

Metabolismo del rame e del ferro nell'uomo



ACS
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PERIODIC TABLE OF ELEMENTS

GROUP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
PERIOD 1	H Hydrogen 1.008																	He Helium 4.003
PERIOD 2	Li Lithium 6.94	Be Beryllium 9.012											B Boron 10.81	C Carbon 12.01	N Nitrogen 14.01	O Oxygen 16.00	F Fluorine 18.99	Ne Neon 20.18
PERIOD 3	Na Sodium 22.99	Mg Magnesium 24.31											Al Aluminum 26.98	Si Silicon 28.09	P Phosphorus 30.97	S Sulfur 32.06	Cl Chlorine 35.45	Ar Argon 39.95
PERIOD 4	K Potassium 39.10	Ca Calcium 40.08	Sc Scandium 44.96	Ti Titanium 47.88	V Vanadium 50.94	Cr Chromium 52.00	Mn Manganese 54.94	Fe Iron 55.85	Co Cobalt 58.93	Ni Nickel 58.69	Cu Copper 63.55	Zn Zinc 65.39	Ga Gallium 69.72	Ge Germanium 72.64	As Arsenic 74.92	Se Selenium 78.96	Br Bromine 79.90	Kr Krypton 83.79
PERIOD 5	Rb Rubidium 85.47	Sr Strontium 87.62	Y Yttrium 88.91	Zr Zirconium 91.22	Nb Niobium 92.91	Mo Molybdenum 95.96	Tc Technetium (98)	Ru Ruthenium 101.1	Rh Rhodium 102.9	Pd Palladium 106.4	Ag Silver 107.9	Cd Cadmium 112.4	In Indium 114.8	Sn Tin 118.7	Sb Antimony 121.8	Te Tellurium 127.6	I Iodine 126.9	Xe Xenon 131.3
PERIOD 6	Cs Cesium 132.9	Ba Barium 137.3	57-71 Lanthanides	Hf Hafnium 178.5	Ta Tantalum 180.9	W Tungsten 183.8	Re Rhenium 186.2	Os Osmium 190.2	Ir Iridium 192.2	Pt Platinum 195.1	Au Gold 197.0	Hg Mercury 200.5	Tl Thallium 204.38	Pb Lead 207.2	Bi Bismuth 208.9	Po Polonium (209)	At Astatine (210)	Rn Radon (222)
PERIOD 7	Fr Francium (223)	Ra Radium (226)	89-103 Actinides	Rf Rutherfordium (261)	Db Dubnium (264)	Sg Seaborgium (263)	Bh Bohrium (264)	Hs Hassium (277)	Mt Meitnerium (266)	Ds Darmstadtium (271)	Rg Roentgenium (272)	Cn Copernicium (285)	Nh Nihonium (284)	Fl Flerovium (289)	Mc Moscovium (288)	Lv Livermorium (293)	Ts Tennessine (294)	Og Oganesson (294)
	La Lanthanum 138.9	Ce Cerium 140.1	Pr Praseodymium 140.9	Nd Neodymium 144.2	Pm Promethium (145)	Sm Samarium 150.4	Eu Europium 152.0	Gd Gadolinium 157.2	Tb Terbium 158.9	Dy Dysprosium 162.5	Ho Holmium 164.9	Er Erbium 167.3	Tm Thulium 168.9	Yb Ytterbium 173.0	Lu Lutetium 175.0			
	Ac Actinium (227)	Th Thorium 232.0	Pa Protactinium 231.0	U Uranium 238.0	Np Neptunium (237)	Pu Plutonium (244)	Am Americium (243)	Cm Curium (247)	Bk Berkelium (247)	Cf Californium (251)	Es Einsteinium (252)	Fm Fermium (257)	Md Mendelevium (258)	No Nobelium (259)	Lr Lawrencium (262)			

- Alkali Metals
- Alkaline Earth Metals
- Transition Metals
- Other Metals
- Metalloids
- Non-metals
- Halogens
- Noble Gases
- Lanthanides
- Actinides

78 — Atomic Number
Pt — Symbol
Platinum — Name
195.1 — Average Atomic Mass

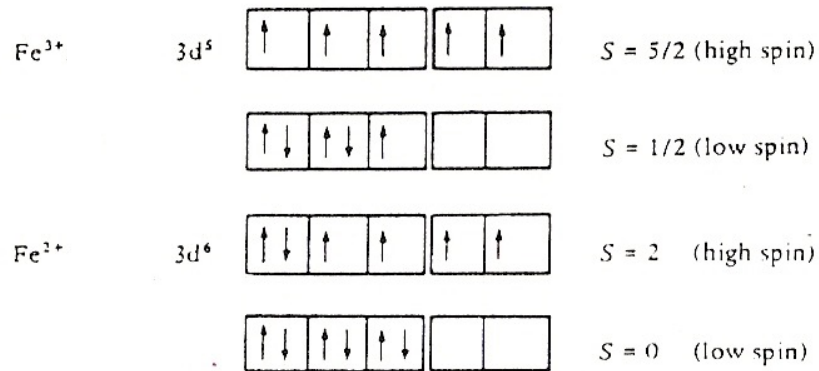
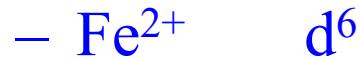
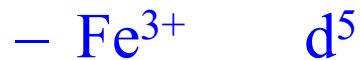
Ruolo biologico del ferro e del rame

Ferro e rame sono metalli **essenziali** per tutti gli organismi perchè sono legati a proteine indispensabili per la vita della cellula: respirazione, fotosintesi, fissazione dell'azoto, ciclo di Krebs, trasporto dell'ossigeno, sintesi di DNA, regolazione genica etc.

- Fe
 - citocromo ossidasi – proteine a **eme** (citocromi, emoglobina) – proteine con **cluster Fe-S** (catena di trasporto degli elettroni) – proteine a ferro non-eme
- Cu
 - citocromo ossidasi – superossido dismutasi – ceruloplasmina – lisil ossidasi – tirosinasi – dopamina β idrossilasi - metallotioneina

Il ferro

- In condizioni fisiologiche il ferro si trova in due stati di ossidazione:

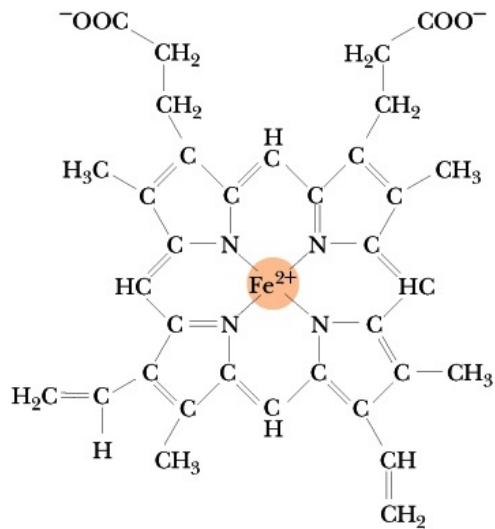


Il potenziale redox del ferro nelle proteine varia da -300 mV a +700 mV

Fe^{2+} è instabile in aerobiosi ed è rapidamente ossidato a pH 7

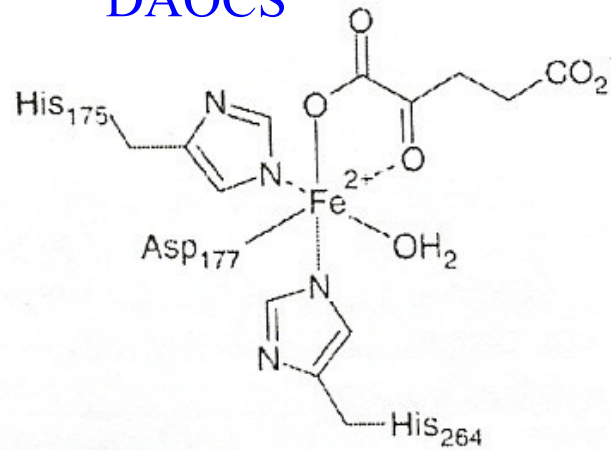
Fe^{3+} è insolubile a pH 7 (10^{-18} M)

Strutture di siti di legame del ferro nelle proteine

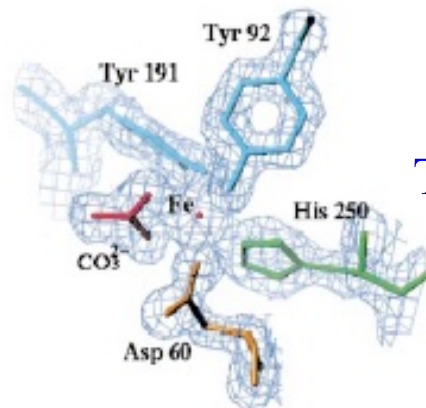
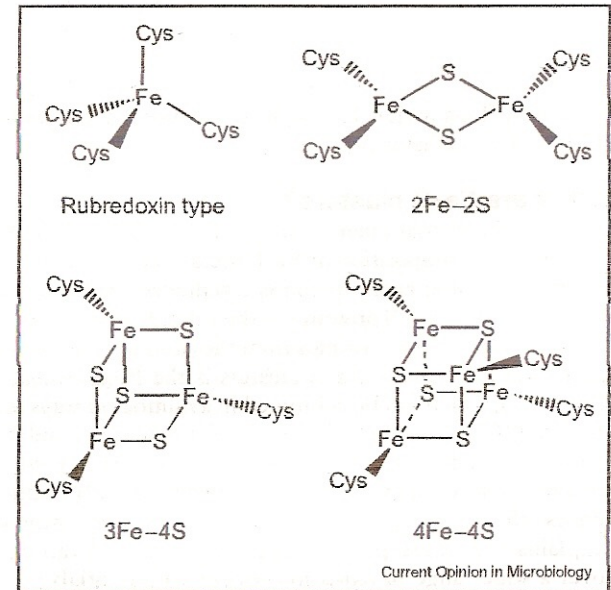


Heme
(Fe-protoporfirina IX)

