and tissues are stated, and the approximate daily fluxes of iron are also indicated. Note that these values are approximate and subject to significant person-to-person variation. Iron losses result from sloughing of skin and mucosal cells as well as blood loss. Importantly, there exists no regulated excretion pathway to control systemic iron homeostasis.

Cell biology of iron metabolism in mammals

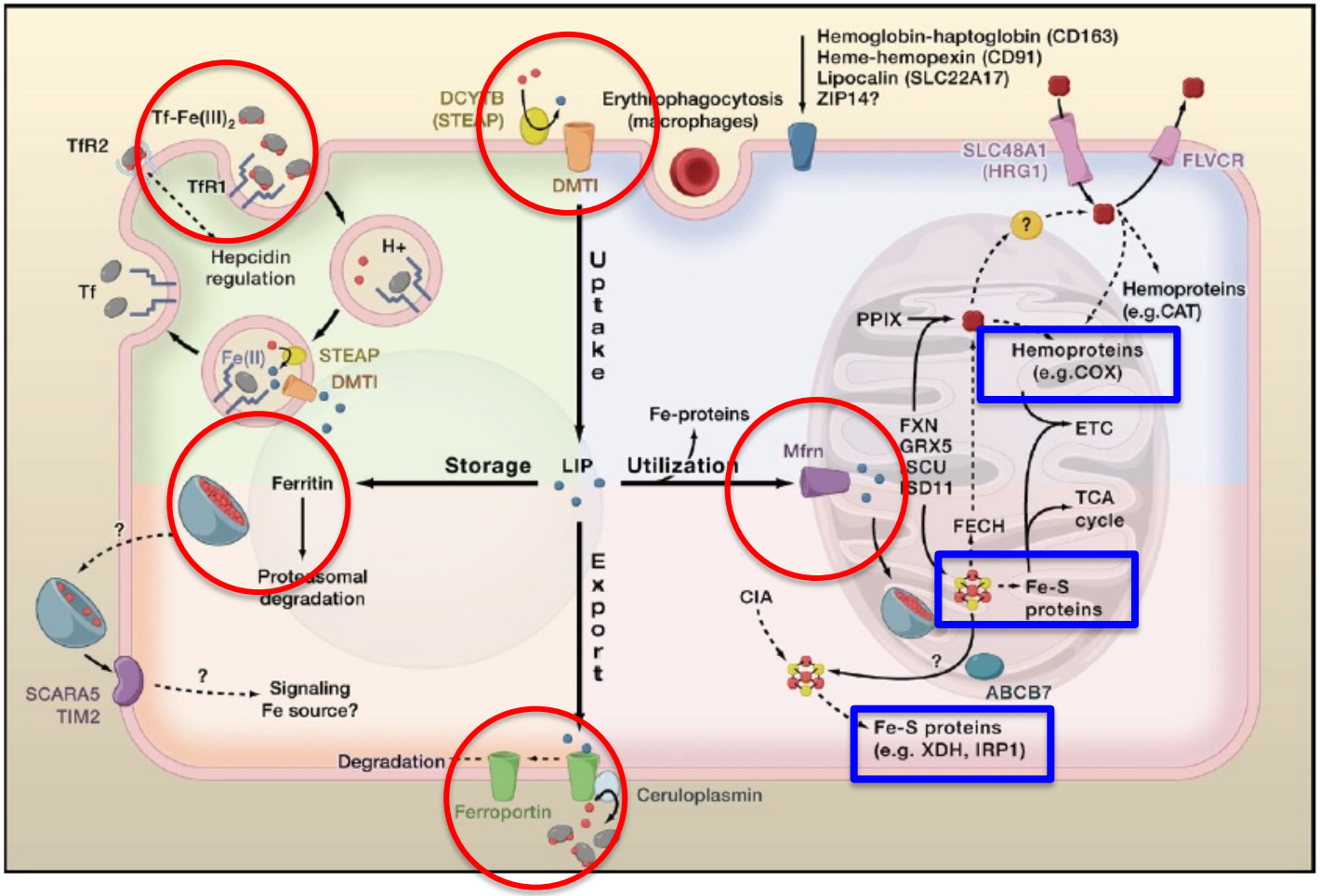


Figure 3. Cell Biology of Iron Metabolism

A generic cell is depicted. Most cells acquire plasma iron via transferrin receptor 1 (TfR1)-mediated endocytosis of transferrin-bound iron. In endosomes, iron is freed from transferrin and reduced to Fe(II) by STEAP metalloreductases prior its release into cytosol via divalent metal transporter 1 (DMT1); transferrin and TfR1 return to the plasma membrane to be used for further cycles. DMT1 also functions in the apical absorption of dietary iron after reduction by DCYTB and possibly other ferrireductases. Other iron acquisition pathways are symbolized (e.g., acquisition of heme iron from red blood cells by macrophages). Iron uptake systems feed the so-called labile iron pool (LIP). The LIP is utilized for direct incorporation into iron proteins or iron transport to mitochondria via mitoferrin (Mfrn), where the metal is inserted into heme and Fe/S cluster prosthetic groups. Proteins promoting heme transport into and out of cells have been identified. The fraction of the LIP that is not utilized for metalation reactions can be exported via ferroportin, which works together with ferroxidases for iron loading onto transferrin, or stored in a nontoxic form in ferritin shells. Ferritin can be released into the extracellular milieu by unknown mechanisms and interact with specific receptors on the cell surface. Some cells also express a mitochondrial form of ferritin to protect the organelle against iron-induced toxicity. The size of the LIP is determined by the rate of iron uptake, utilization, storage, and export; these processes must be coordinately regulated to avoid detrimental iron deficiency and prevent iron excess.

Heme biosynthesis

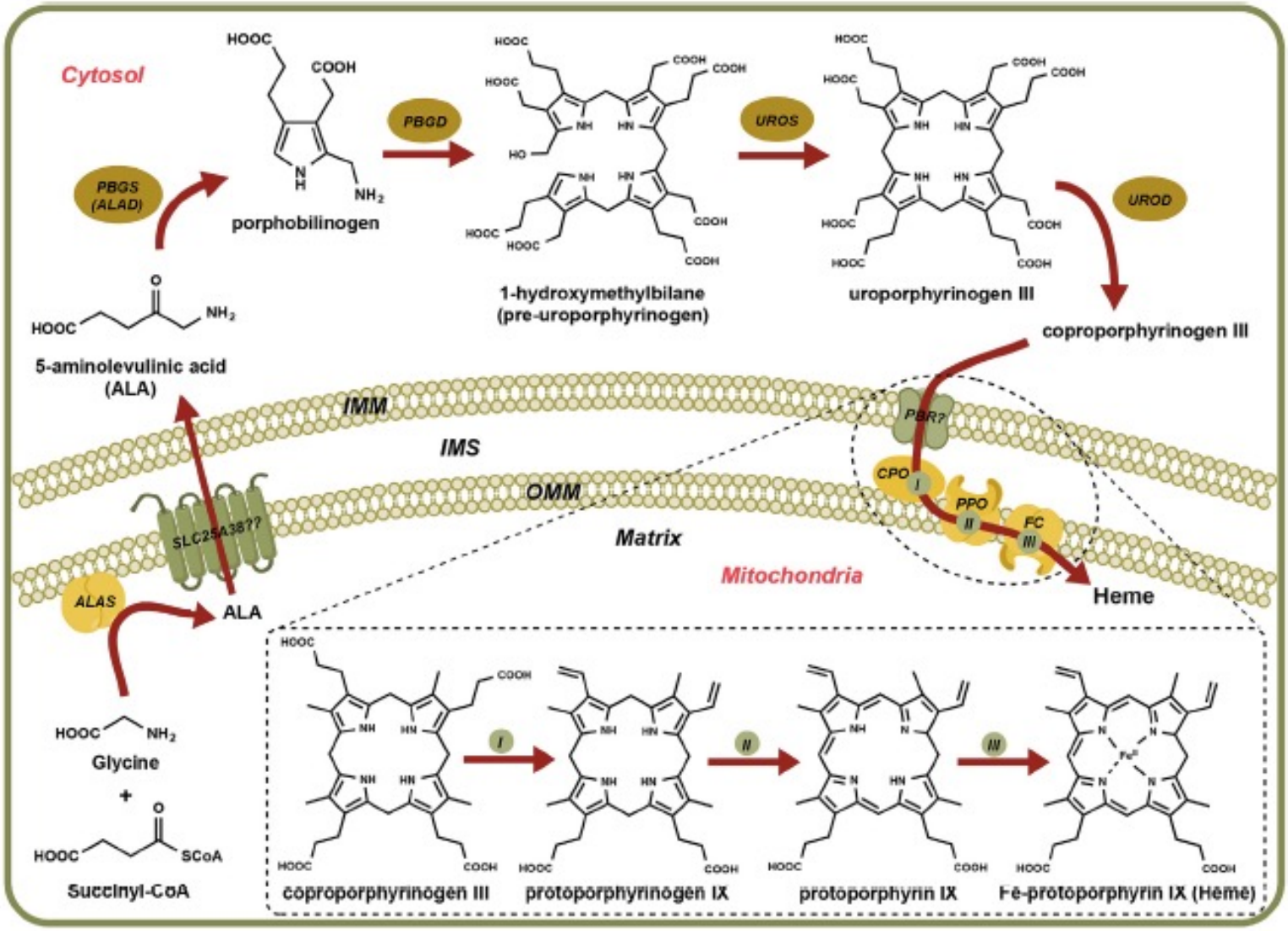
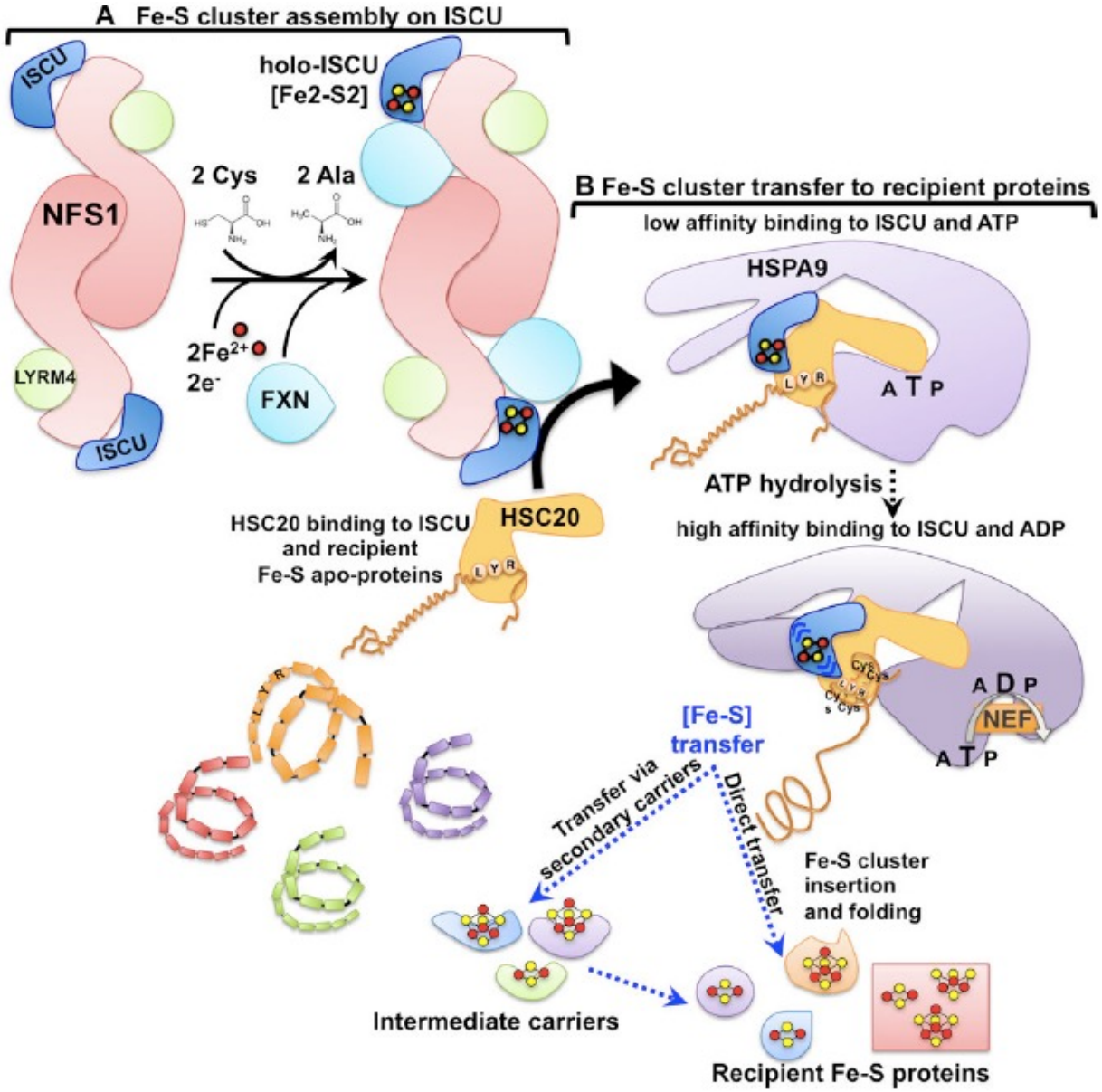


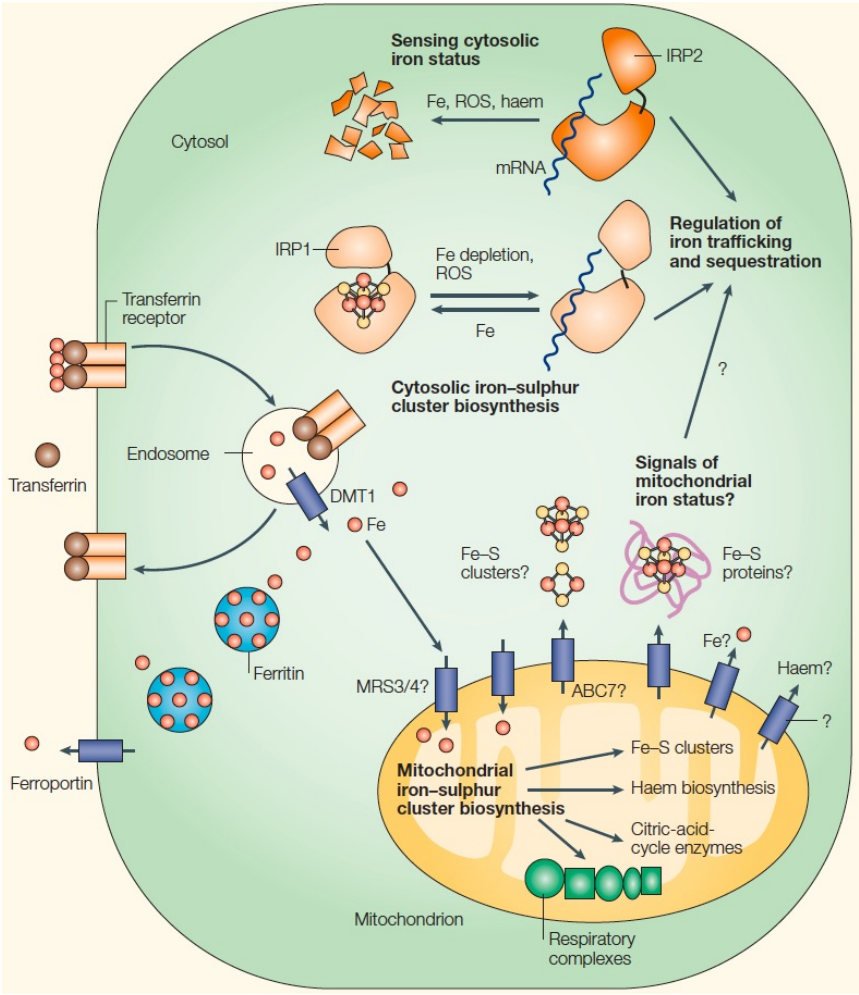
Fig. 4. Heme biosynthesis pathway. ALA: 5-aminolevulinic acid, ALAS: ALA synthase, PBGS: porphobilinogen synthase, ALAD: ALA dehydratase, PBGD: porphobilinogen deaminase, UROS: uroporphyrinogen synthase, UROD: uroporphyrinogen decarboxylase, PBR: peripheral-type benzodiazepine receptor, CPO: coproporphyrinogen III oxidase, PPO: protoporphyrinogen IX oxidase, FC: Ferrochelatase, IMM: inner mitochondrial membrane, IMS: intermembrane space, OMM: outer mitochondrial membrane.