DAOCS



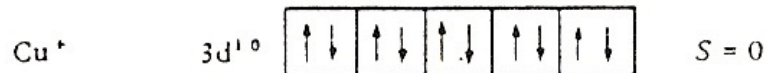
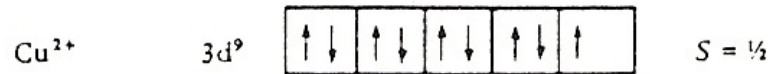
Cluster Fe-S



Transferrina

Il rame

- In condizioni fisiologiche il rame si trova in due stati di ossidazione :

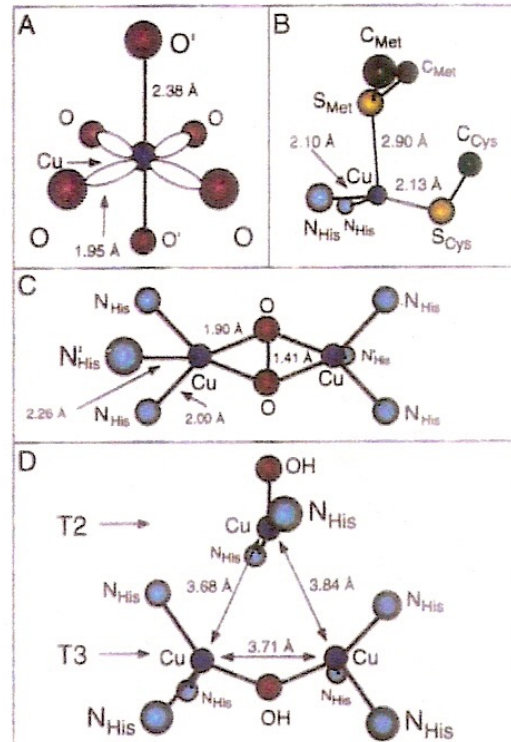
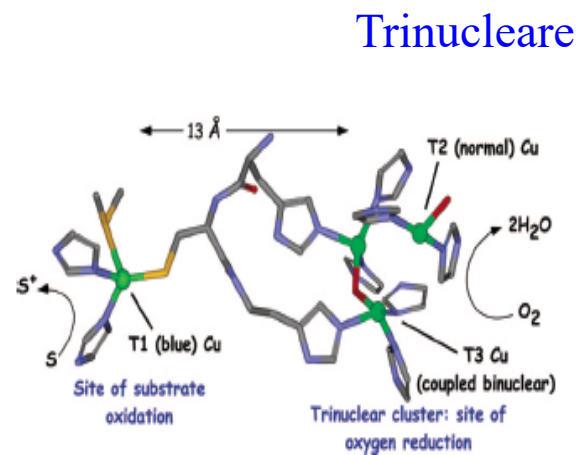
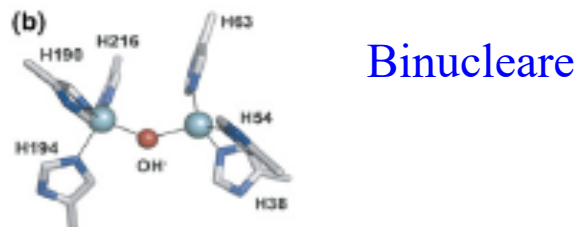
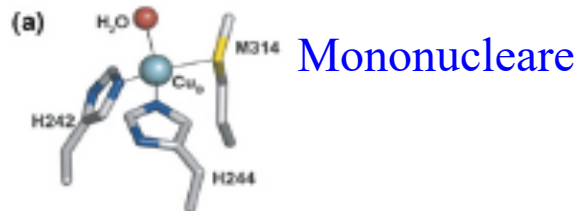


Il potenziale redox del rame nelle proteine varia da +200 mV a +750 mV

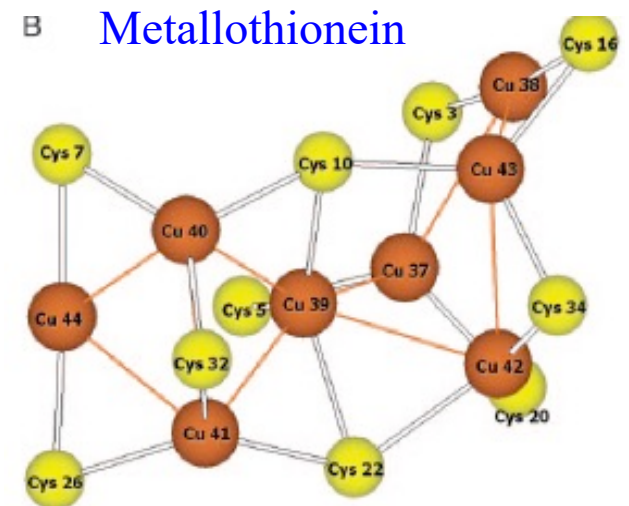
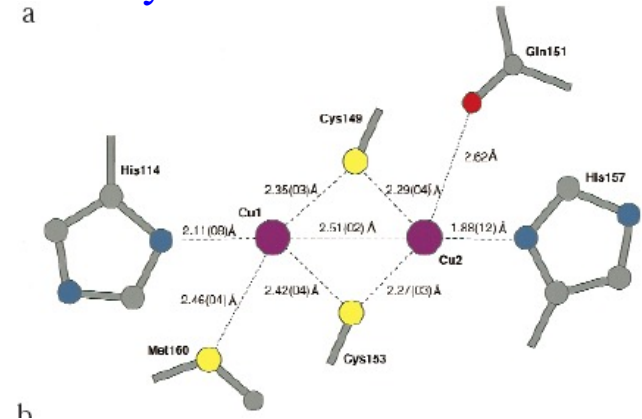
Cu^+ è instabile in aerobiosi ed è rapidamente ossidato a pH 7

Sia Cu^{2+} che Cu^+ sono solubili a pH 7

Strutture di siti di legame per il rame nelle proteine



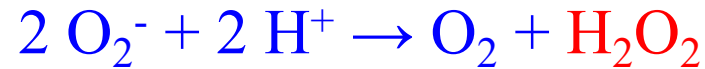
CuA cytochrome oxidase



Ruolo biologico del ferro e del rame

Ferro e rame sono **tossici** se presenti a livelli elevati e se non sono complessati a proteine perché reagiscono con O_2 generando specie reattive dell'ossigeno (ROS) che danneggiano proteine, DNA e lipidi (membrane)

Reazione di Haber-Weiss catalizzata da metalli



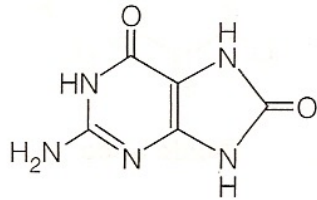
Reazioni di ossidazione catalizzate da metalli

- Proteine: modificazioni degli ammino acidi

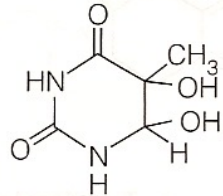
Ammino acido	Prodotto di ossidazione
Cysteine	Disulfide, cysteic acid
Methionine	Methionine sulfoxide, sulfone
Tryptophan	Hydroxytryptophan, nitrotryptophan, kynurenine
Phenylalanine	2,3-dihydroxyphenylalanine, hydroxyphenylalanine
Tyrosine	3,4 dihydroxyphenylalanine, tyrosine dimers, dinitrotyrosine
Histidine	2-oxohistidine, asparagine, aspartate
Arginine	Glutamic semialdehyde
Lysine	α -amino adipic semialdehyde
Proline	2-pyrrolidone, hydroxyproline, glutamic semialdehyde
Threonine	2- amino- 3- ketobutirrate
Glutamate/glutamine	Oxalate, piruvate

Reazioni di ossidazione catalizzate da metalli

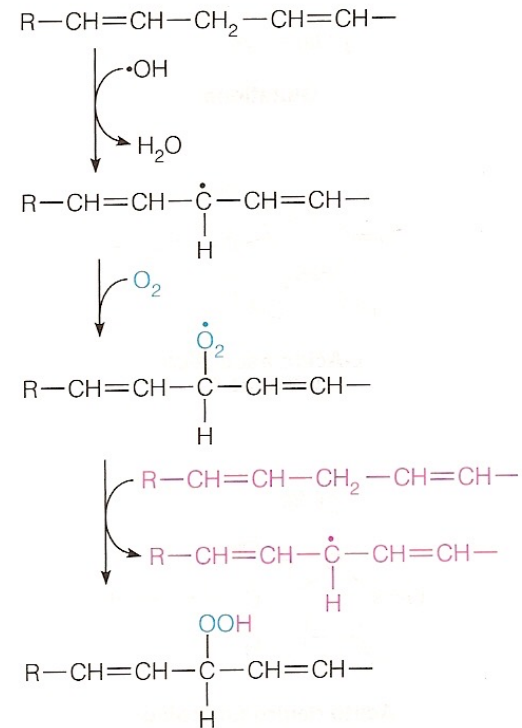
- DNA: ossidazione delle basi
- Lipidi: perossidazione



8-Ossoguanina



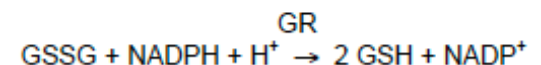
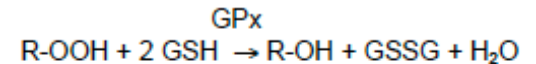
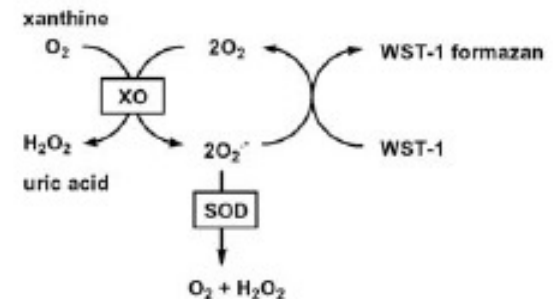
Glicole della timina



Perossidazione dei lipidi

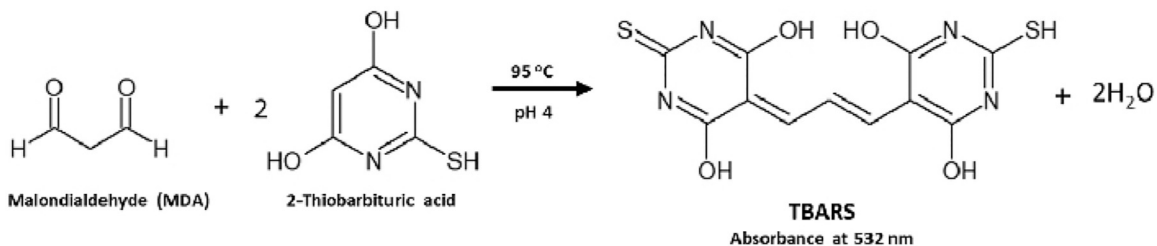
Come si misura lo stress ossidativo?

- Indicatori di stress ossidativo
 - Enzimi antiossidanti (SOD, catalasi, perossidasi): misura dell'attività enzimatica o dei livelli di espressione
 - Antiossidanti non-enzimatici (glutathione, vitamina E)
- SOD
 - Misura spettrofotometrica a 440 nm dell'inibizione della produzione di formazano in presenza di superossido
- Catalase
 - Misura spettrofotometrica di H_2O_2 a 240 nm
- Glutathione peroxidase
 - Saggio accoppiato che misura il consumo di NADPH a 340 nm



Come si misura lo stress ossidativo?

- Misura di specie reattive dell'ossigeno e dell'azoto con sonde fluorescenti come DCFDA (dichlorofluorescein diacetate) o DAF (diaminofluorescein) o cromogeni come NBT (nitroblue tetrazolium)
- Saggi di misura di molecole danneggiate da stress ossidativo
 - Malondialdeide (MDA), proteine ossidate



Controllo della tossicità di ferro e rame

- Sistemi di detossificazione delle specie reattive dell'ossigeno (ROS)
 - Enzimatici: superossido dismutasi, catalasi e glutazione perossidasi
 - Non enzimatici: vitamine E e C, glutazione, acido uric, bilirubina ecc.
- Sistemi di riparo e proteine di deposito
 - Ferritina
 - Metallothioneina
- I meccanismi cellulari di acquisizione del ferro e del rame sono finemente regolati per mantenere adeguati livelli intracellulari di questi metalli

Patologie associate a dismetabolismo del rame o del ferro

Patologia	Gene mutato	
Sindrome di Menkes	ATP7A	Carenza di rame (difettoso assorbimento del rame)
Morbo di Wilson	ATP7B	Accumulo di rame nel fegato e nel cervello
Emocromatosi di tipo I-IV	HFE, TfR2, HJV, HAMP, Fpn	Accumulo di ferro in diversi organi
Atassia di Friedreich	Frataxina	Neurodegenerazione e accumulo di ferro in specifiche regioni del cervello
Aceruloplasminemia	Ceruloplasmina	
Sindrome di Hallevorden-Spatz	PKAN-2	
Neuroferritinopatia	L-ferritina	
Corea di Huntington		
Morbo di Alzheimer		
Morbo di Parkinson		

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

^a Human Genome Organization approved symbol

Modelli animali
utili per lo studio di
patologie umane
associate a
dismetabolismo del
ferro

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
β2m ^{-/-}	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
TfR2 ^{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.

Omeostasi sistemica del ferro

Il ferro viene assorbito dagli **enterociti** nel duodeno, rilasciato nel sangue e trasportato ai diversi organi dalla transferrina. I **macrofagi** reticoloendoteliali costituiscono il sito principale di riciclaggio del ferro dagli eritrociti senescenti.

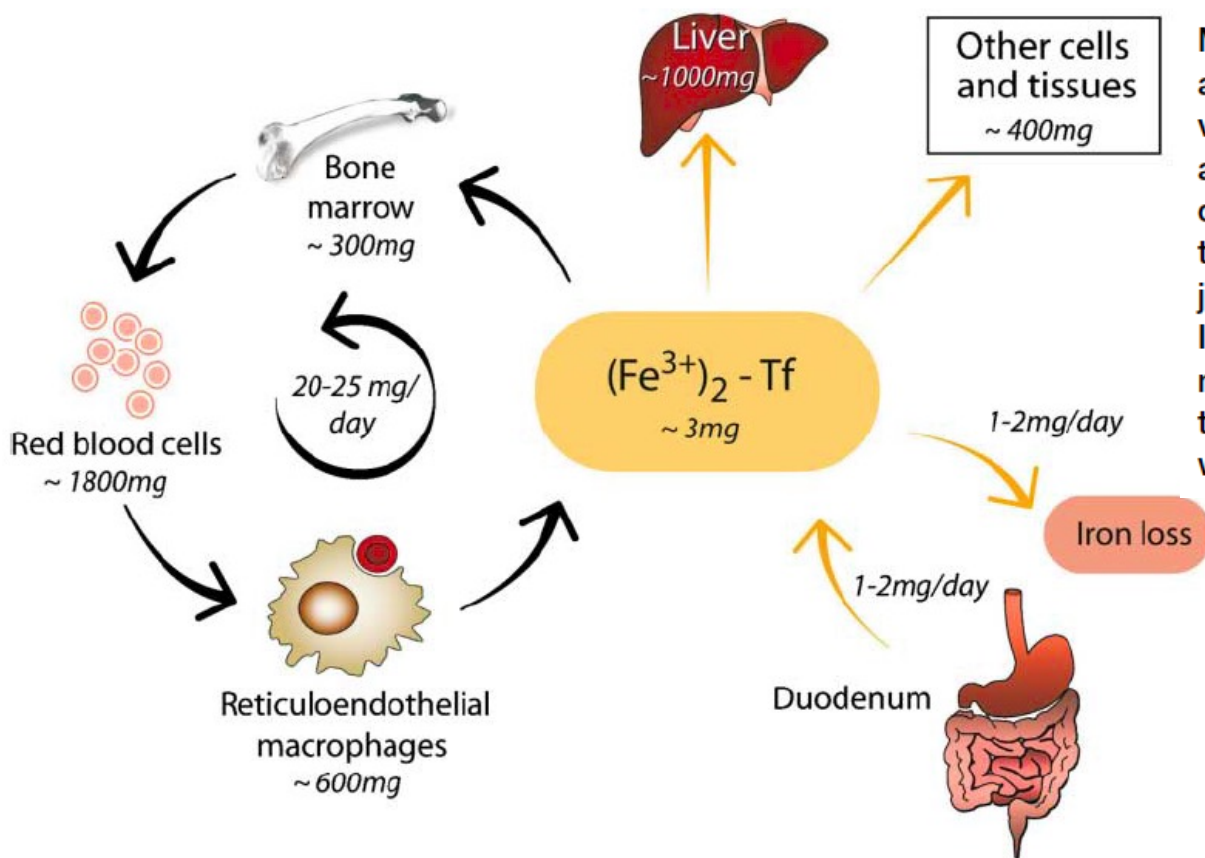


Figure 3. Systemic Iron Homeostasis

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.

Metabolismo cellulare del ferro in mammiferi

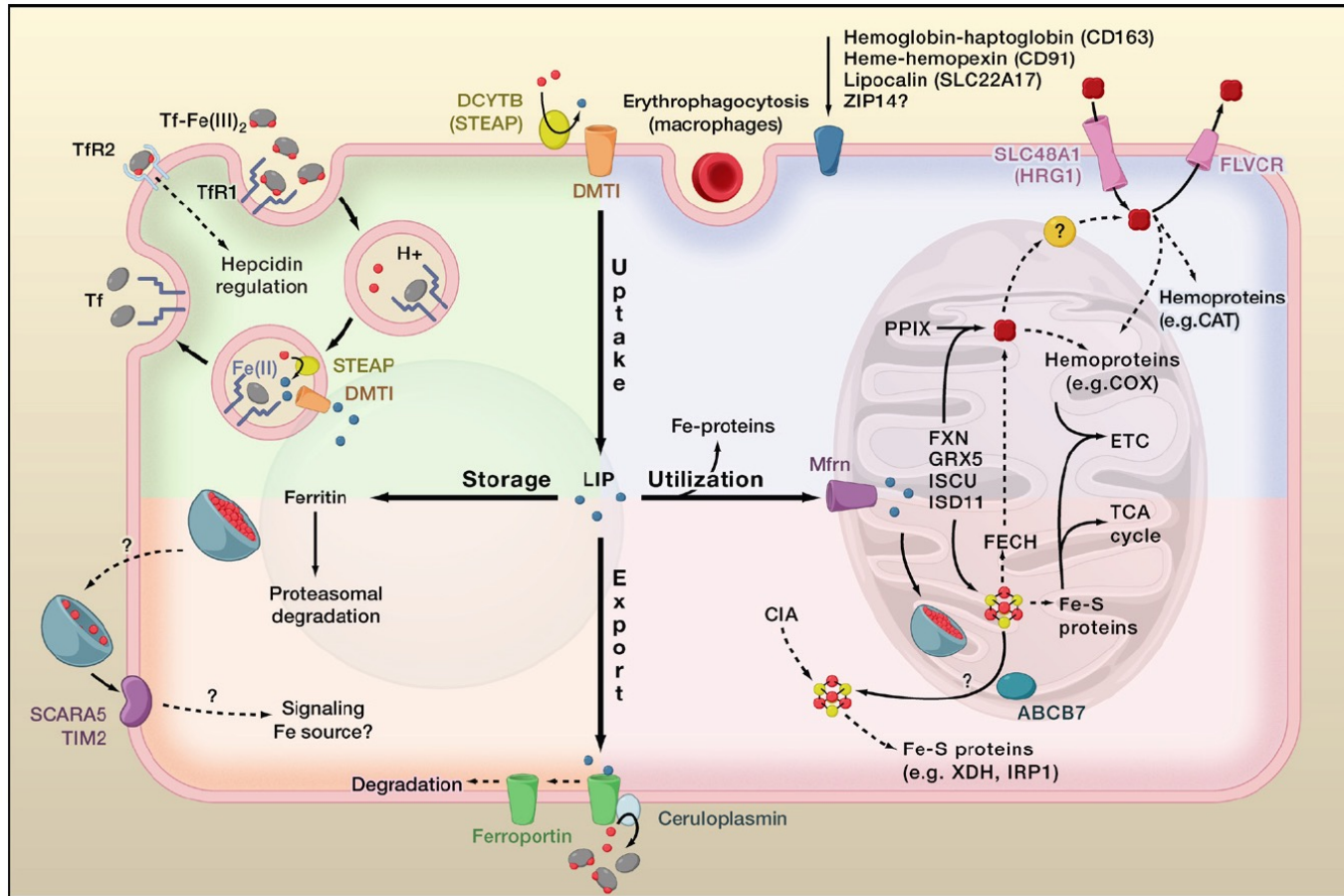


Figure 3. Cell Biology of Iron Metabolism

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.