Mechanism for Fe-S cluster biosynthesis in mammals



Biogenesis of Fe-S clusters. Role of mitochondria in iron metabolism

Figure 4 | **Iron–sulphur (Fe–S) cluster biogenesis in mammalian iron homeostasis.** Extracellular diferric transferrin proteins are bound by the dimeric transferrin receptor and internalized by receptor-mediated endocytosis. Iron is then transported into the cytosol through divalent metal transporter-1 (DMT1). In the cytosol of mammalian cells, the assembly and disassembly of a [4Fe–4S] cluster in iron regulatory protein-1 (IRP1) and the iron-dependent degradation of IRP2 provide the mechanisms for sensing intracellular iron levels. In the tissues of healthy animals, most IRP1 contains a [4Fe–4S] cluster and functions as a cytosolic aconitase. Its homologue, IRP2, is therefore responsible for regulating the levels of the transferrin receptor and the iron storage protein ferritin (which can store up to 4,000 Fe atoms per molecule), as well as probably one isoform of DMT1 and the iron exporter ferroportin, by binding to iron-responsive elements in their mRNAs[48]. IRP1 might contribute to iron regulation in pathophysiological situations. Mitochondrial Fe–S protein biosynthesis seems to require the proteins ISCS, ISCU, NFU, ISCA, HSCA, HSCB, ferredoxin, glutaredoxin and frataxin (not shown; see FIG. 2). ISCS, ISCU and NFU might also function in Fe–S cluster assembly in the cytosol (not shown). The mitochondrial transporters that are involved in iron uptake and in the efflux of Fe, Fe–S clusters, Fe–S cluster proteins and haem groups (ferrochelatase is an Fe–S enzyme that is involved in haem biosynthesis in mitochondria; see TABLE 1) have only been partially characterized[26,70], and the mechanism of sensing and regulation remains unclear. However, genetic studies in *Saccharomyces cerevisiae* indicate that the sensor/regulator of mitochondrial homeostasis is an Fe–S protein or that it senses an Fe–S-cluster-containing protein[32,50]. Please note that because of the sequence and functional similarities, mammalian Fe–S cluster assembly proteins are mentioned here using the nomenclature that was originally proposed for the *Escherichia coli isc* operon. ABC, ATP-binding cassette; ROS, reactive oxygen species.



Disfunction of Fe-S cluster biosynthesis causes iron overload in mitochondria and oxidative stress.

Table 1 Some mammalian proteins of iron transport and its regulation

Protein	Protein abbreviation	Gene symbol[a]	Function/role in iron metabolism	Consequence of mutation or deletion
Iron uptake				
Transferrin	Tf	*TF*	Plasma iron transport	Iron deficiency anemia with tissue iron overload Human disease: atransferrinemia
Transferrin receptor 1	TfR1	*TFRC*	Internalization of diferric transferrin	Embryonic lethality in homozygote. Mild anemia in heterozygotes.
Divalent metal-ion transporter 1	DMT1	*SLC11A2*	Ferrous iron importer	Iron deficiency anemia Human disease: refractory hypochromic, microcytic anemia
Six transmembrane epithelial antigen of prostate protein 3	STEAP3	*STEAP3*	Iron reductase of erythroid cells	Iron deficiency anemia
Exocyst complex component 6	SEC15L1	*EXOC6*	Vesicle trafficking	Iron deficiency anemia
Duodenal cytochrome B	DCYTB	*CYBRD1*	Enterocyte brush border reductase	No overt phenotype
Iron recovery				
Hemopexin	HPX	*HPX*	Heme binding	No phenotype unless stressed by hemolysis; then extensive renal damage
Haptoglobin	HP	*HP*	Hemoglobin binding	No phenotype unless stressed by hemolysis; then extensive renal damage
Iron export				
Ferroportin	FPN	*SLC40A1*	Ferrous iron exporter	Iron overload in heterozygous state; embryonic lethality in homozygotes Human disease: hemochromatosis type 4
Ceruloplasmin	Cp	*CP*	Iron oxidase	Iron overload; CNS dysfunction Human disease: aceruloplasminemia
Hephaestin	Hp	*HEPH*	Iron oxidase (gut and CNS)	Iron deficiency anemia
Feline leukemia virus, type C, receptor	FLVCR	*FLVCR1*	Heme export protein	Embryonic lethality. Erythropoietic and developmental abnormalities
Mitochondrial iron transport				
Mitoferrin	MFRN	*SLC25A37*	Mitochondrial iron importer	Iron deficiency anemia; Erythroid maturation arrest
ABC transporter type B7	ABCB7	*ABCB7*	Mitochondrial Fe–S export	Mitochondrial iron loading Human disease: X-linked sideroblastic anemia
Cellular regulation				
Iron regulatory protein 1	IRP1	*ACO1*	Iron-dependent RNA binding protein	Negligible. Some abnormalities in brown fat and kidney.
Iron regulatory protein 2	IRP2	*IREB2*	Iron-dependent RNA binding protein	Anemia; CNS abnormalities of varying severity
Systemic regulation				
Hepcidin	HEPC	*HAMP*	Regulator of iron release into plasma	Severe iron overload Human disease: hemochromatosis type 2B
Hemochromatosis protein	HFE	*HFE*	Regulator of hepcidin	Iron overload Human disease: hemochromatosis type 1
Transferrin receptor 2	TfR2	*TFR2*	Regulator of hepcidin	Iron overload Human disease: hemochromatosis type 3
Hemojuvelin	HJV	*HFE2*	Regulator of hepcidin	Severe iron overload Human disease: hemochromatosis type 2A

[a] Human Genome Organization approved symbol

The Transferrin-TfR1 cycle for cell aquisition of iron

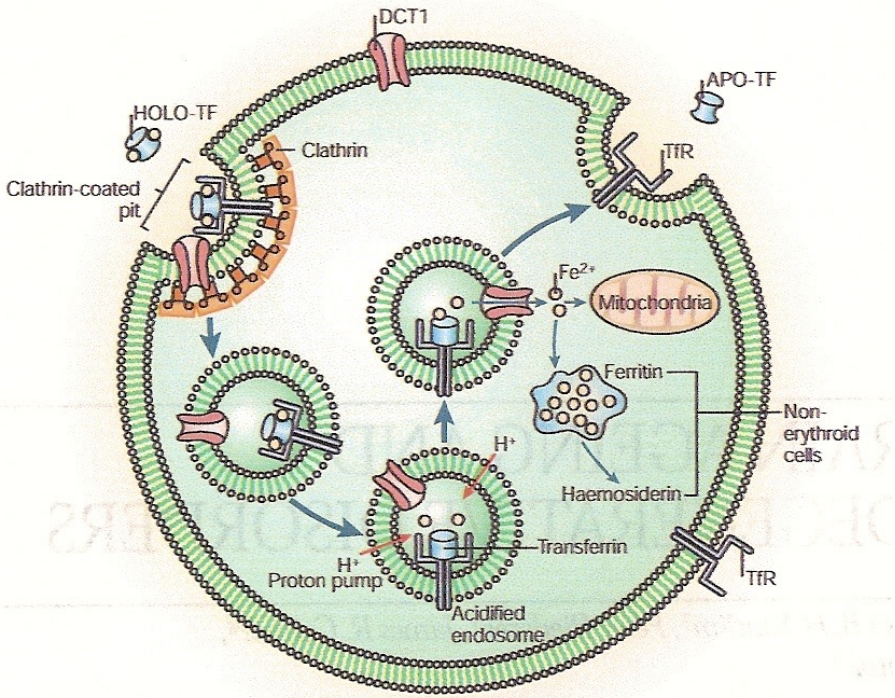
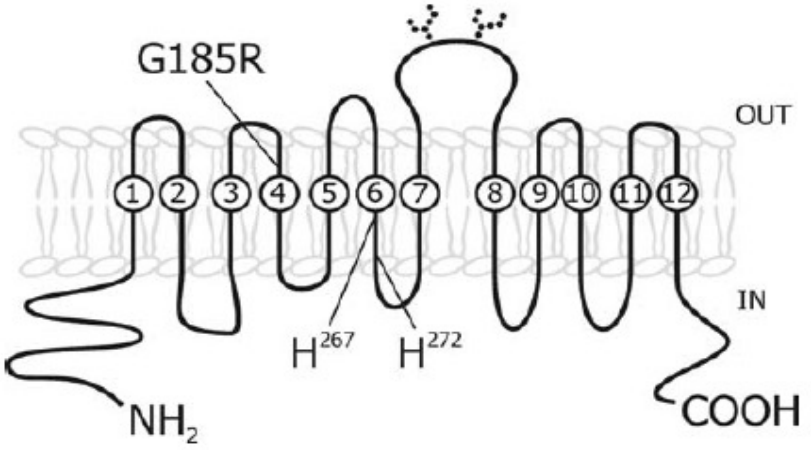


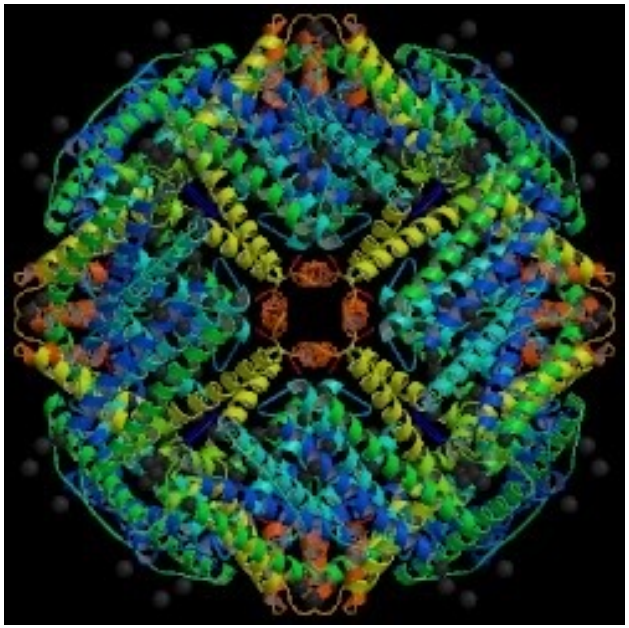
Figure 1 | **The Tf cycle.** Holotransferrin (HOLO-TF) binds to the transferrin receptor (TfR) at the cell surface. These complexes localize to clathrin-coated pits, which invaginate to initiate endocytosis. Specialized endosomes form, which are acidified by a proton pump. When the required acidic pH is reached, iron is released from transferrin (Tf) and is co-transported, with the protons, out of the endosomes by the divalent cation transporter DCT1. Apotransferrin (APO-TF) is returned to the cell membrane bound to TfR, where, at neutral pH, they dissociate to participate in further rounds of iron delivery. The iron can be targeted to the mitochondria. In non-erythroid cells, iron is stored in the form of ferritin and haemosiderin.

Proteins of cellular iron metabolism

- DMT1 co-transports divalent cations and H^+, it is expressed in many tissues and cells (enterocytes, erythroid cells, kidney, lung, brain…).
- It plays a role in **absorption** of iron in duodenum and in the machanism of iron release from transferrin.
- DMT1 expression is induced by iron deficiency and different isoforms of the protein have been identified.

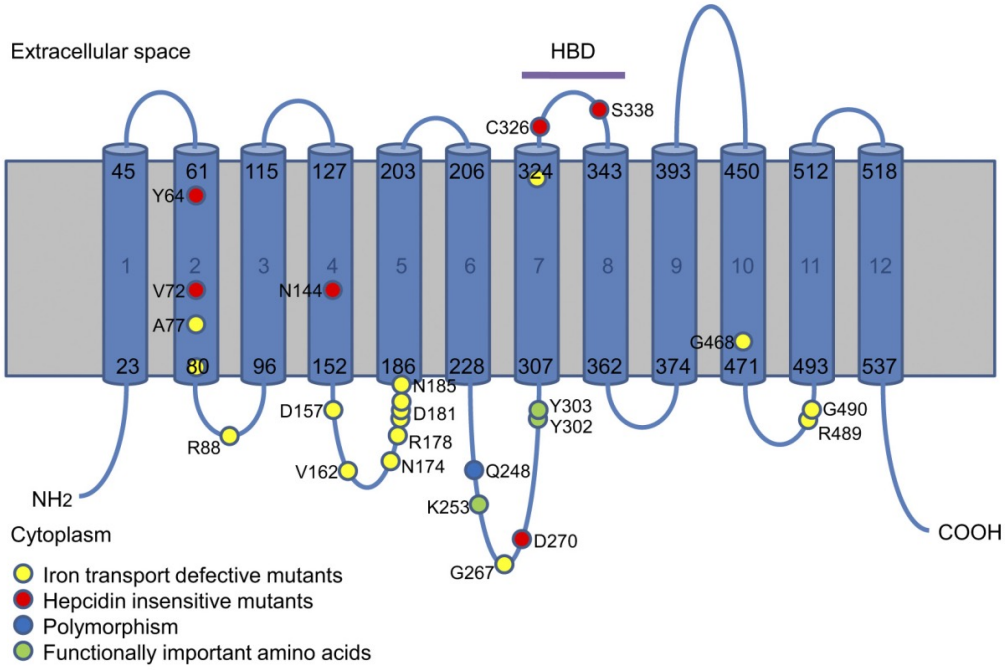


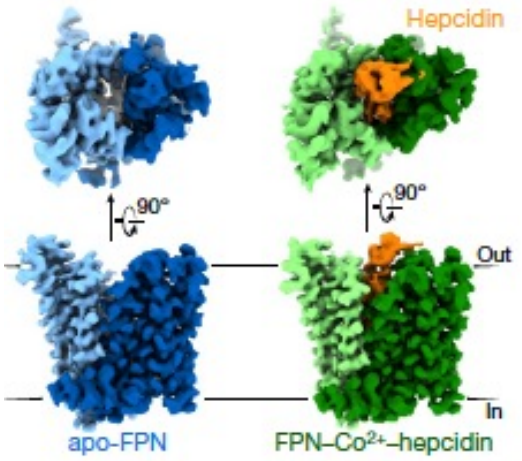
- Ferritin is the major intracellular iron **storage** protein. It is formed by 24 subunits of H- and L-type and it can bind up to 4500 iron atoms.