Biosintesi dell'eme

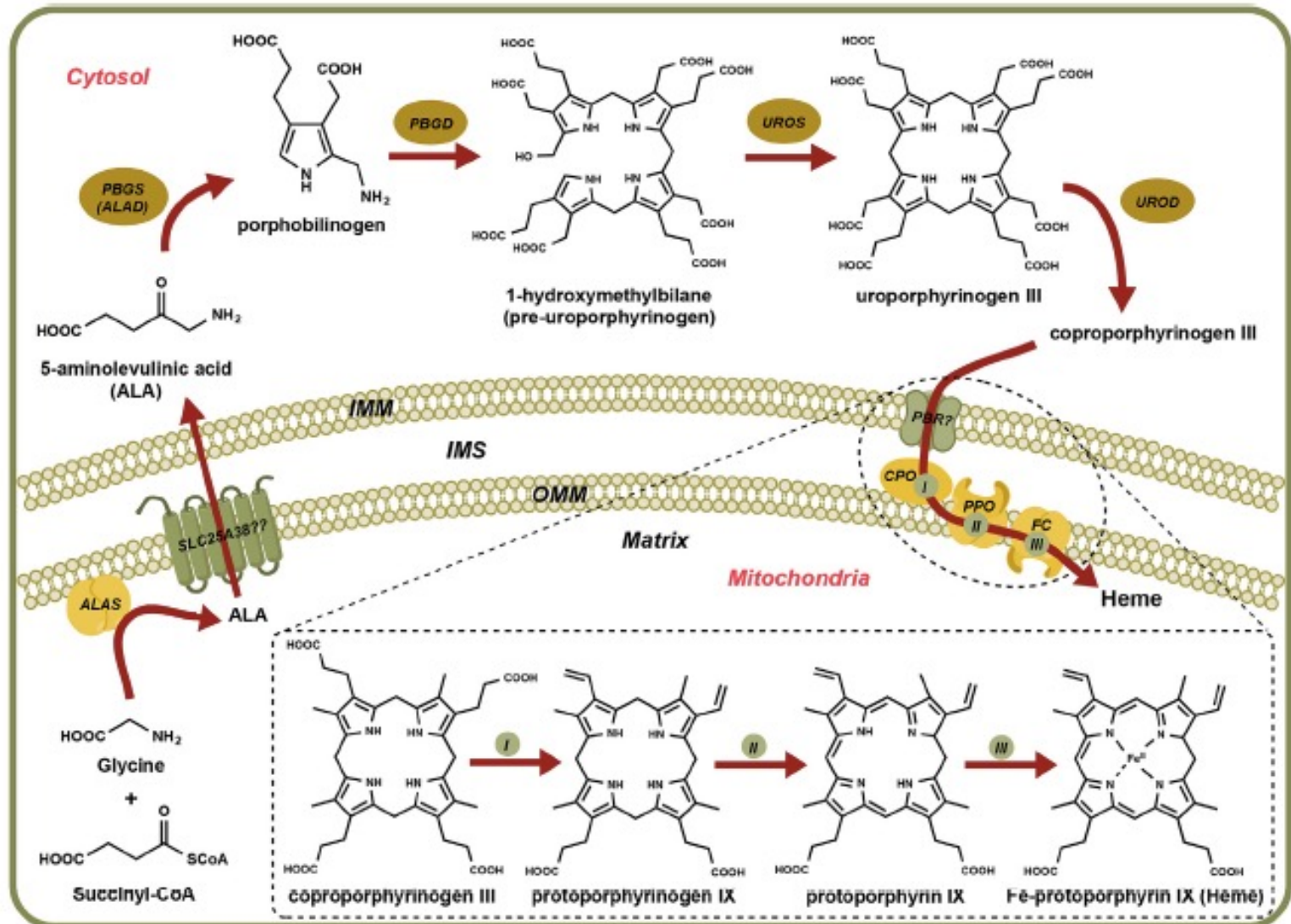
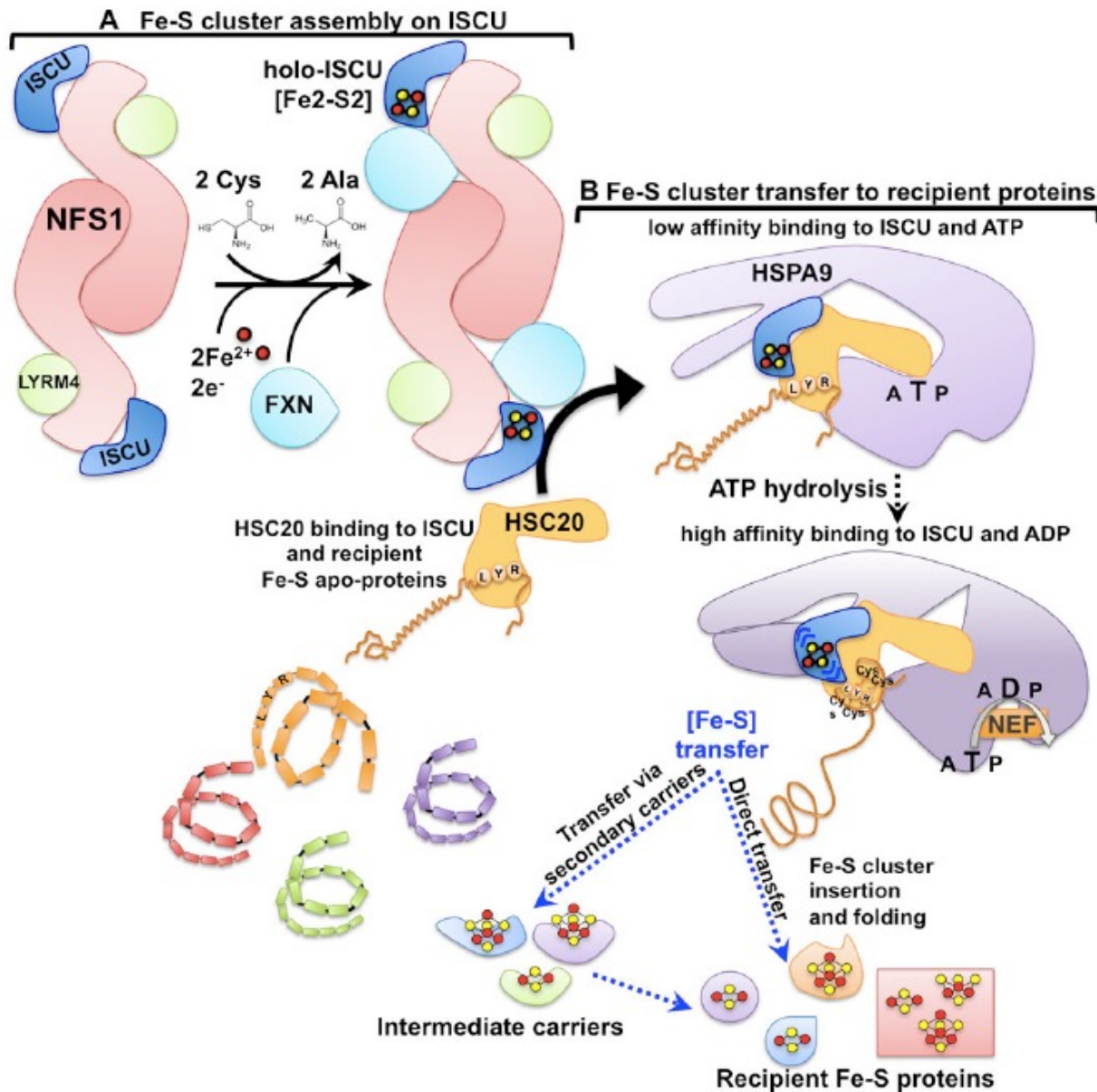


Fig. 4. Heme biosynthesis pathway. ALA: 5-aminolevulinic acid, ALAS: ALA synthase, PBGS: porphobilinogen synthase, ALAD: ALA dehydratase, PBGD: porphobilinogen deaminase, UROS: uroporphyrinogen synthase, UROD: uroporphyrinogen decarboxylase, PBR: peripheral-type benzodiazepine receptor, CPO: coproporphyrinogen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