Export of iron from cells: ferroportin

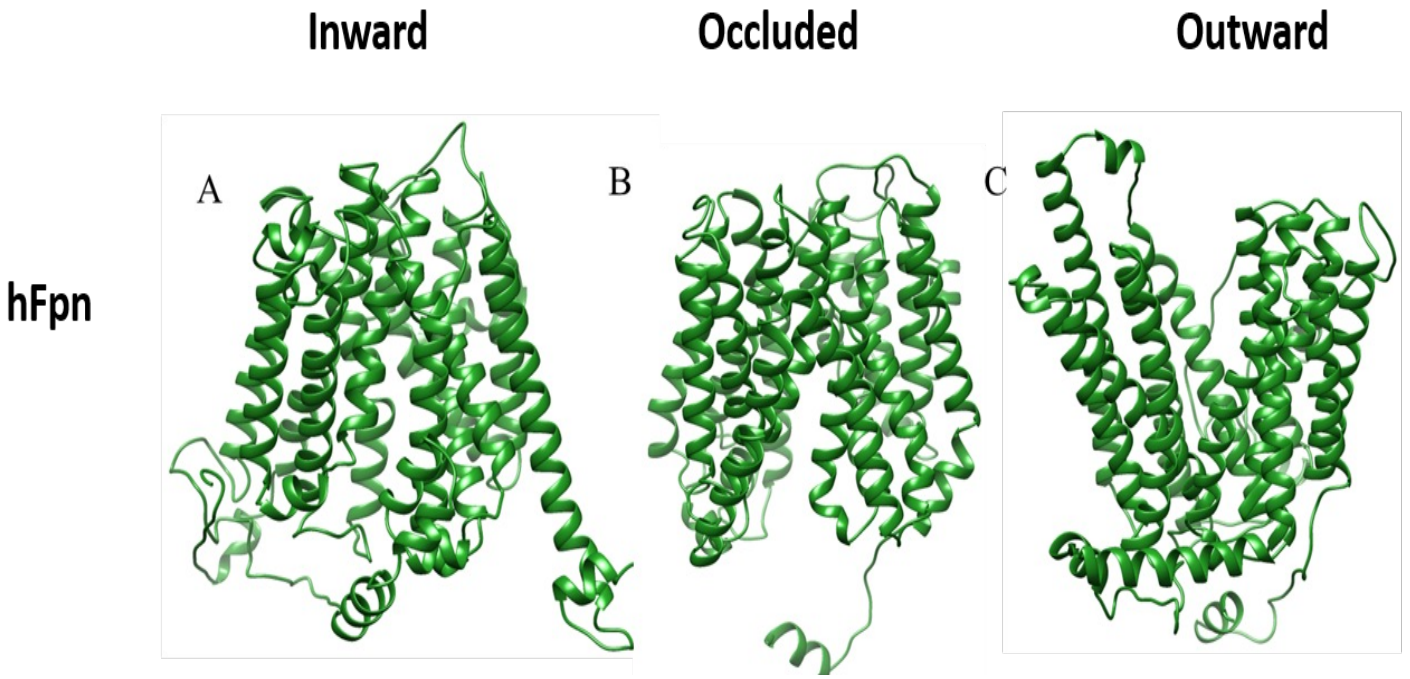
- Ferroportin is the only cellular iron exporter identified to date.
- It is expressed on the basolateral membrane of enterocytes, in macrophages, astrocytes and hepatocytes.
- Ferroportin mutations cause iron overload in liver or in reticuloendothelial macrophages (hemochromatosis type 4).





Export of iron from cells: ferroportin

Ferroportin belongs to the Major Facilitator Superfamily of membrane transporters, that cycle between different conformations during substrate translocation across the membrane.



Ferroxidases: ceruloplasmin and hephaestin

- The ferroxidases ceruloplasmin and hephaestin belong to the family of blue multicopper oxidases (MCO) and catalyze oxidation of Fe^{2+} to Fe^{3+} with reduction of O_2 to H_2O.
- Ceruloplasmin collaborates with ferroportin by oxidizing Fe^{2+} exported and facilitating incorporation into transferrin.
- Hephaestin is a membrane protein expressed mainly in enterocytes where it participates in intestinal iron absorption.

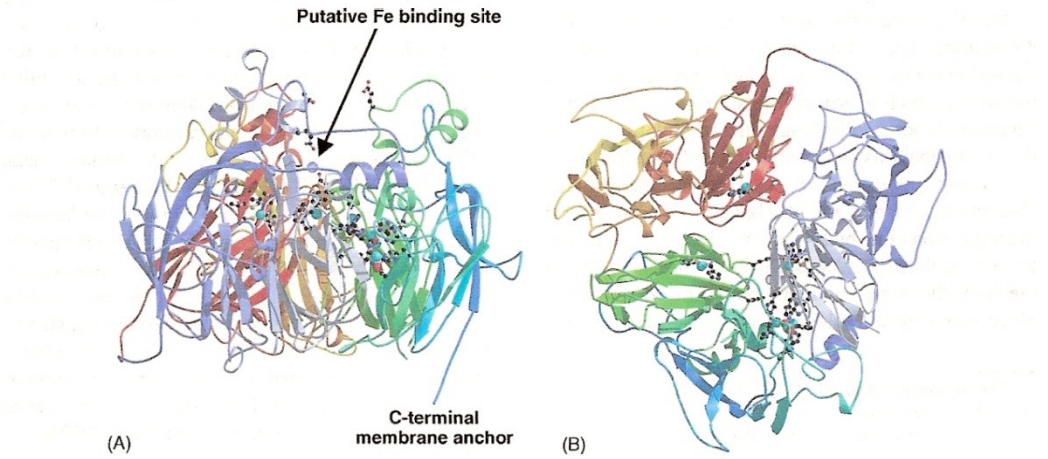
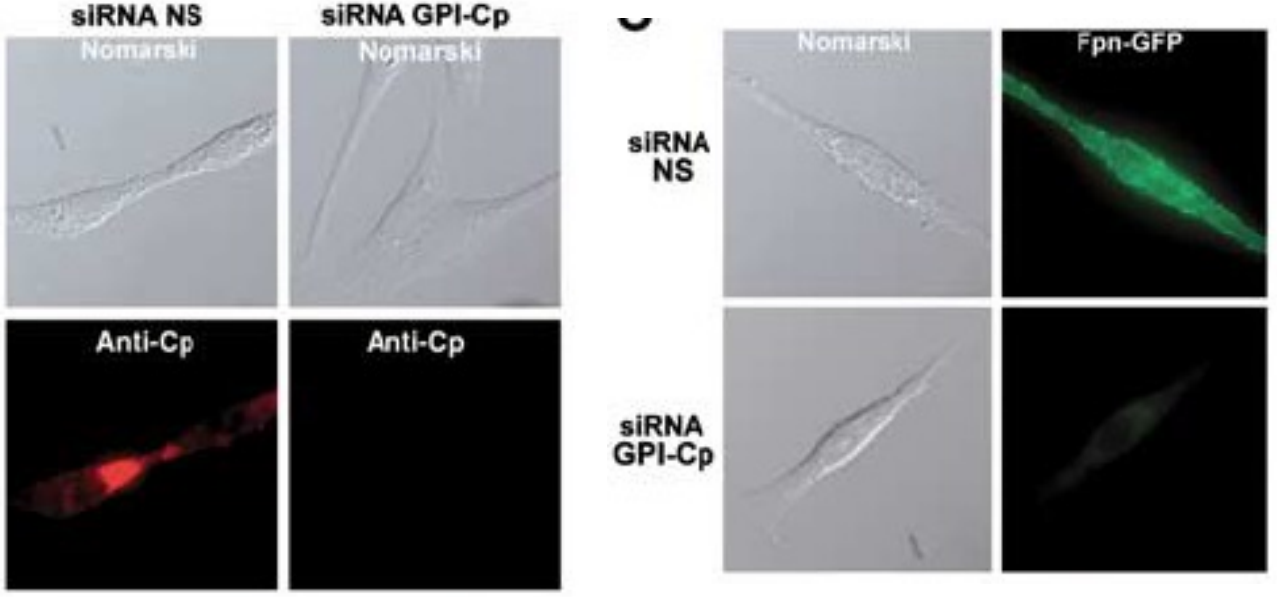


Fig. 1. Predicted molecular structure of hephaestin. Used with permission (Syed et al., 2002). (A) Side view of human hephaestin molecule. (B) Top view of the molecule. The copper atoms are represented as light blue spheres, oxygen atoms are red.

Ferroxidase activity of ceruloplasmin (and hephaestin) is required to stabilize ferroportin at the cell surface

A membrane GPI-ceruloplasmin isoform is expressed mainly in astrocytes. Lack of ceruloplasmin in these cells causes disappearance of ferroportin from the cell surface and this could explain the brain iron overload in patients affected by aceruloplasminemia.



Cellular regulation of iron homeostasis
The IRE/IRP regulatory system.

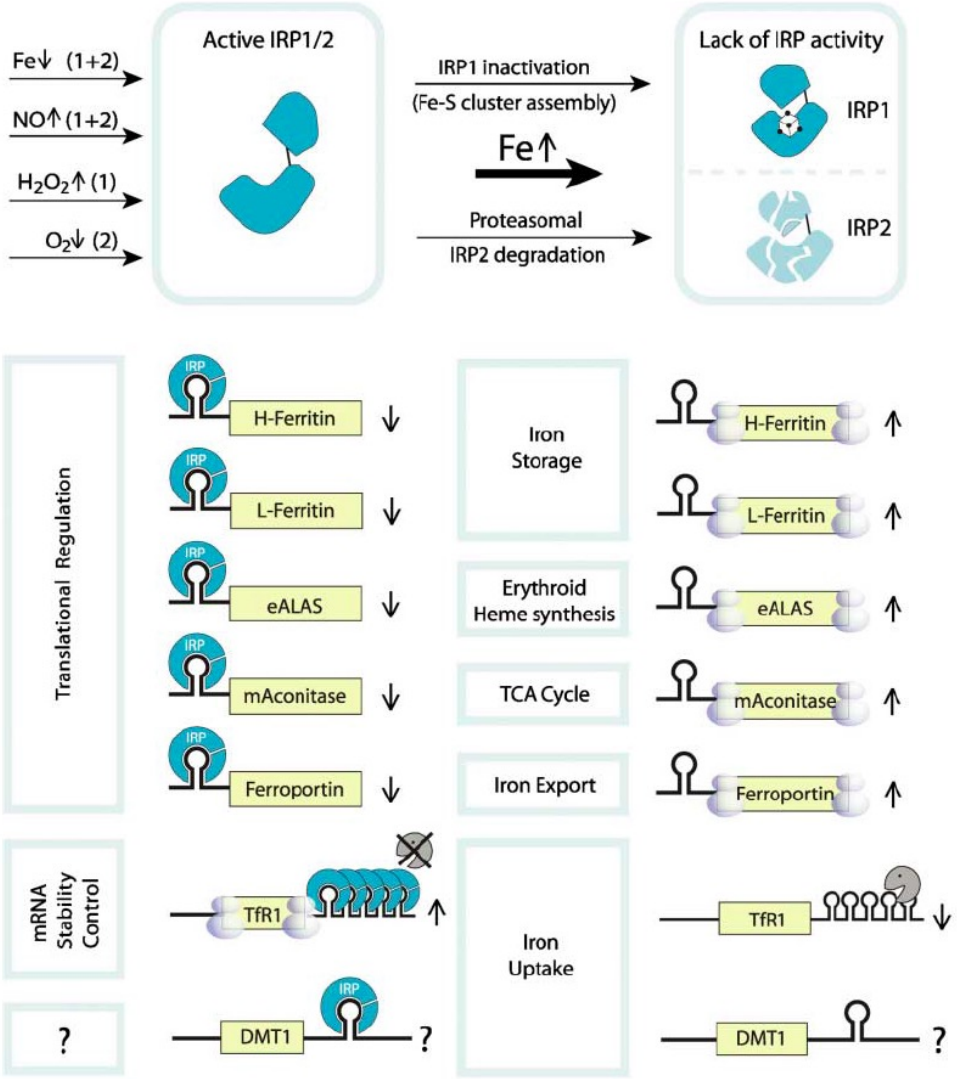


Figure 2. The IRE/IRP Regulatory System

Proteins involved in iron storage, erythroid heme synthesis, the TCA cycle, iron export, and iron uptake are coordinately regulated by the interaction of the iron regulatory proteins (IRPs) with conserved RNA secondary structures, the iron-responsive elements (IREs). The binding of IRPs to single IREs in the 5′-untranslated regions (UTRs) of mRNAs blocks their translation, while IRP binding to multiple IREs in the 3′ UTR stabilizes the TfR-1 mRNA. IRPs exist in two isoforms, IRP1 and IRP2. Increased iron levels favor the conversion of IRP1 from its active RNA binding form into an Fe-S cluster containing cytoplasmic aconitase that lacks IRE binding activity as well as the proteasomal degradation of IRP2. Low iron levels or the action of NO promote accumulation of the active apoprotein form of IRP1 and stabilize IRP2. In contrast, H_2O_2 only activates IRP1, while hypoxia interferes with IRP2 degradation.

Systemic regulation of iron homeostasis mediated by hepcidin

Hepcidin is a 25 amino acid peptide produced by the liver. It binds to ferroportin causing internalization and degradation of the transporter, reducing export of iron from enterocytes and macrophages to the circulation.

Expression of hepcidin is induced by iron excess and inflammation (IL-6), and it is repressed by anemia and hypoxia.

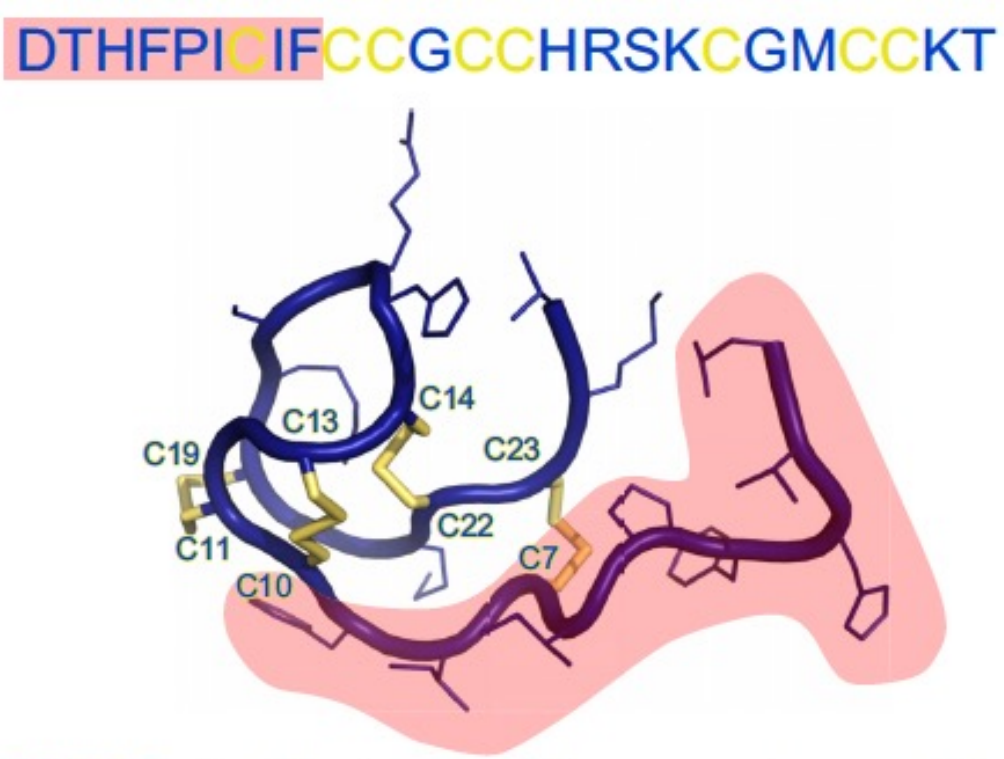


FIGURE 3. Hepcidin: the amino acid sequence and structure. The NH_2-terminal segment known to interact with ferroportin (193) is shaded in light red. The characteristic cysteines and their disulfide bonds are shown in yellow.

Regulation of hepcidin synthesis

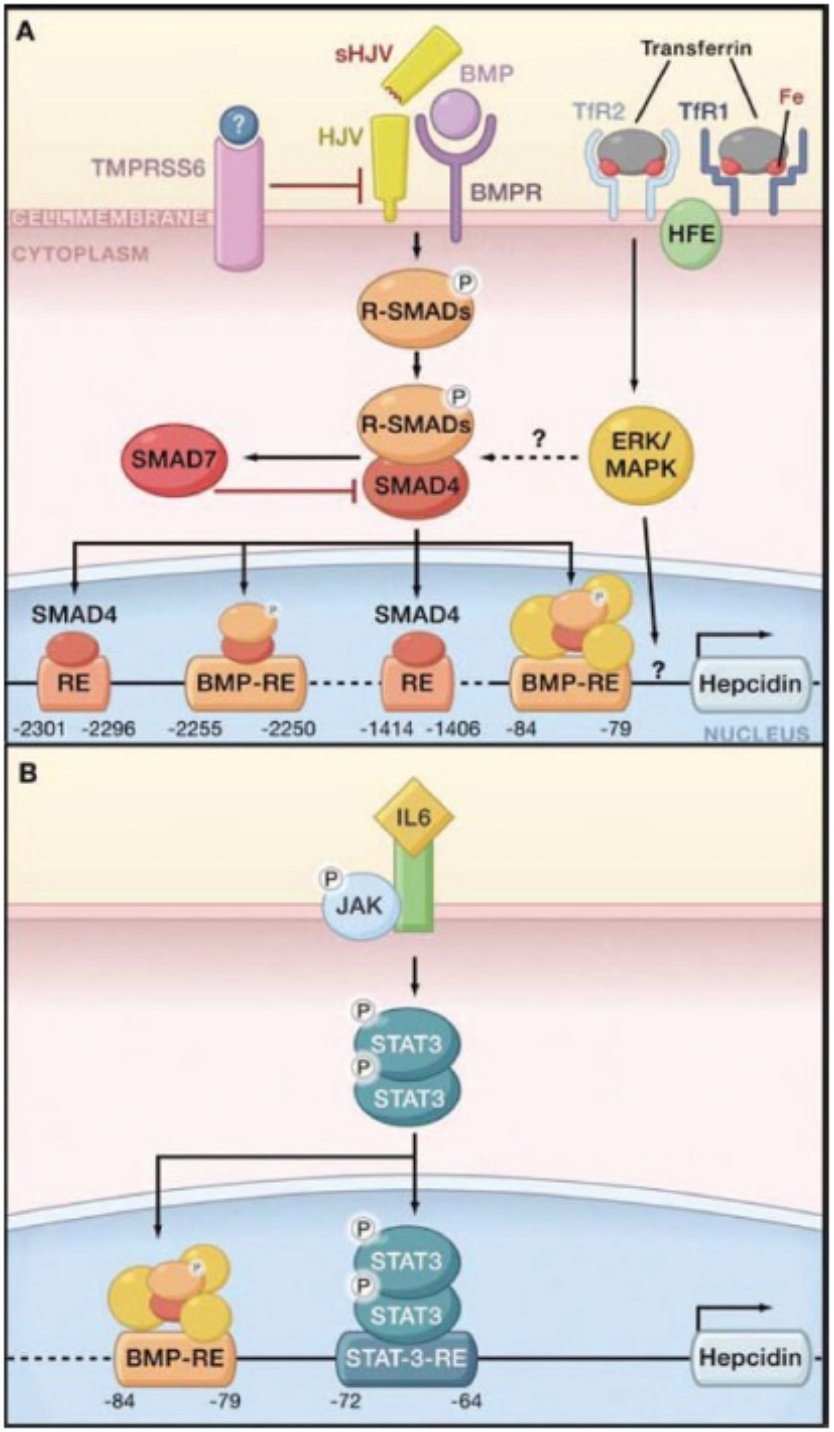


Figure 3. Regulation of hepcidin by iron and inflammation. (A) Hepcidin regulation by iron. Binding of BMP6 to BMP receptor complex on the hepatocyte surface, in the presence of the coreceptor HJV, activates the receptor kinase to phosphorylate SMAD1, SMAD5, and SMAD8 proteins. Phosphorylated SMADs, together with SMAD4, translocate into the nucleus to induce transcription of hepcidin and of other target genes. HFE displaced from TFR1 by high circulating iron binds to TFR2 to activate hepcidin through uncertain mechanisms. TMPRSS6 cleaves HJV from the cell membrane to dampen BMP receptor signaling. Relevant sequence motifs of the hepcidin promoter are shown. (B) IL-6 and other cytokines activate the JAK2 and STAT signaling pathway to activate hepcidin via a STAT-binding motif in the hepcidin promoter. Modified and used with permission from Hentze et al.[1]

Systemic regulation of iron homeostasis

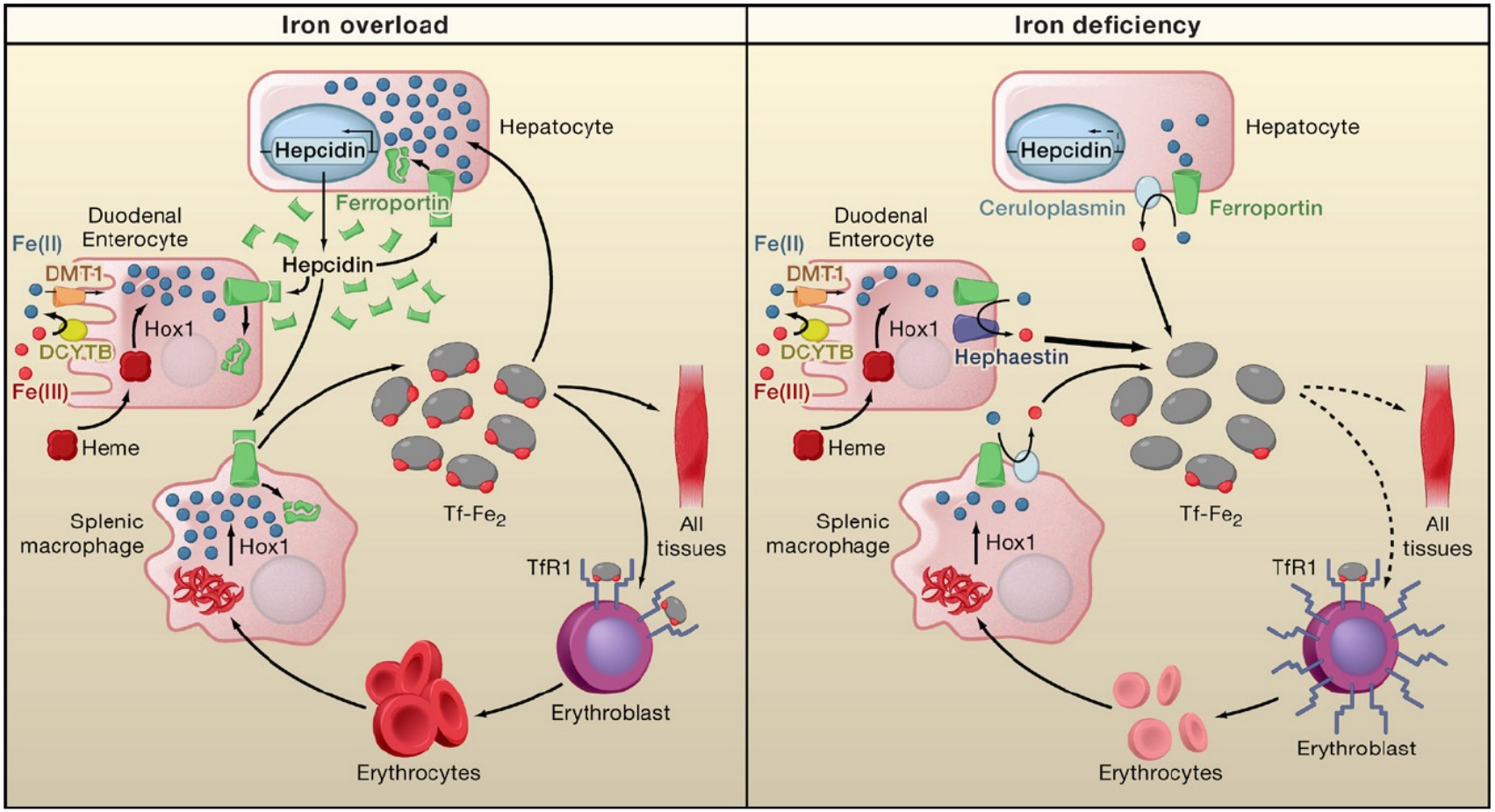


Figure 1. Regulation of Systemic Iron Homeostasis

Cells involved in systemic iron regulation are shown. Divalent metal transporter 1 (DMT1) at the apical membrane of enterocytes takes up iron from the lumen of the duodenum after DCYTB reduces Fe(III) to Fe(II). Ferroportin at the basolateral membrane cooperates with hephaestin that oxidizes Fe(II) to Fe(III). Iron-loaded (diferric) transferrin (Tf-Fe_2), indicated by red dots, supplies iron to all cells by binding to the transferrin receptor 1 (TfR1) and subsequent endocytosis. TfR1 is highly expressed on hemoglobin-synthesizing erythroblasts. Hepatocytes sense transferrin saturation/iron stores and release hepcidin accordingly. Red cell iron is recycled by macrophages via ferroportin and the ferroxidase ceruloplasmin. In iron overload (left), high hepcidin levels inhibit ferroportin-mediated iron export by triggering internalization and degradation of the complex to reduce transferrin saturation. Hepcidin expression is high. In iron deficiency (right), iron is released by ferroportin into the circulation. Hemoglobin-derived heme is catabolized in macrophages by hemoxygenase-1 (HOX1). Hepcidin expression is low.

Copper metabolism

- The mechanisms at the basis of cellular homeostasis of copper are conserved in bacteria (partly), in yeast and in higher eukaryotes.
- Studies in yeast have been particularly useful to identify and/or characterize structurally and functionally homologous human proteins by complementation of defective strains.
- Copper metabolism in eukaryotes is tightly linked with iron metabolism through the Cu-dependent ferroxidases: defects in copper incorporation in ferroxidases will lead to iron dysmetabolism.

Copper resistance in *Escherichia coli*

- *E. coli* does not possess cytosolic cuproproteins so it does not need transport systems for delivery of copper to the cytosol
- Copper is exported to the periplasm and outside the cell by the *cue* and *cus* (chromosomal) and *pco* (plasmidic) systems.
- *cue (CopA, CueO), cus (CusCFBA)* and *pco (PcoABCDE)* operons are induced at different concentrations of copper by transcription factors CueR, CusR and PcoR.