Meccanismo di biosintesi dei cluster Fe-S nei mammiferi



Il ciclo Transferrina-TfR per l'acquisizione cellulare del ferro

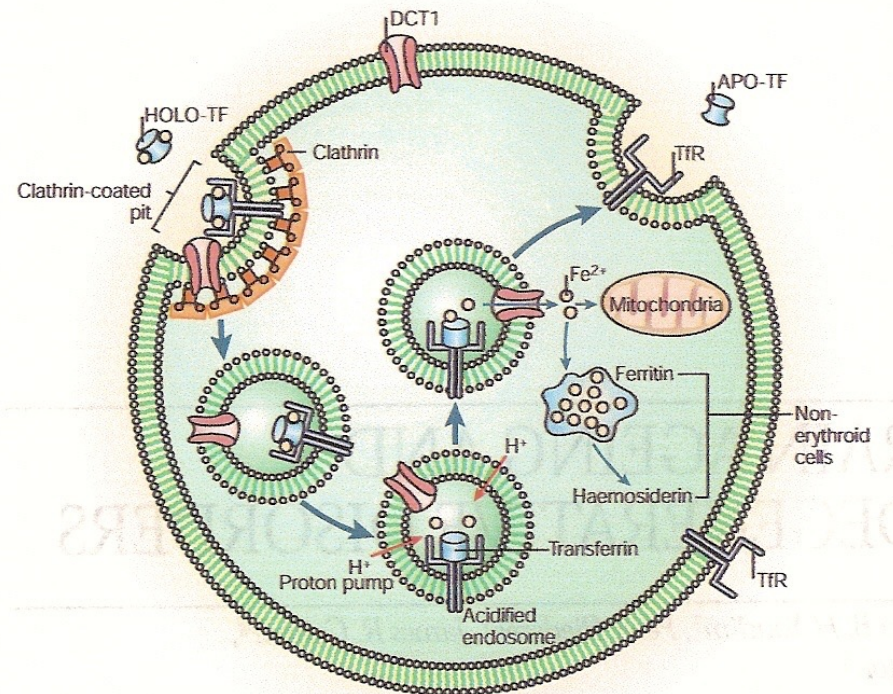
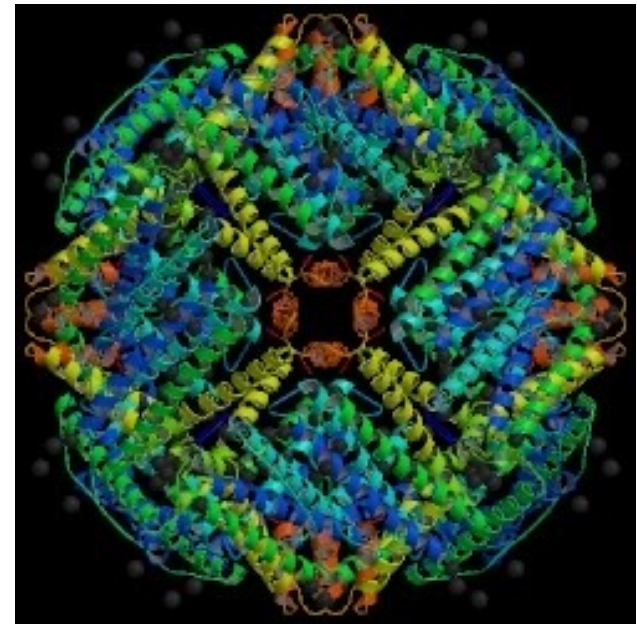
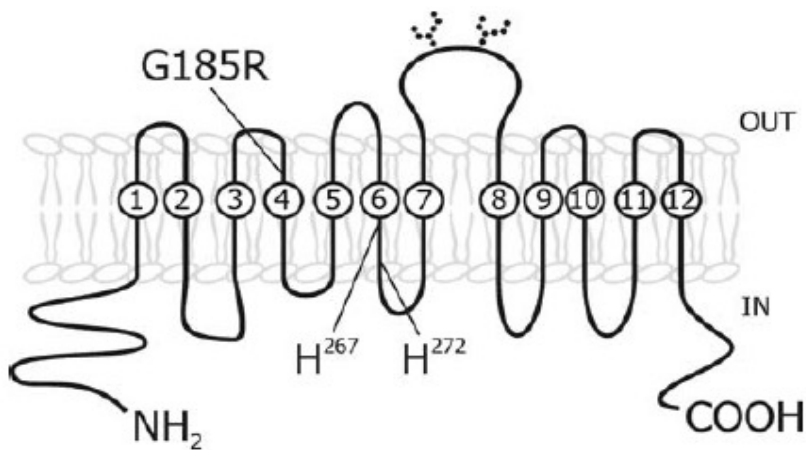


Figure 1 | **The Tf cycle.** Holotransferrin (HOLO-TF) binds to the transferrin receptor (TfR) at the cell surface. These complexes localize to clathrin-coated pits, which invaginate to initiate endocytosis. Specialized endosomes form, which are acidified by a proton pump. When the required acidic pH 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.

Proteine del metabolismo cellulare del ferro

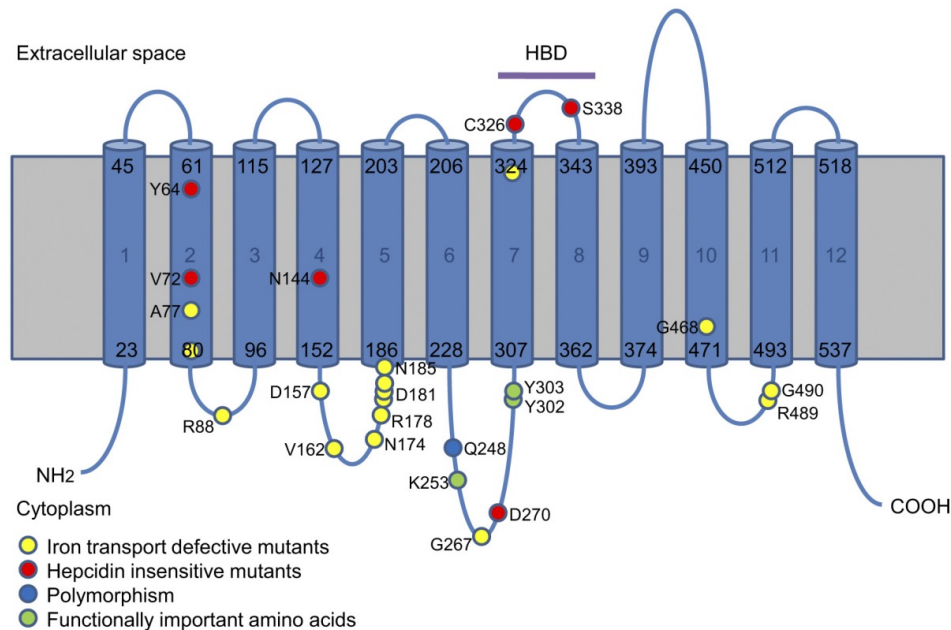
- **DMT1** è un co-trasportatore di cationi bivalenti e H^+ espresso in molti tessuti (enterociti, cellule eritroidi, rene, polmone, cervello...).
- Ha un ruolo nell'**assorbimento** del ferro nel duodeno e nel meccanismo di rilascio del ferro dalla transferrina.
- L'espressione di DMT1 è indotta da carenza di ferro e sono state identificate diverse isoforme della proteina.

- La **Ferritina** è la principale proteina di **deposito** intracellulare del ferro. E' formata da 24 subunità di tipo H e di tipo L ed è in grado di legare fino a 4500 atomi di ferro.



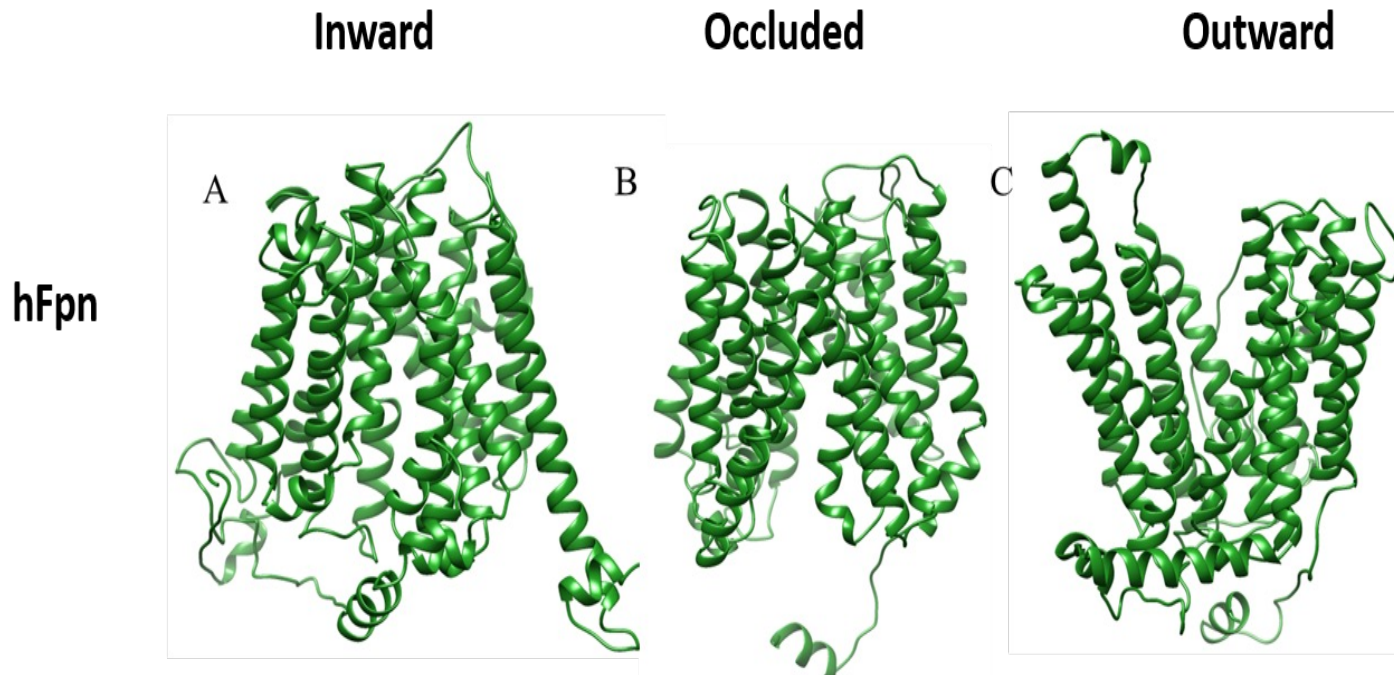
Esporto del ferro dalle cellule: la ferroportina

- La **Ferroportina** è l'unico esportatore del ferro dalle cellule finora identificato.
- È espressa sulla membrana basolaterale degli enterociti, nei macrofagi, negli astrociti e negli epatociti.
- Mutazioni della ferroportina causano accumulo di ferro nel fegato o nei macrofagi reticoloendoteliali.



Esporto del ferro dalle cellule: la ferroportina

La ferroportina appartiene alla Major Facilitator Superfamily, trasportatori di membrana che ciclano tra diverse conformazioni per traslocare il substrato attraverso la membrana.



Ferrossidasi: ceruloplasmina ed efestina

- Le ferrossidasi **ceruloplasmina** ed **efestina** appartengono alla famiglia delle ossidasi blu multinucleari a **rame** (MCO) e catalizzano l'ossidazione del Fe^{2+} a Fe^{3+} con riduzione dell' O_2 ad H_2O .
- La ceruloplasmina collabora con la ferroportina ossidando il Fe^{2+} esportato da quest'ultima e facilitandone l'incorporazione nella transferrina.
- L'efestina è una proteina di membrana intracellulare, è espressa principalmente negli enterociti ed ha un ruolo nell'assorbimento intestinale del ferro.