Detoxification of copper in the periplasm in *Escherichia coli*

Mechanism of homeostasis	Regulated by	Function
CopA Cu export pump	CueR (Cu^+ sensor in the cytosol)	Detoxification Cu in the cytosol
CusCFBA Cu export	CusRS (Cu^+ sensor in the periplasm)	Detoxification Cu in the periplasm
CueO Cu oxidase	CueR (Cu^+ sensor in the cytosol)	Protection of periplasmatic proteins
PcoABCD Cu export	PcoRS (Cu^+ sensor in the periplasm)	Protection of the periplasm from elevated Cu stress
PcoE	CusRS and PcoRS	Cu chaperone in the periplasm

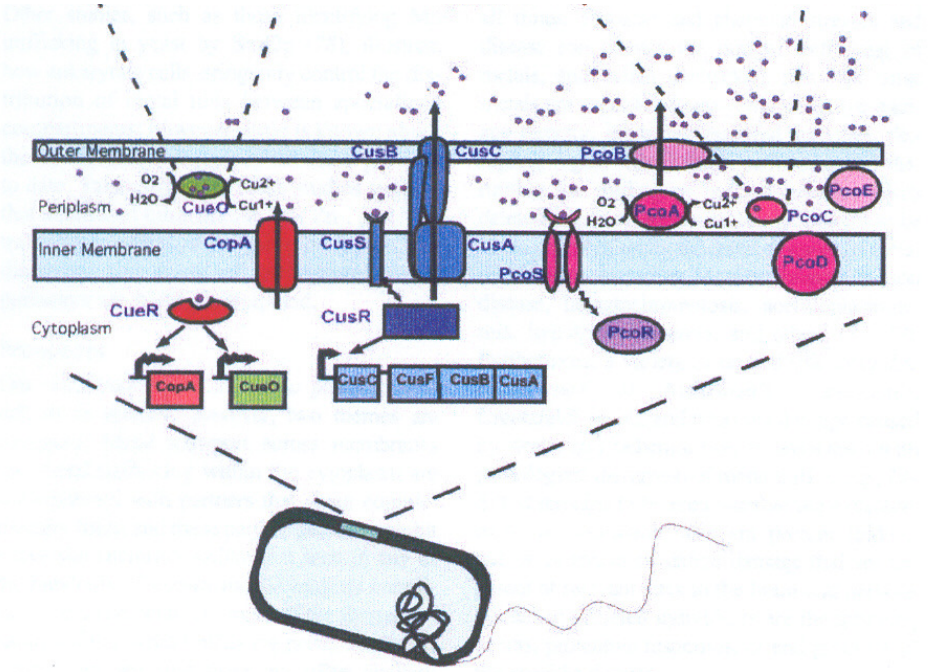
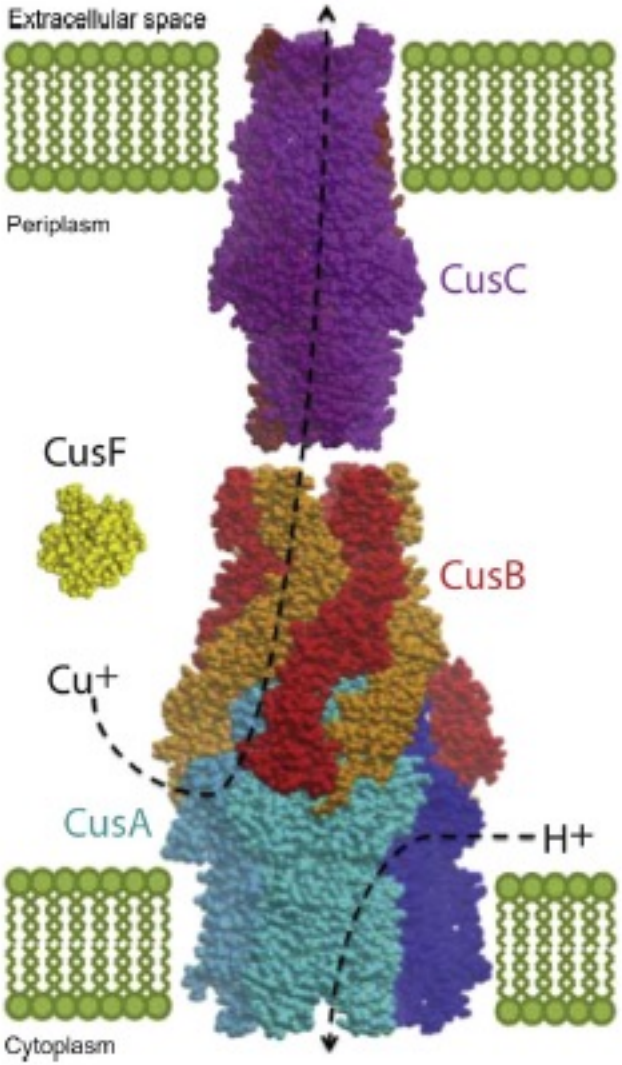
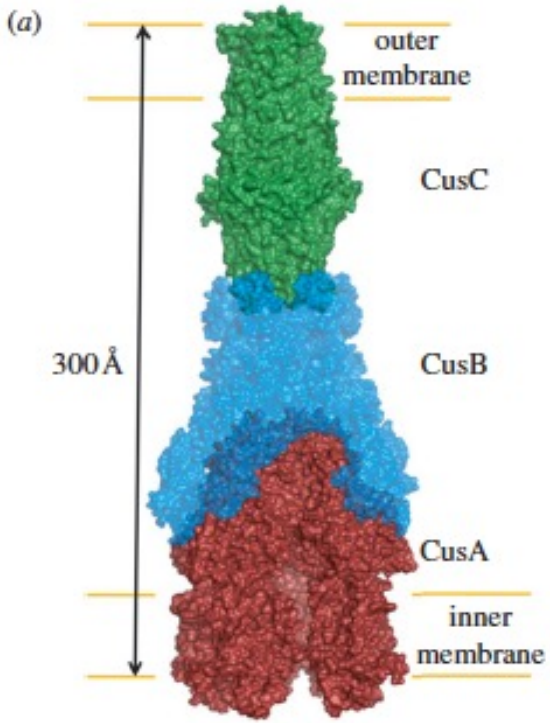
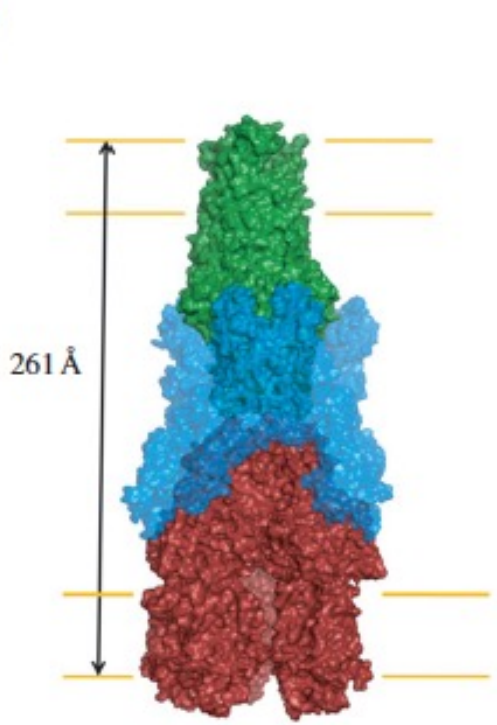


Fig. 2. Copper trafficking in the periplasm. The periplasm, a compartment of the cell envelope of Gram-negative bacteria, is proving to be an important site of Cu trafficking and utilization. Cellular Cu efflux is controlled in *E. coli* by the *cue*, *cus*, and *pco* operons, each of which is induced at different levels of Cu stress by separate metalloregulatory proteins. Recent structural insights for CueO and PcoC are highlighted. The cartoons of Cu ions (purple balls) represent various levels of total Cu content in the periplasm.

Structure of the $cusC_3B_6A_3$ system for export of copper







Copper transport in *Enterococcus hirae*

- Copper resistance operon

copY – copZ – copA – copB

- copY regulates transcription of the operon, transcriptional repressor binds the promoter in the absence of copper
- copZ intracellular copper transporter, transfers the metal to cop Y
- copA protein of 727 aa with ATPase activity responsible for copper uptake in the cell
- copB protein of 745 aa with ATPase activity responsible for copper export from the cell

- Determination of the role of copA and copB by studies with strains with inactivated copA and copB genes
- Characterization of transport activity of the proteins by competition with other metals (Ag, Cd), use of inhibitors (vanadate) and use of radioactive isotopes

Copper homeostasis in *Enterococcus hirae*

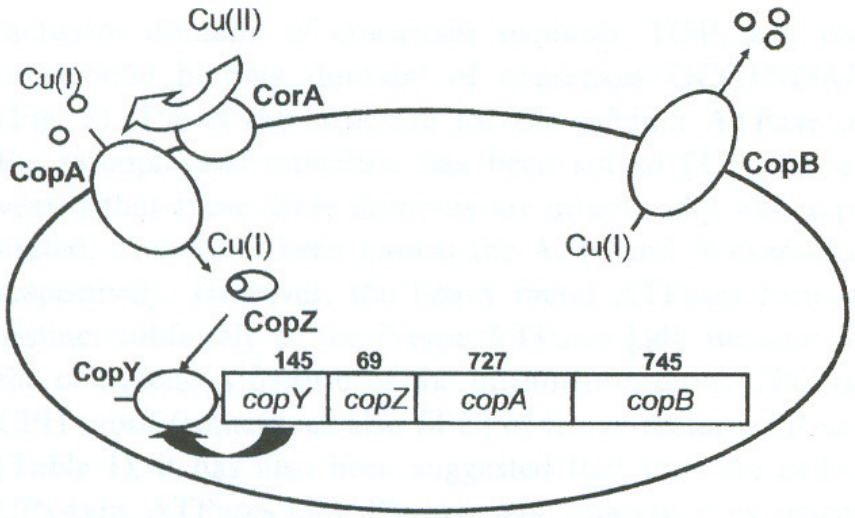


Fig. 1. Model of the *cop* operon and copper homeostasis in *E. hirae*. The extracellular reductase CorA supplies copper(I) for uptake by CopA. Inside the cell, copper is routed from the CopA ATPase to the CopY repressor by the CopZ copper chaperone. The copper form of CopY is released from the promoter, thereby allowing expression of the four *cop* genes. In the case of excess copper, CopZ may deliver copper to the CopB copper export ATPase. The numbers indicate the number of amino acids of the respective proteins.

Copper transport in eukaryotic cell. The membrane transporter Ctr1

- Ctr1 is the main Cu^{+} transporter in the cell (Km 1-5 μM).
- Ctr1 has three transmembrane domains and various 'Met' ($MX_{1-3}M$) sequences necessary for Cu^{+} binding.
- Functional complementation in *ctr1ctr3Δ* yeast strains allowed to isolate human Ctr1. The importance of Ctr1 is demonstrated by the finding that Ctr1 knock-out is embryonically lethal in mice.
- Ctr1 expression is transcriptionally regulated by Mac1 in yeast and post-translationally by copper-mediated endocytosis and degradation (in yeast and humans).

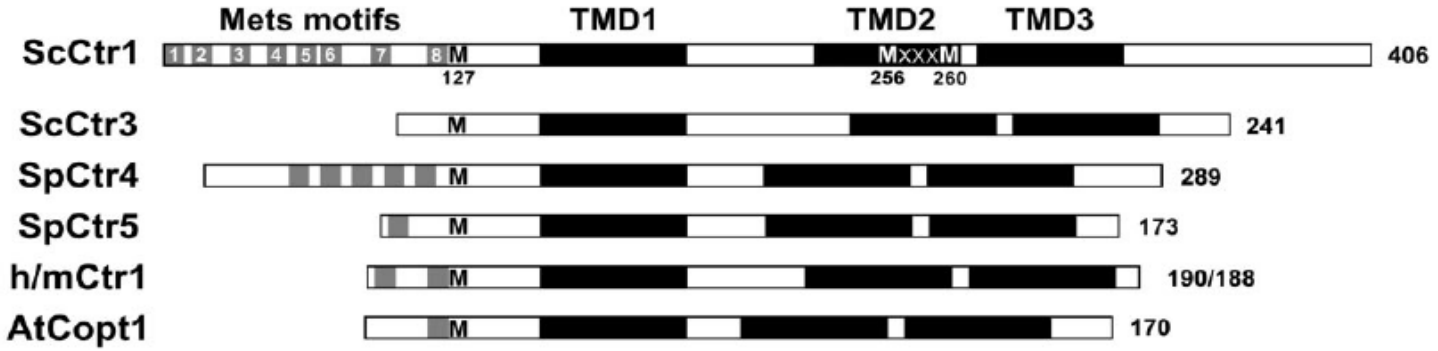
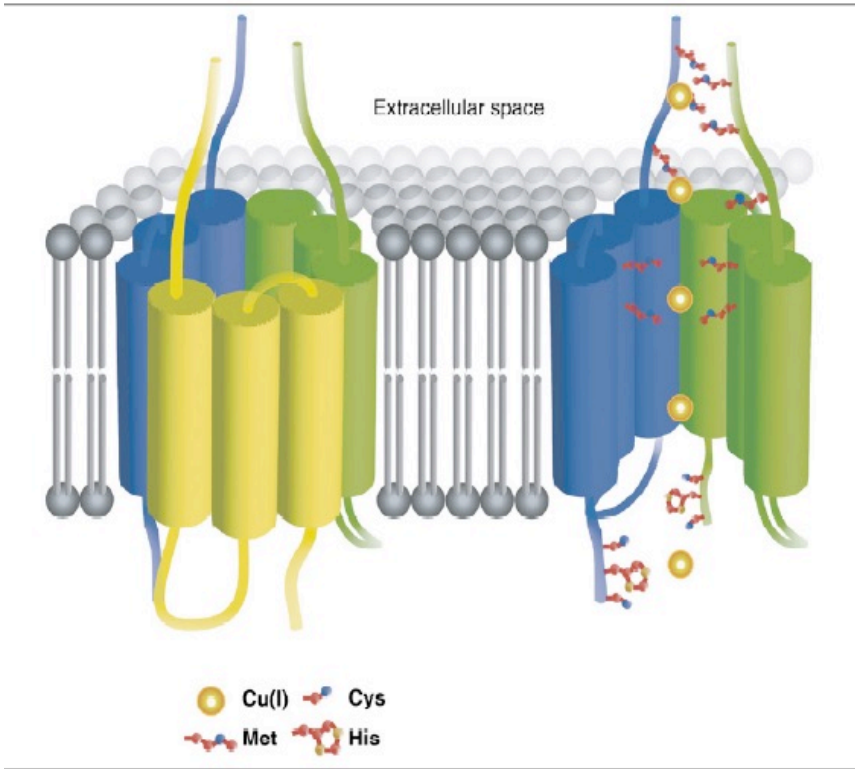
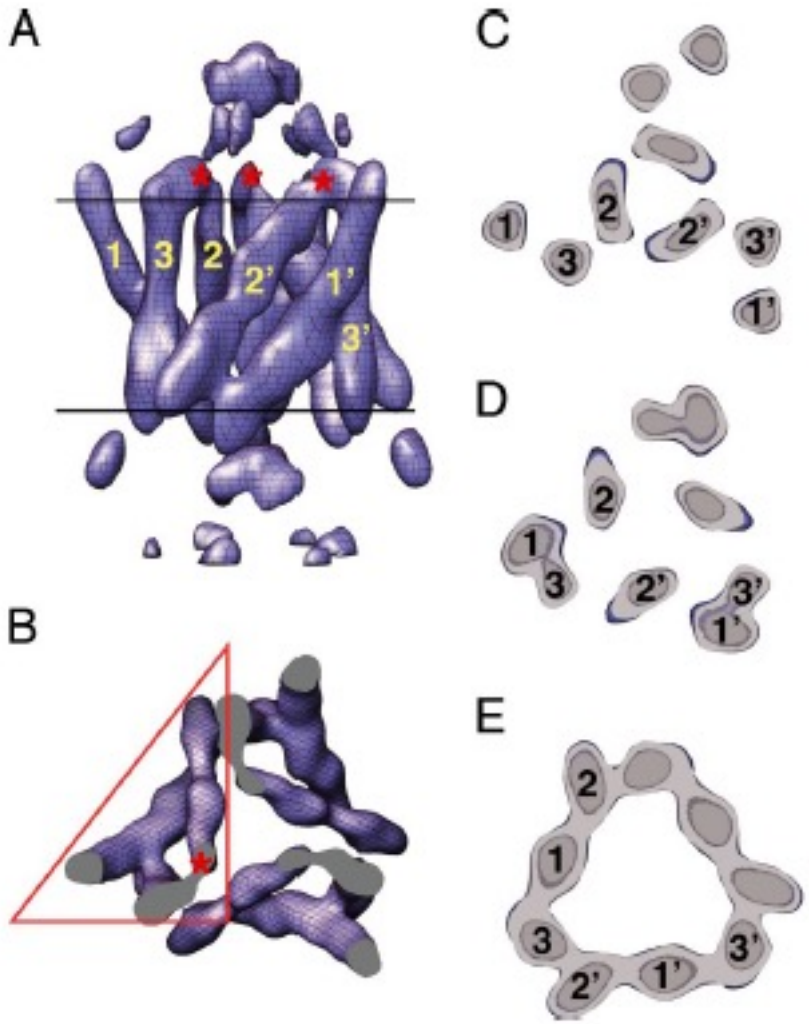


FIG. 1. **Model for the primary structure of Ctr high affinity copper transport proteins.** Alignment of copper transport proteins from *S. cerevisiae* (ScCtr1, ScCtr3), *S. pombe* (SpCtr4, SpCtr5), human and mouse (h/mCtr1), and *A. thaliana* (AtCopt1). Conserved features in the primary structure are represented from the amino terminus (*left*) to carboxyl terminus (*right*). All proteins contain three putative transmembrane domains (TMD1–3) shown in *black*. With the exception of yeast Ctr3, all members of the Ctr family of copper transporters contain putative copper binding motifs, called Mets motifs (*gray boxes*), consisting of 3–5 methionine residues arranged as M*X*X*M* and/or M*X*M. Yeast and human Ctr1 proteins contain eight and two Mets motifs, respectively. Other conserved features, Ctr1 Met-127 and the M*XXX*M motif in TMD2, are represented in *black* and *white* characters, respectively. The length of each protein in amino acids is shown on the *right*.

Trimeric structure of the transporter Ctr1.



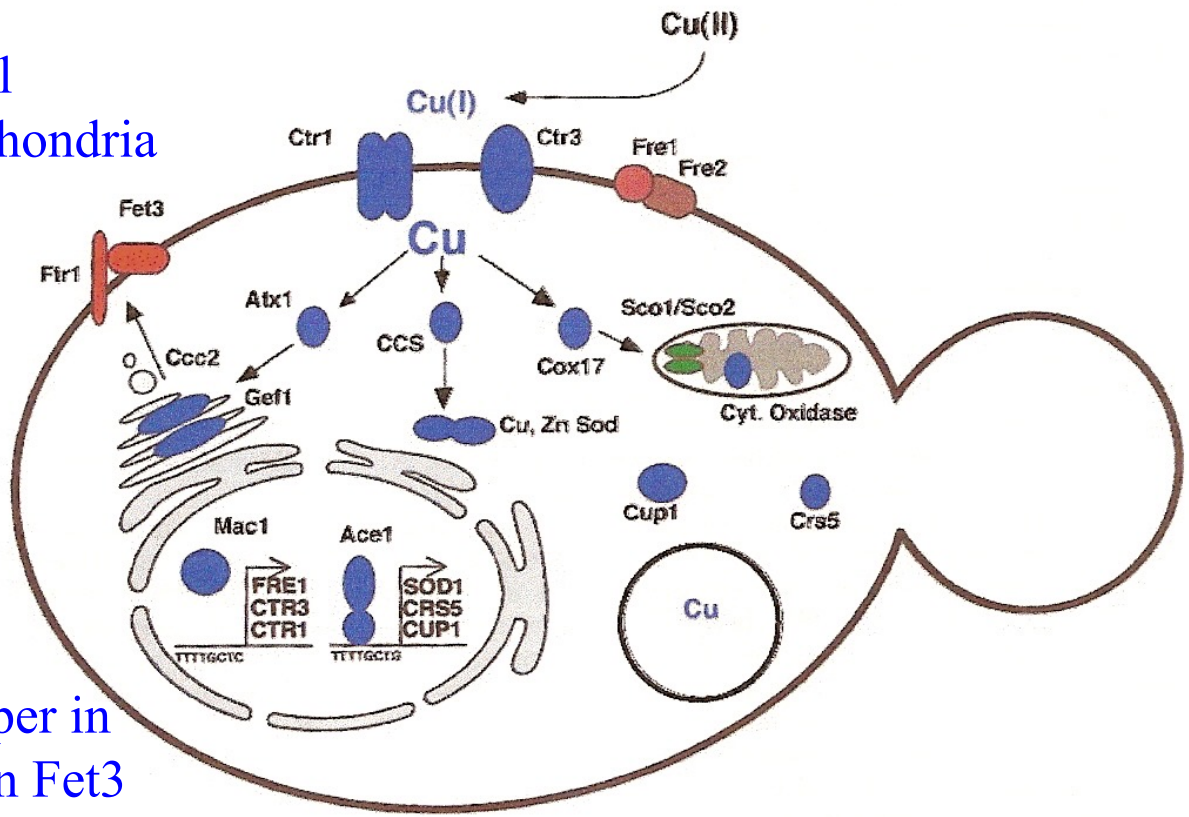


Intracellular copper transport in yeast. Chaperones and pumps for metal delivery

Cuproproteins
- Cu,ZnSOD and MT cytosol
- Cytochrome oxidase mitochondria
- Fet3 plasma membrane

Copper chaperones
- CCS cytosol
- Cox17 mitochondria
- Atx1 Golgi

Copper pumps
ATPase Ccc2 transports copper in the Golgi for incorporation in Fet3



Intracellular copper transport in hepatocytes and neurons. Chaperones and pumps for metal delivery

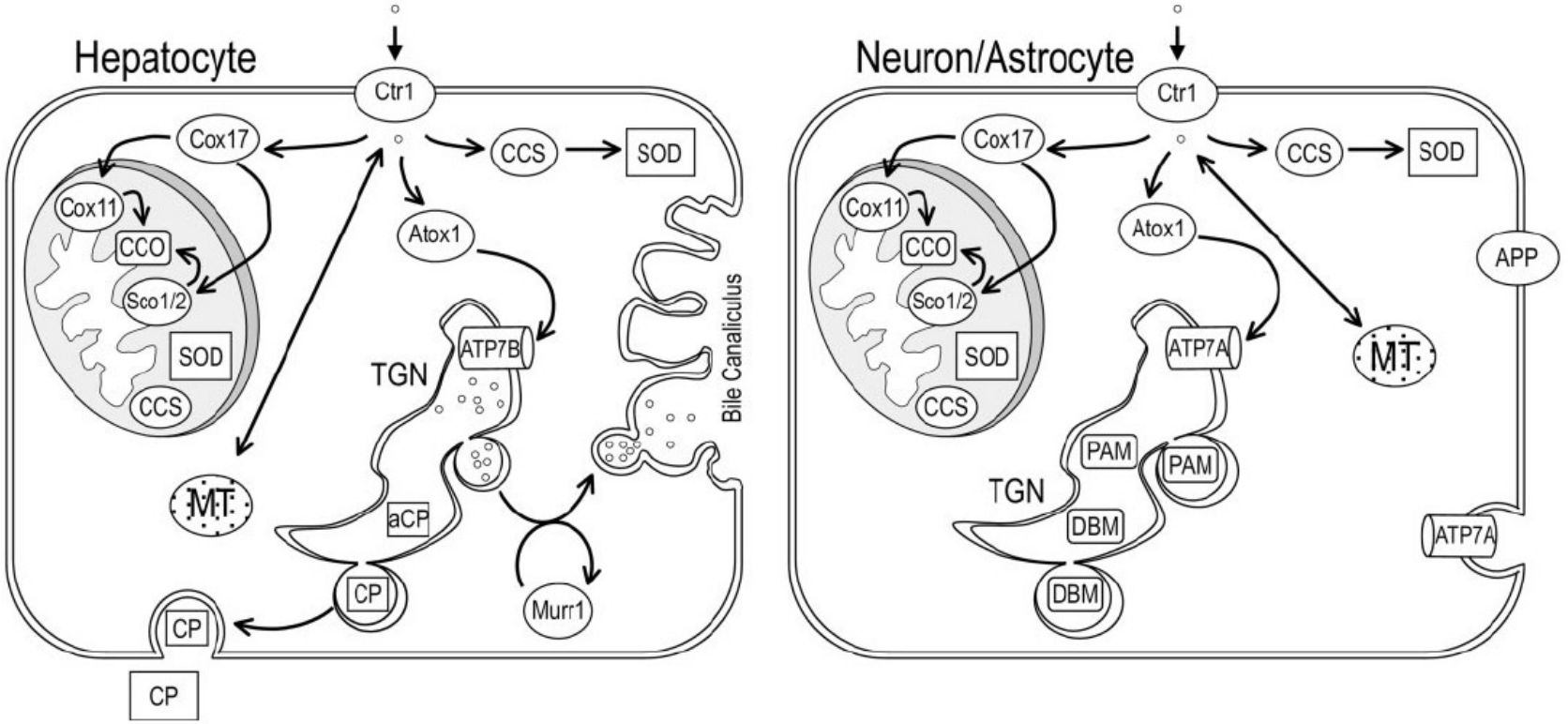


FIGURE 1 Copper transport in hepatocytes, neurons, and astrocytes. Cuproenzymes (rectangles) are dependent on copper chaperones (ellipses) and copper-transporting ATPases (ATP7A and ATP7B) for the delivery of imported copper to biosynthesis and metal-transfer sites. Chaperones and ATPases, which normally reside in the transgolgi network (TGN), are also necessary for the transport of copper to the bile (hepatocytes) or the plasma membrane for copper efflux from the cell (neurons and astrocytes).

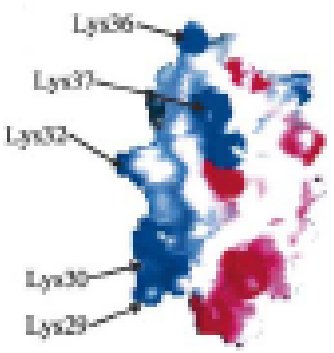
Enzymes that receive copper in the Golgi

Table 1 Mammalian enzymes, which receive Cu in the Golgi, and their suggestive relationship with symptoms in Menkes or Wilson disease

Enzymes	Biological activity	Symptoms
Dopamine β-hydroxylase	Catecholamine production	Ataxia, Hypothermia, Hypotension
Peptidylglycine α-amidating monooxygenase	Activation of peptide hormones	Widespread aberrations in nervous and endocrine system
Lysyl oxidase	Collagen and elastin cross-linking	Loose skin and joints, Emphysema, Hernias, Bladder diverticula, Arterial aneurysms, Loose skin and joints, Osteoporosis, Petechial hemorrhage, Poor wound healing
Tyrosinase	Pigment formation	Hypopigmentation
Ceruloplasmin	Iron and copper transport	Anemia
Hephaestin	Iron transport	Anemia
Peptidylglycine α-amidating monooxygenase	Activation of peptide hormones	Widespread aberrations in nervous and endocrine system
Sulfhydryl oxidase	Cross-linking of keratin	Abnormal hair, Dry skin

Copper chaperones

Copper chaperones are small proteins (about 70-80 amino acids) conserved from bacteria to humans. Copper is bound as Cu^{+} by two cysteine residues with an atypical coordination.



CopZ

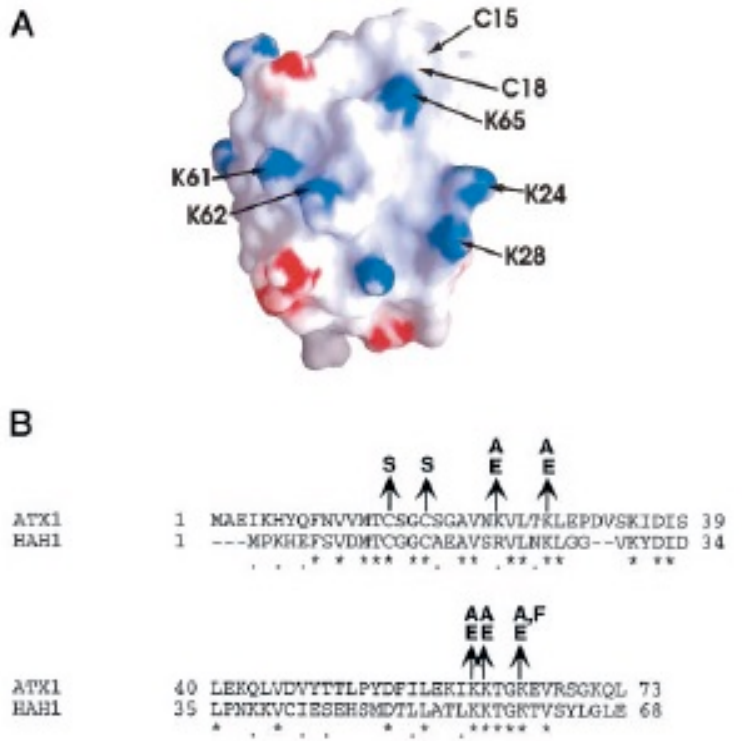


FIG. 1. **X-ray crystal structure and sequence alignment of Atx1p.** *A*, An electrostatic surface representation of the Hg(II) form of Atx1p with residues selected for mutagenesis indicated. The positively, negatively charged and neutral amino acids are represented in blue, red and white, respectively. *B*, amino acid alignment of Atx1p and HAH1. Stars indicate amino acid identity, dots indicate amino acid similarity and arrows designate mutational substitutions.

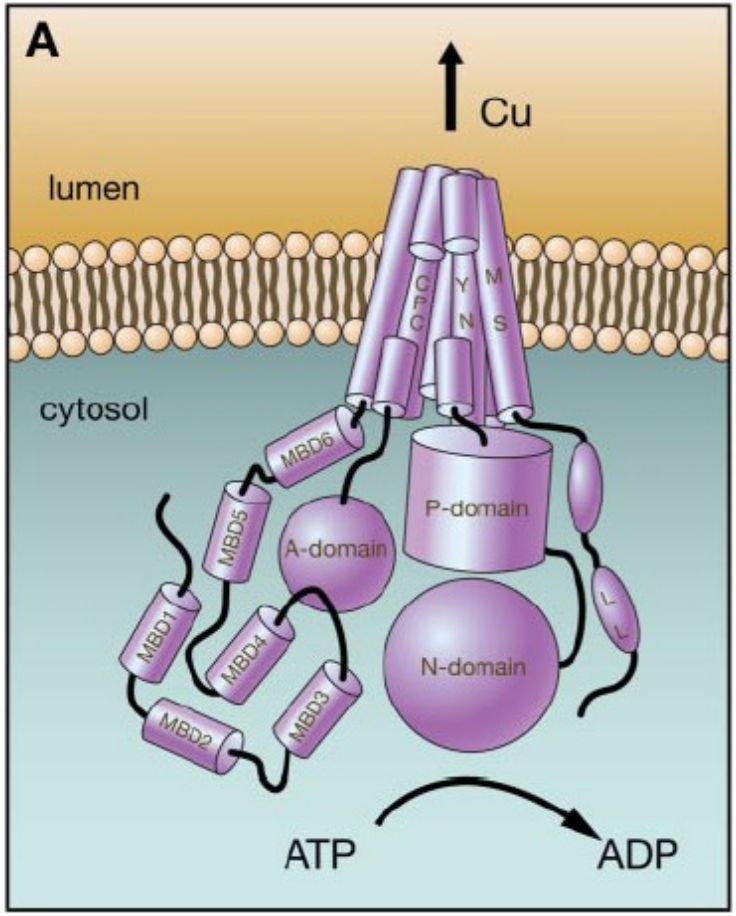
Copper transport ATPases

Copper transport pumps belong to the family of P-type or CPx ATPase and are found in bacteria, yeast and higher eukaryotes. They couple metal trasport tc ATP hydrolysis forming an acyl-phosphate intermediate on an aspartate residue.