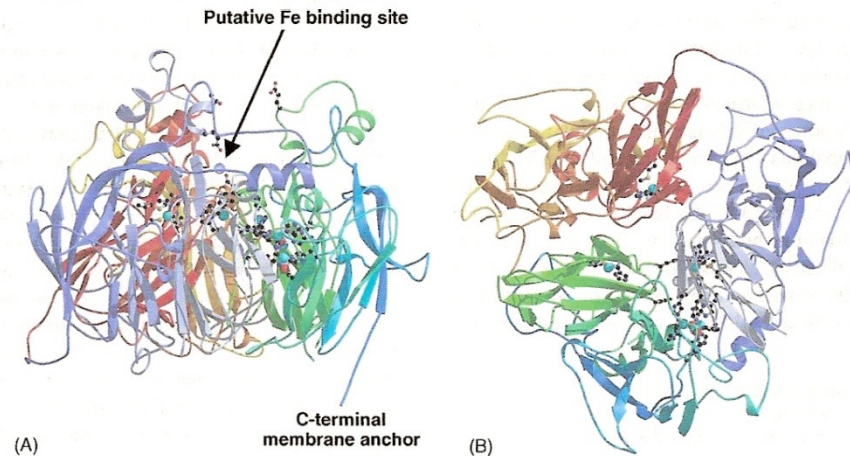
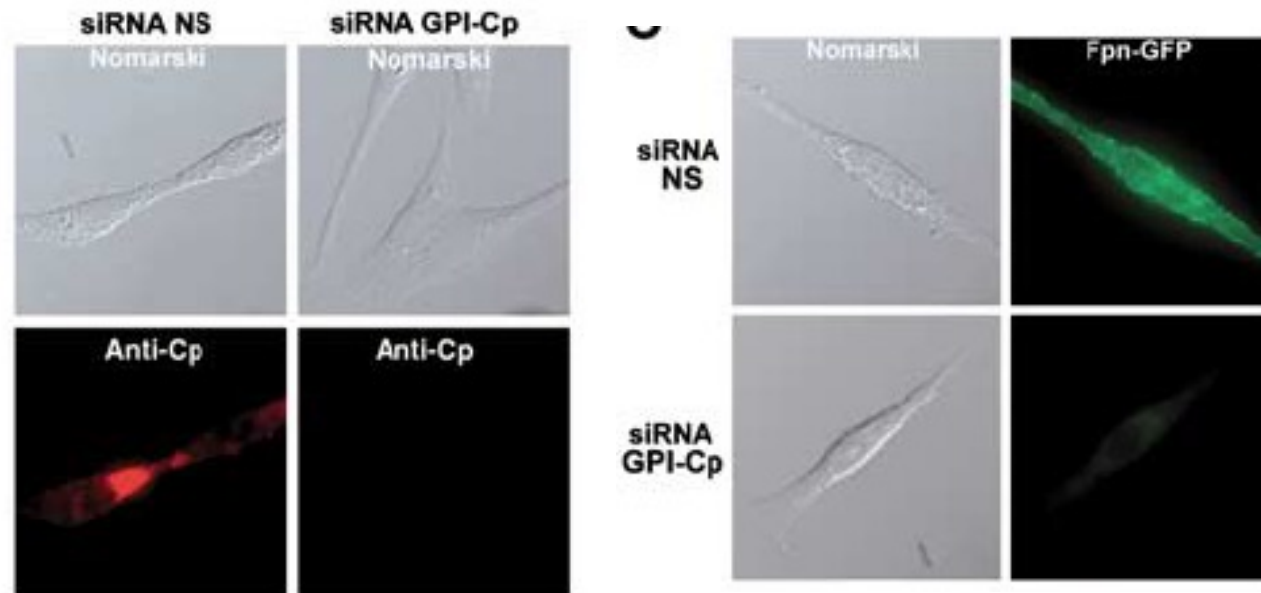


Fig. 1. Predicted molecular structure of hephaestin. Used with permission (Syed et al., 2002). (A) Side view of human hephaestin molecule. (B) Top view of the molecule. The copper atoms are represented as light blue spheres, oxygen atoms are red.

L'attività ferrossidasica della ceruloplasmina (e dell'efestina) è necessaria per mantenere la ferroportina sulla superficie della cellula

Una isoforma della ceruloplasmina è ancorata alla membrana mediante un'ancora GPI ed è espressa principalmente negli astrociti.

La mancanza di ceruloplasmina in queste cellule causa la scomparsa della ferroportina dalla superficie cellulare e ciò potrebbe spiegare l'accumulo di ferro riscontrato in pazienti affetti da aceruloplasminemia.



Omeostasi cellulare del ferro.

Il sistema IRE/IRP: regolazione post-trascrizionale

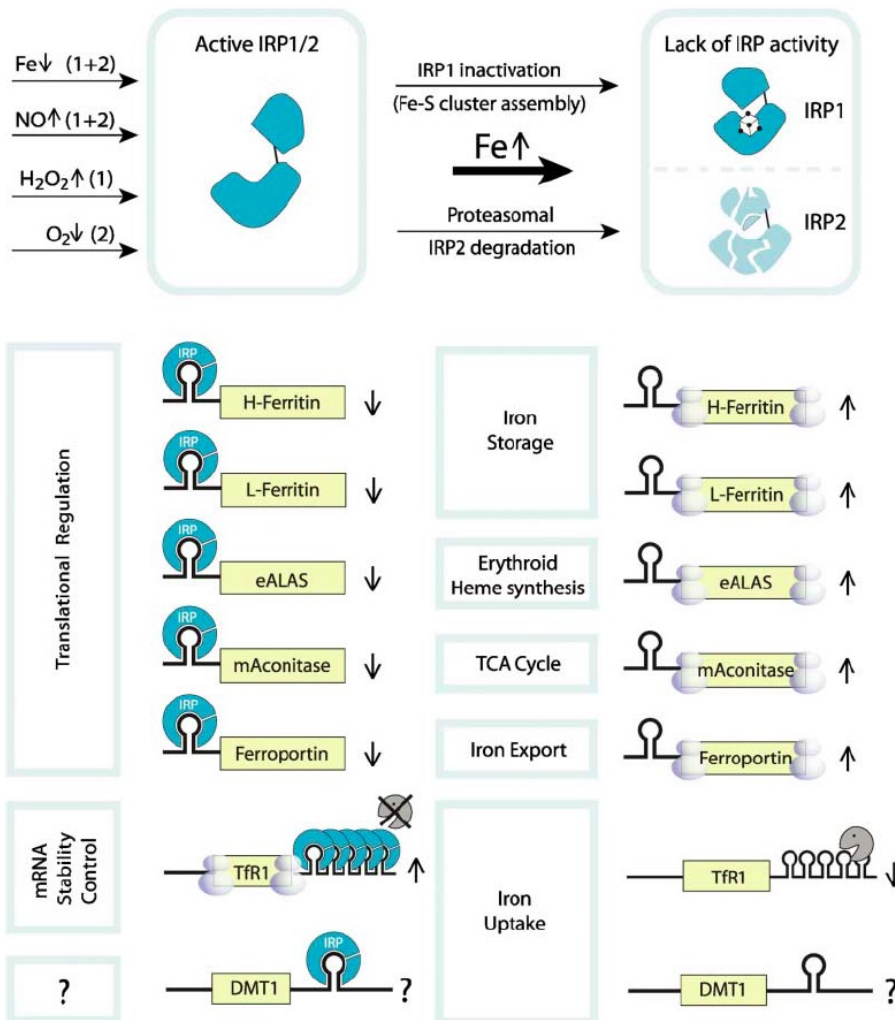


Figure 2. The IRE/IRP Regulatory System
Proteins involved in iron storage, erythroid heme synthesis, the TCA cycle, iron export, and iron uptake are coordinately regulated by the interaction of the iron regulatory proteins (IRPs) with conserved RNA secondary structures, the iron-responsive elements (IREs). 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 apo-protein form of IRP1 and stabilize IRP2. In contrast, H₂O₂ only activates IRP1, while hypoxia interferes with IRP2 degradation.

Regolazione **sistemica** dell'omeostasi del ferro mediata dall'epcidina

L'**epcidina** è un peptide di 25 aminoacidi prodotto dal fegato. Si lega alla ferroportina causandone l'internalizzazione e la degradazione, in questo modo diminuisce l'esporto del ferro dagli enterociti e dai macrofagi nella circolazione sanguigna. L'espressione dell'epcidina è indotta da eccesso di ferro e infiammazione (IL-6), e repressa da anemia e ipossia.