They present 8 transmembrane regions and an N-terminal region for copper binding in domains with conserved MXCXXC sequences.

In the P domain and in the N domain aspartate phosphorylation and nucleotide binding take place. In the A domain aspartate is dephosphorylated.

Bacteria	CopA, copB
Yeast	Ccc2
Mammals	ATP7A, ATP7B



Mechanism of copper transport ATPases

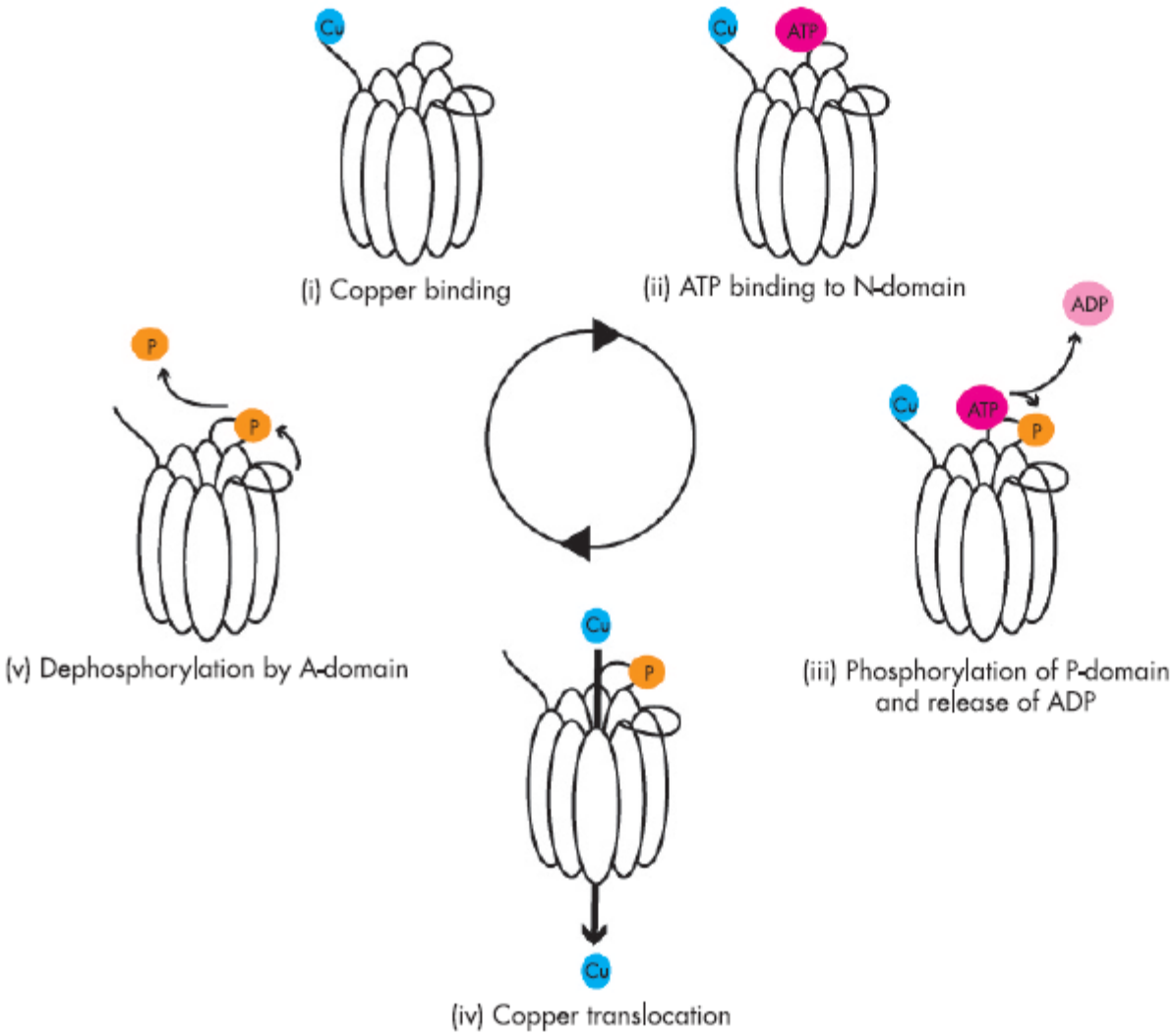
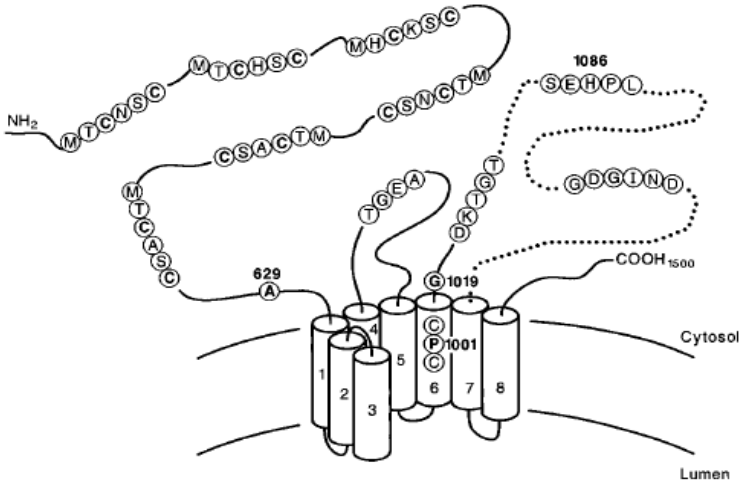


Figure 1 Schematic representation of the general ATPase catalytic cycle. Copper translocation by ATP7A and ATP7B is believed to occur through a general cycling model involving several discrete stages. These stages include (i) binding of the target ion, (ii) binding of ATP to the N-domain, (iii) ATP hydrolysis and phosphorylation of the P-domain, (iv) translocation of the target ion, and (v) dephosphorylation of the P-domain by the A-domain.

Efflux of copper from cells: Menkes (ATP7A) and Wilson (ATP7B) proteins

- ATP7A contains six copper binding domains
- It is localized in the trans-Golgi and it is expressed in enterocytes, in the endothelium of the blood-brain barrier and many other tissues.



- ATP7B contains six copper binding domains
- It is localized in the trans-Golgi and it is expressed in the liver and at lower levels in the kidney, placenta, brain and heart.

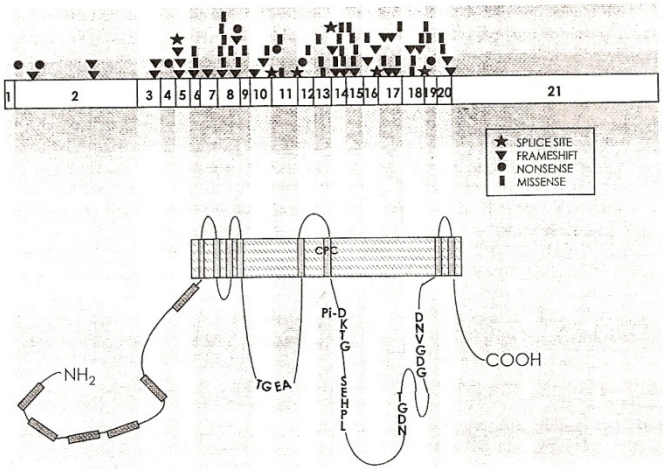


Figure 3 Summary of all published WD mutations in exonic and splice-site-junction sequences (Bull et al. 1993; Tanzi et al. 1993; Figus et al. 1995; Thomas et al. 1995; Loudianos et al. 1996; Waldenström et al. 1996; Kemppainen et al. 1997).

Subcellular localization of ATP7A and ATP7B changes as a function of copper concentration

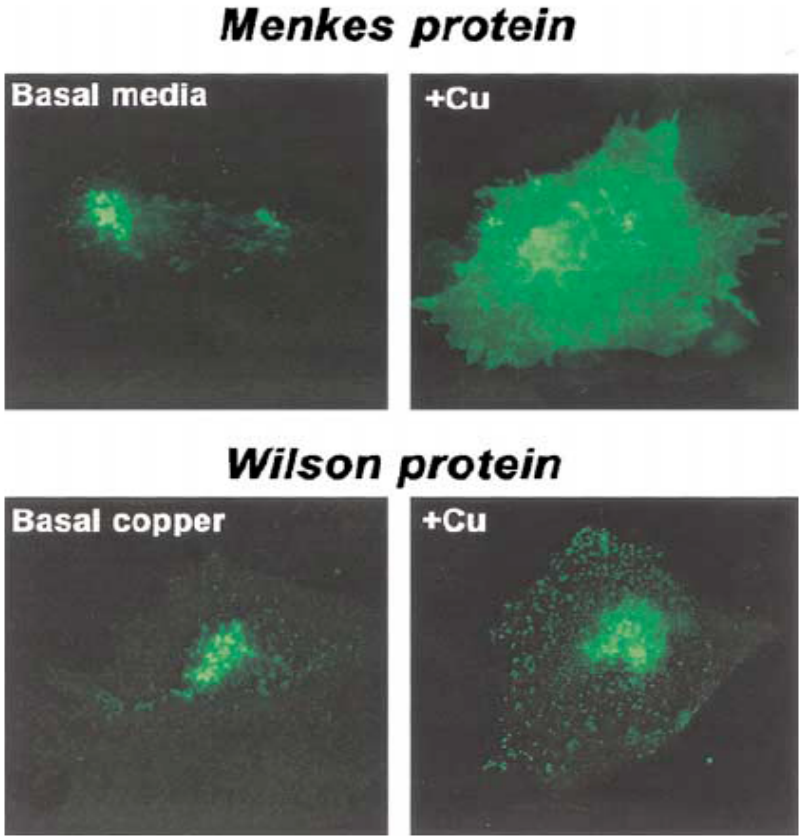
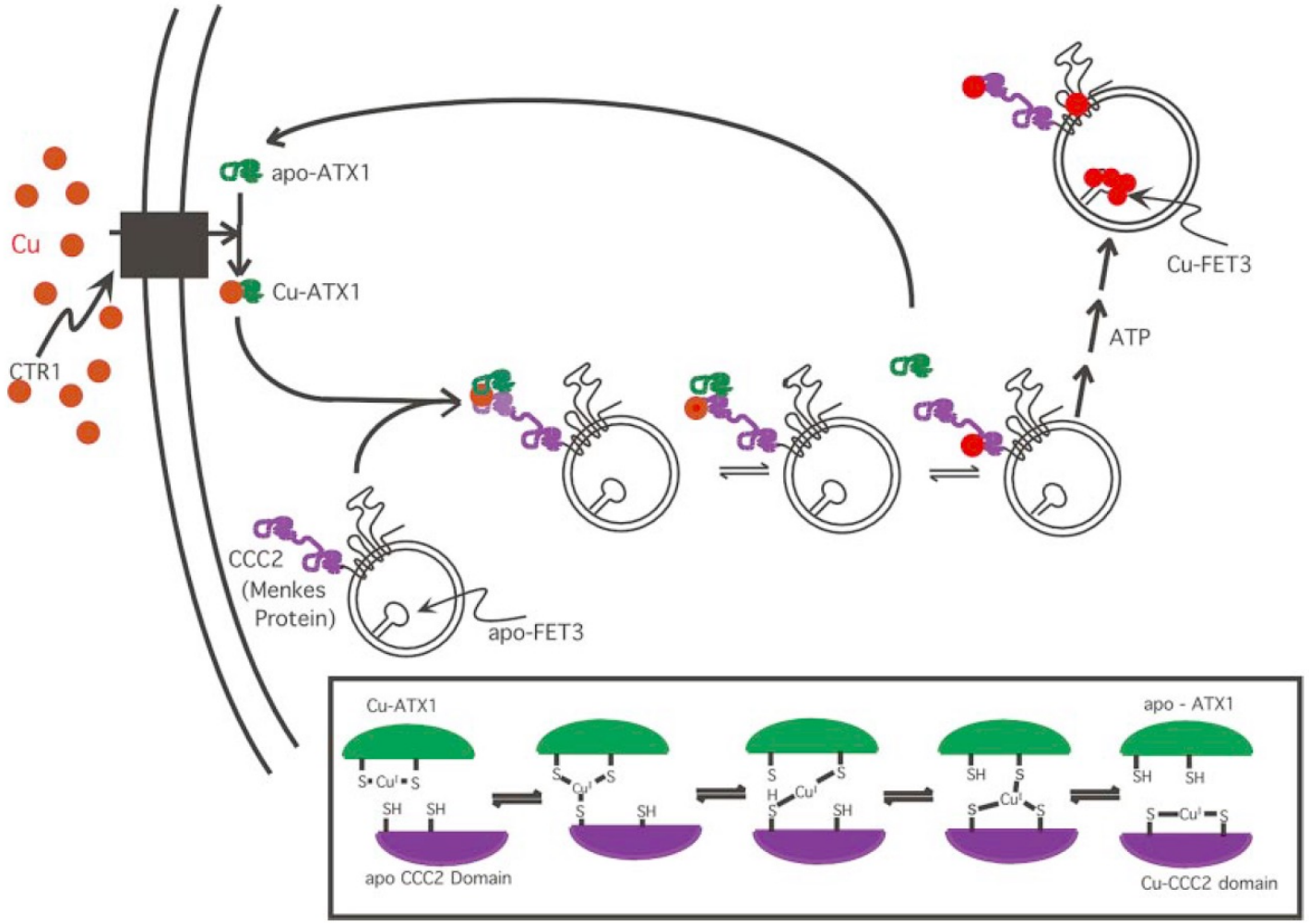


Fig. 5. Copper-dependent localization of the MNKP and WNDP. Immunostaining with specific antibody and fluorescence microscopy demonstrates typical perinuclear localization of MNKP and WNDP in the trans-Golgi network of CHO cells cultured in basal minimal media (*left photos*). Under elevated copper conditions, 2 hr 100 μM $CuCl_2$ (+*Cu*), both proteins traffic to post-TGN compartments. MNKP relocalizes to the plasma membrane (*top right*), whereas WNDP is recruited to the vesicles (*bottom right*).

Copper chaperones and pumps. Mechanism for metal transfer



Chaperones and pumps have similar structures

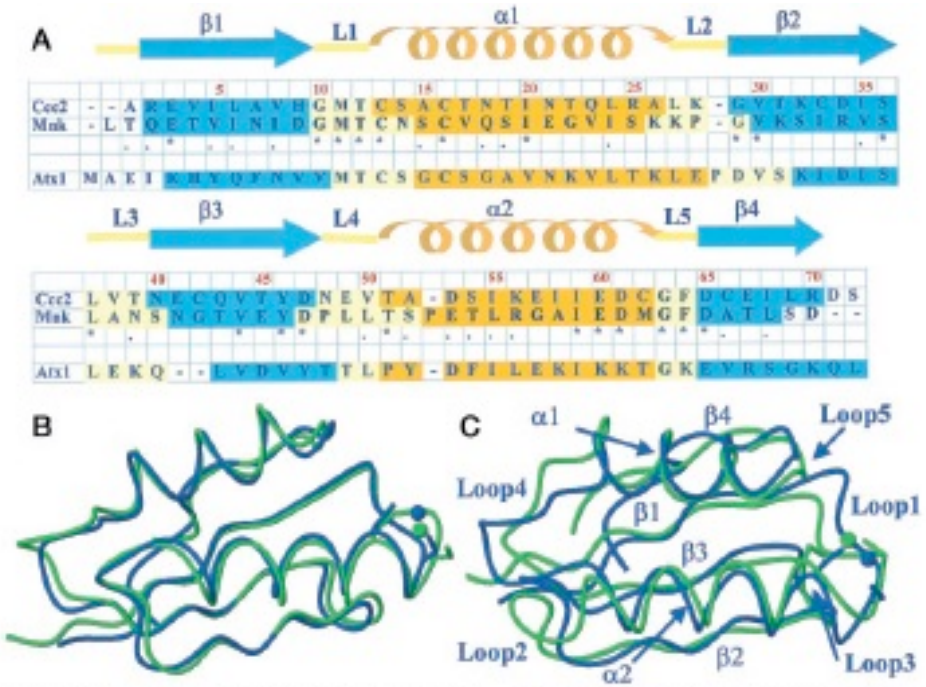
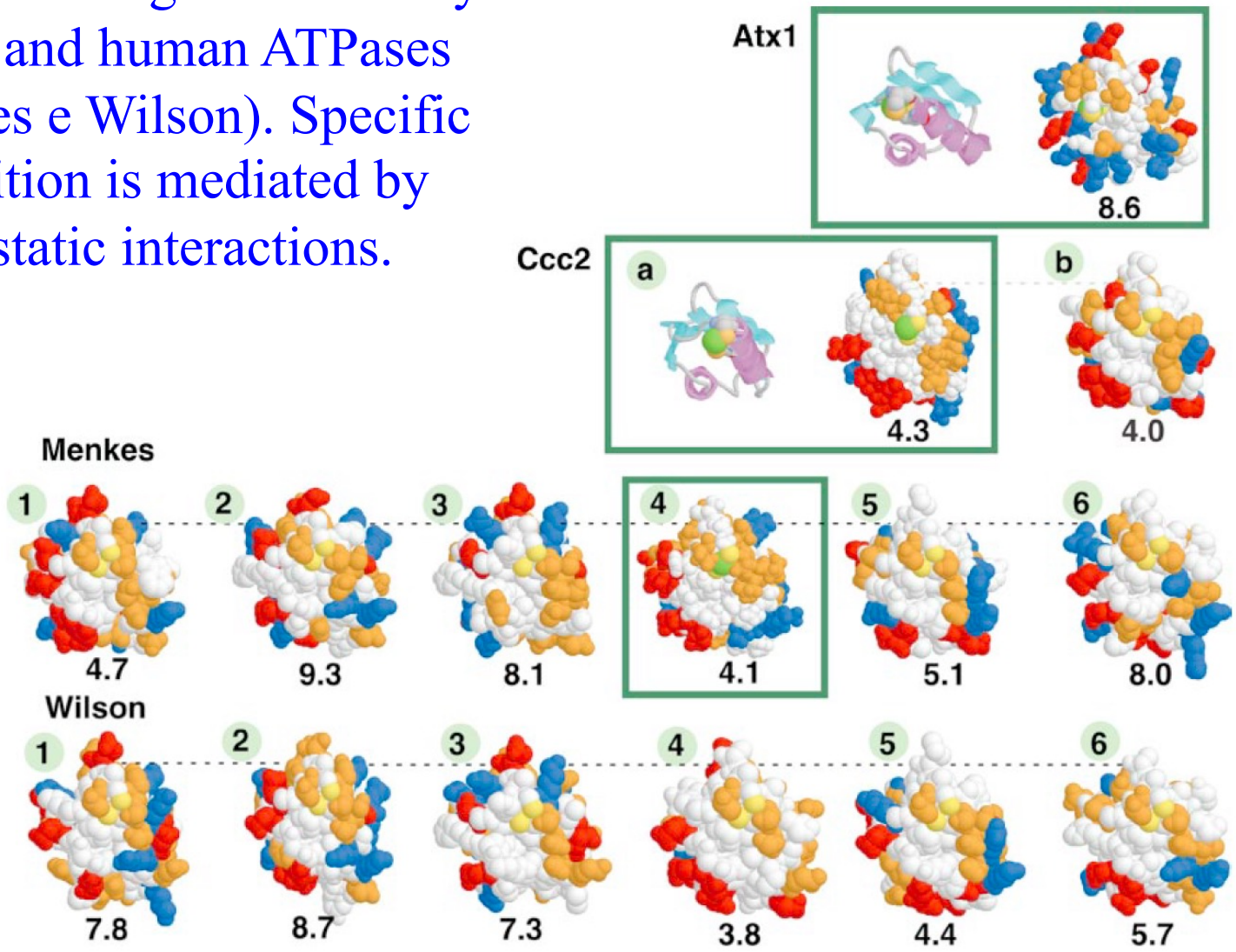


FIG. 11. *A*, sequence alignment of the Ccc2a amino acid sequence from *S. cerevisiae* with the sequences of the fourth metal-binding domain from Menkes-transporting ATPase (mbd4) (Protein Data Bank accession number 1mw0) (20) and of Atx1. The positions of the Ccc2a secondary structure elements (as found in the mean Cu(I) structure) are shown at the *top*. β-strands are in *blue*, α helices are shown in *orange*, and loop regions are in *yellow*. Each sequence is *color-shaded* according to secondary structure element, as found in their metal-bound structures. Residues that are highly similar or conserved are indicated, respectively, by the ● and * *below* the *sequences*. *B*, comparison of the backbone of Cu(I)-Ccc2a (*blue*) and Ag(I)-mbd4 (*green*) structures (20). *C*, comparison of backbone of Cu(I)-Ccc2a (*blue*) and Cu(I)-Atx1 (*green*). The copper ion and the cysteine ligands are also shown. The secondary structure elements are indicated.

Structure of Atx1 and of the copper-binding domains of yeast (Ccc2) and human ATPases (Menkes e Wilson). Specific recognition is mediated by electrostatic interactions.



Charge complementarity between chaperones and pumps

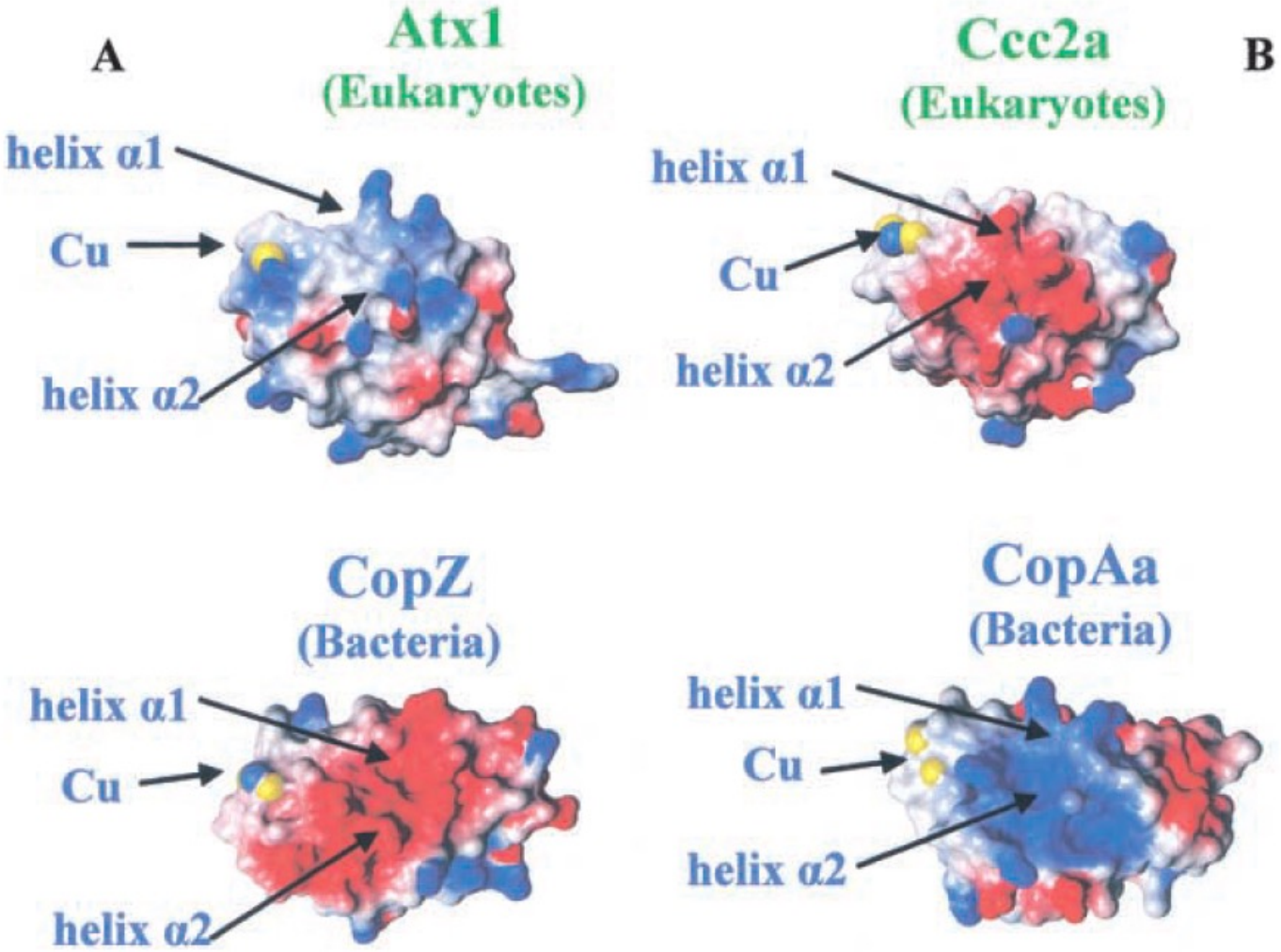
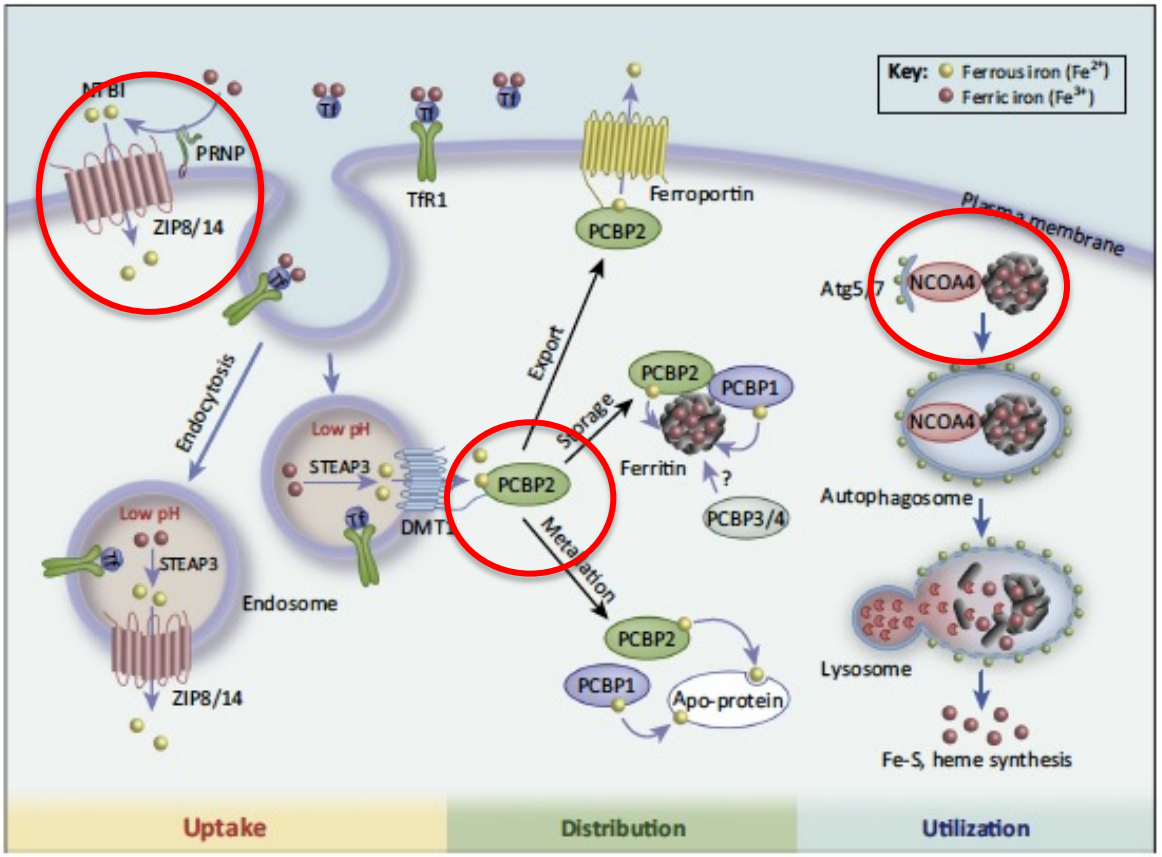


Figure 9 Surface potentials of *S. cerevisiae* Atx1 and *B. subtilis* CopZ structures (*A*). Surface potentials of *S. cerevisiae* Ccc2a and *B. subtilis* CopAa structures (*B*).

Novel proteins involved in intracellular iron metabolism



Trends in Biochemical Sciences

Figure 1. New Mechanisms Regulating Intracellular Iron Metabolism. The ZRT/IRT-like protein (ZIP) family transporters, ZIP8 and ZIP14, were recently identified as crucial for transporting non-transferrin bound iron (NTBI) after reduction of NTBI by prion protein (PRNP). In the acidic endosome, Fe^{3+} is released from transferrin (Tf) and free Fe^{3+} is reduced to Fe^{2+} by six-transmembrane epithelial antigen of prostate 3 (STEAP3) and transported to the cytoplasm by divalent metal transporter 1 (DMT1) and ZIP8/14. Poly-(rC)-binding protein 1 (PCBP1) and PCBP2 are cytosolic iron chaperones that deliver Fe^{2+} to apo-proteins (metallation), such as hypoxia-inducible factor (HIF) prolyl hydroxylases), ferroportin (iron export), and ferritin (oxidation to Fe^{3+} and storage). Nuclear receptor coactivator 4 (NCOA4)-mediated autophagy of iron-loaded ferritin releases iron for utilization in cellular processes (see text).