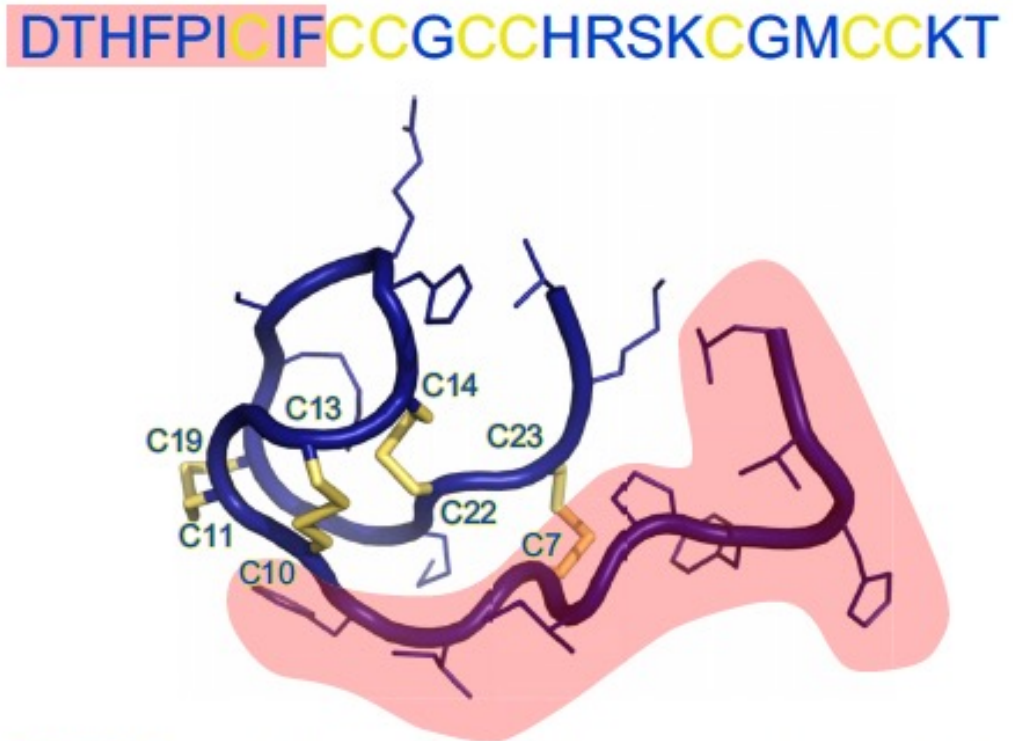
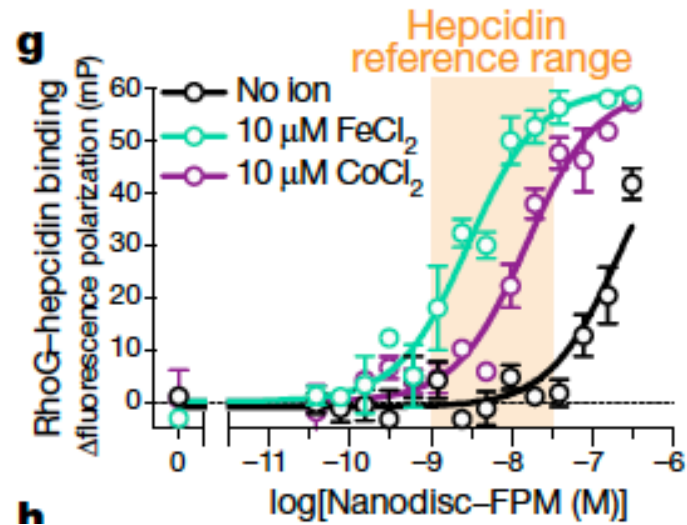
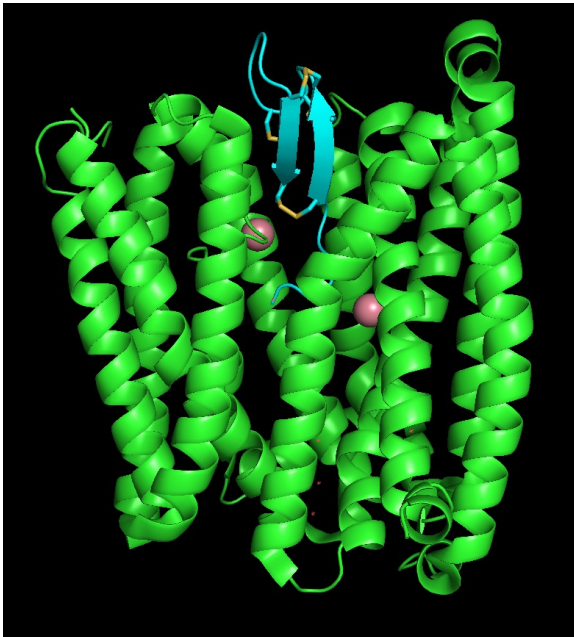


FIGURE 3. Hepcidin: the amino acid sequence and structure. The NH₂-terminal segment known to interact with ferroportin [193] is shaded in light red. The characteristic cysteines and their disulfide bonds are shown in yellow.

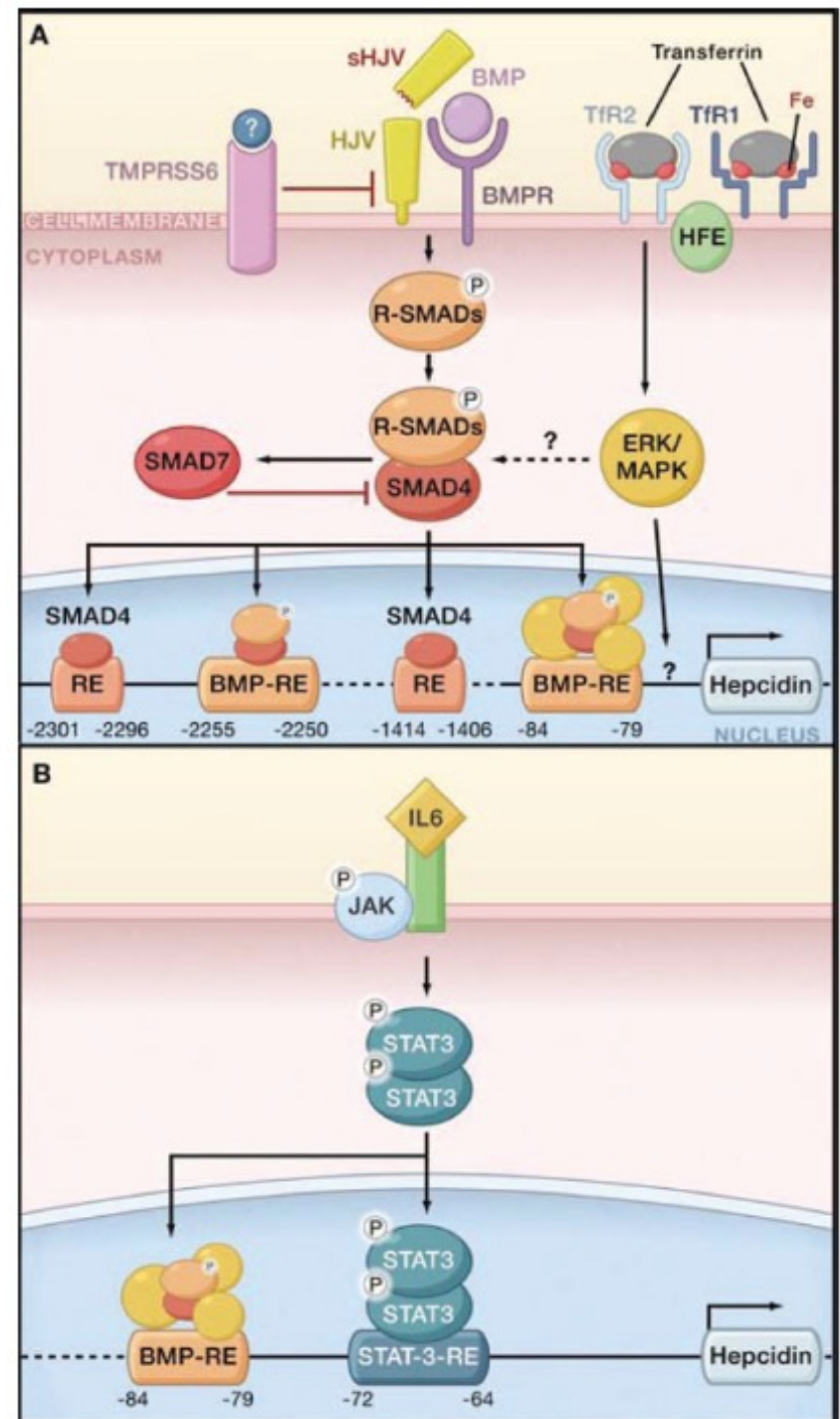
Epcidina lega ferroportina nel canale centrale occludendolo



La presenza di ferro o cobalto aumenta l'affinità di epcidina per ferroportina rendendola compatibile con i livelli fisiologici del peptide

Regolazione della sintesi dell'epcidina

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



Mutazioni nei geni **HFE**, **HAMP** e **HJV** causano una riduzione della sintesi di epcidina ed emocromatosi ereditaria a trasmissione autosomica recessiva

Regolazione sistemica dell'omeostasi del ferro

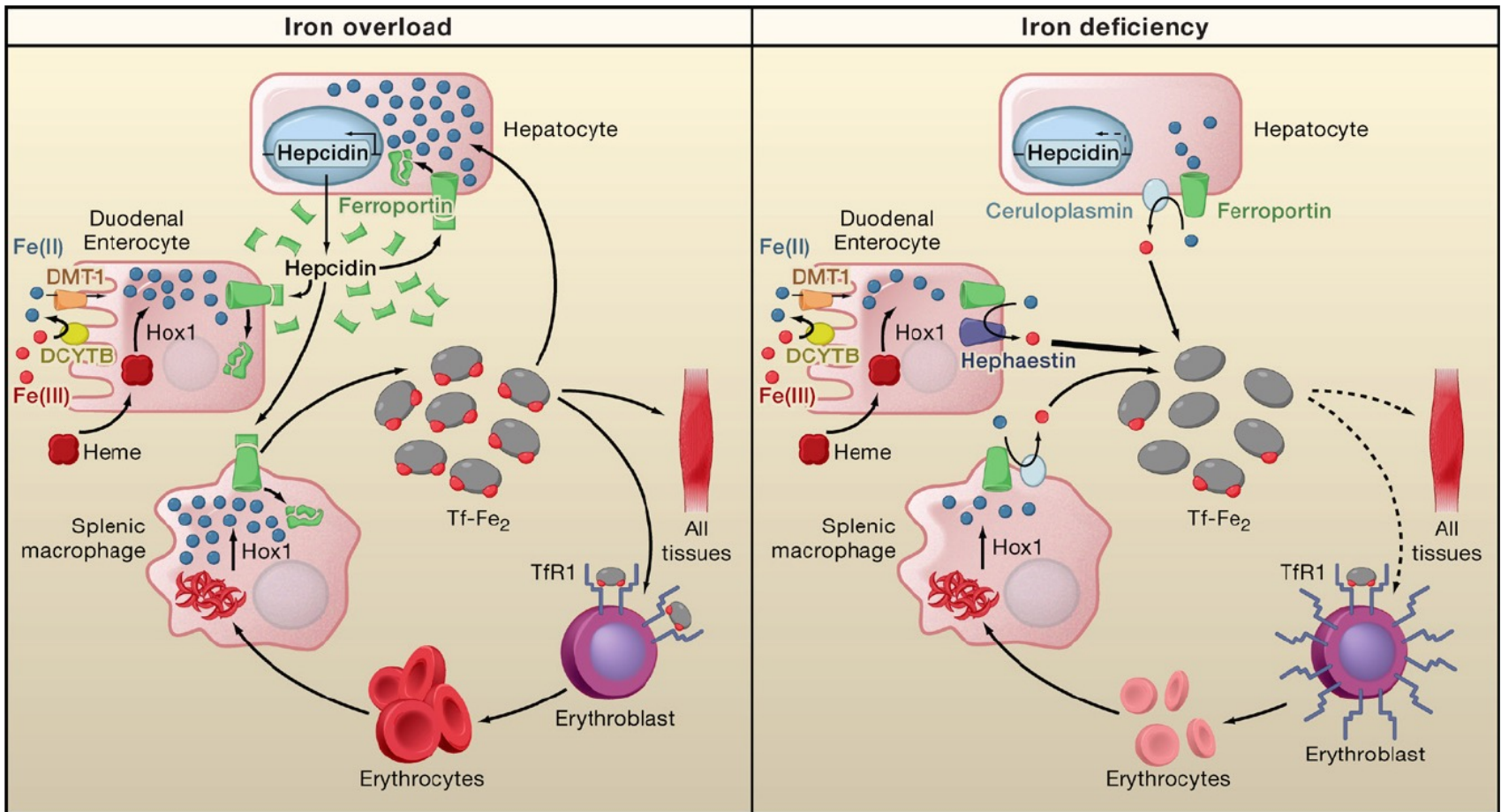


Figure 1. Regulation of Systemic Iron Homeostasis

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-Fe₂), 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.

Metabolismo del rame

- I meccanismi alla base dell'omeostasi cellulare del rame sono conservati in batteri (in parte), in lievito e negli eucarioti superiori.
- Gli studi in lievito si sono rivelati particolarmente utili per identificare e/o caratterizzare proteine umane strutturalmente o funzionalmente omologhe.
- Il metabolismo del rame negli eucarioti è strettamente connesso con il metabolismo del ferro attraverso le **ferrossidasi Cu-dipendenti**: difetti nell'incorporazione del rame nelle ferrossidasi provocano dismetabolismo del ferro.

Trasporto del rame in cellule eucariotiche.

Il trasportatore di membrana Ctr1

- Ctr1 è il principale trasportatore di Cu^+ nella cellula (K_m 1-5 μM).
- Ctr1 possiede tre domini transmembrana e una serie di sequenze 'Met' (MX_{1-3}M) necessarie per il legame del Cu^+ .
- La complementazione funzionale in ceppi di lievito *ctr1ctr3Δ* ha permesso di isolare Ctr1 umano. L'importanza di Ctr1 è dimostrata dal fatto che topi knock-out per Ctr1 muoiono allo stadio embrionale.
- L'espressione di Ctr1 è regolata a livello trascrizionale in lievito dal fattore Mac1 e a livello post-traduzionale attraverso endocitosi e degradazione mediata dal rame (in lievito e nei mammiferi).

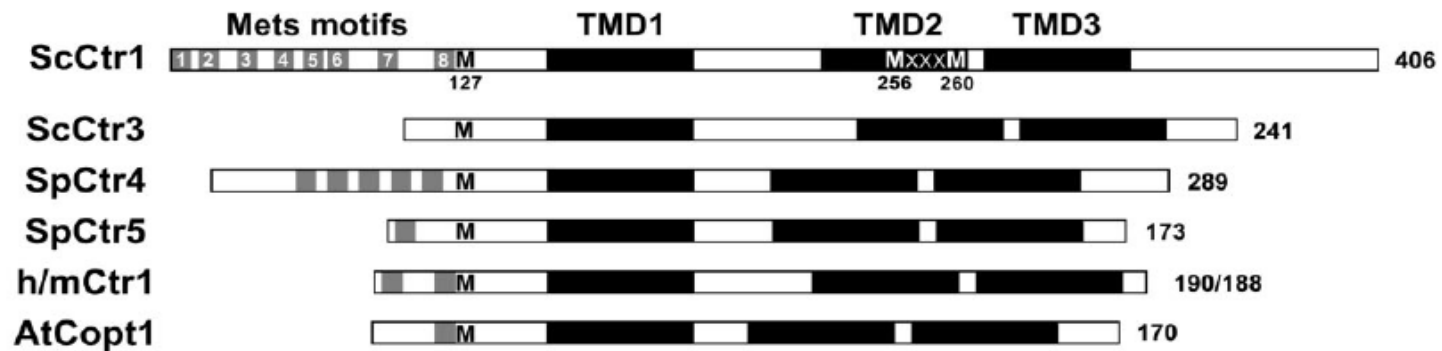
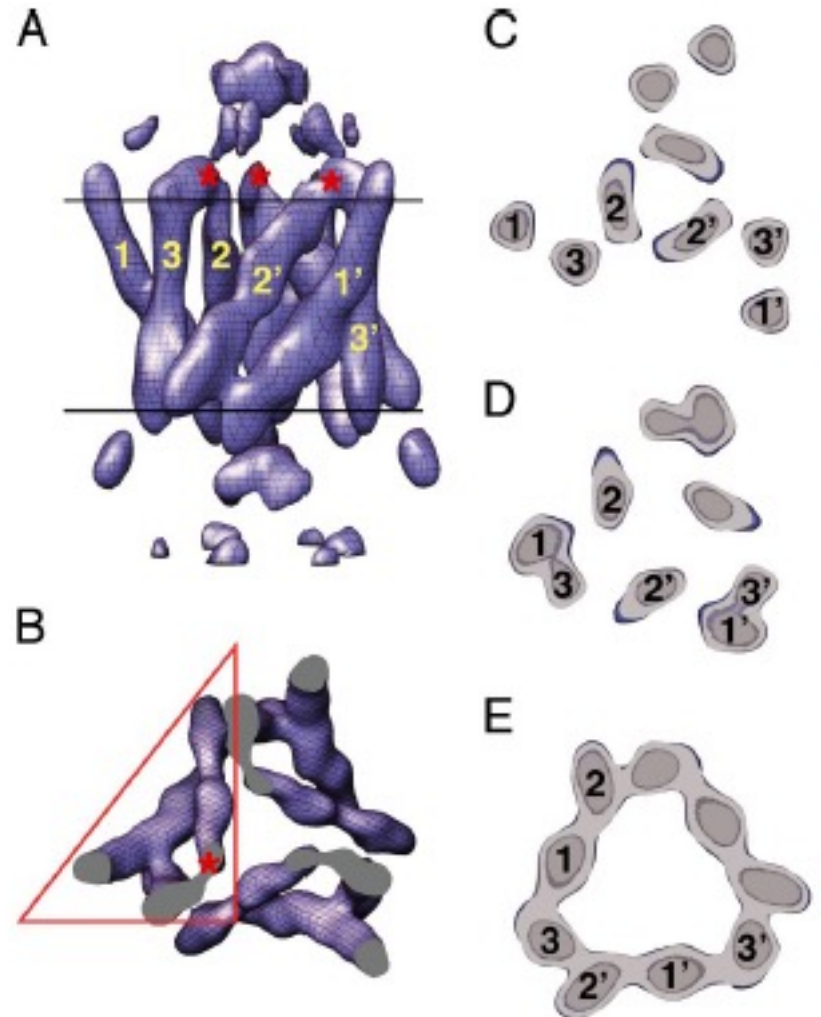
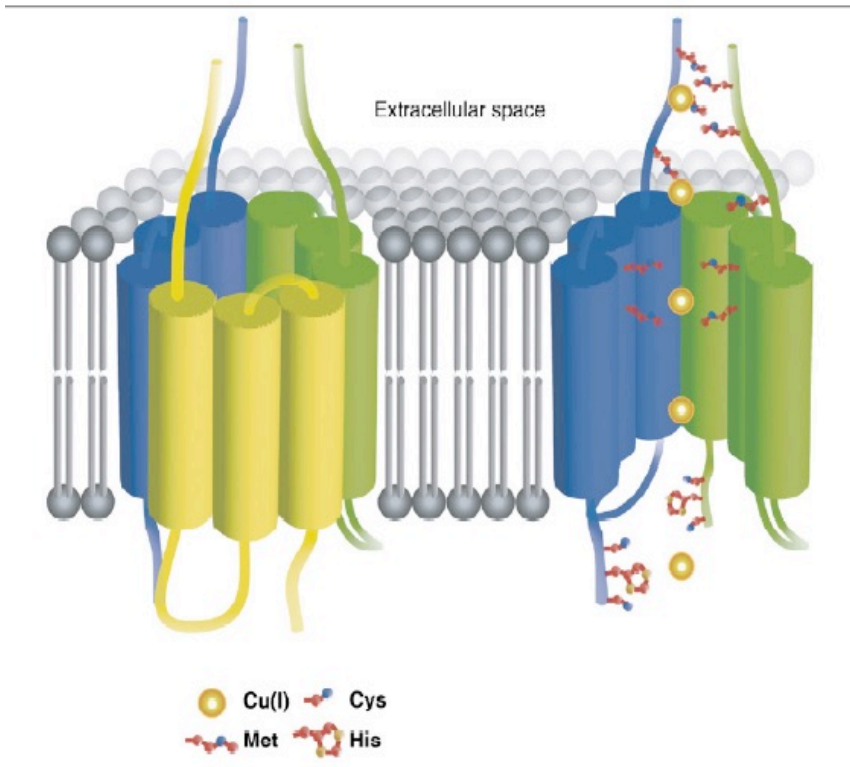


FIG. 1. Model for the primary structure of Ctr high affinity copper transport proteins. Alignment of copper transport proteins from *S. cerevisiae* (ScCtr1, ScCtr3), *S. pombe* (SpCtr4, SpCtr5), human and mouse (h/mCtr1), and *A. thaliana* (AtCopt1). Conserved features in the primary structure are represented 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 MXXM and/or MXM. Yeast and human Ctr1 proteins contain eight and two Mets motifs, respectively. Other conserved features, Ctr1 Met-127 and the MXXXM motif in TMD2, are represented in *black* and *white* characters, respectively. The length of each protein in amino acids is shown on the *right*.

Struttura trimerica del trasportatore Ctr1.



Trasporto intracellulare del rame in epatociti e neuroni. Chaperoni e pompe per lo smistamento del metallo

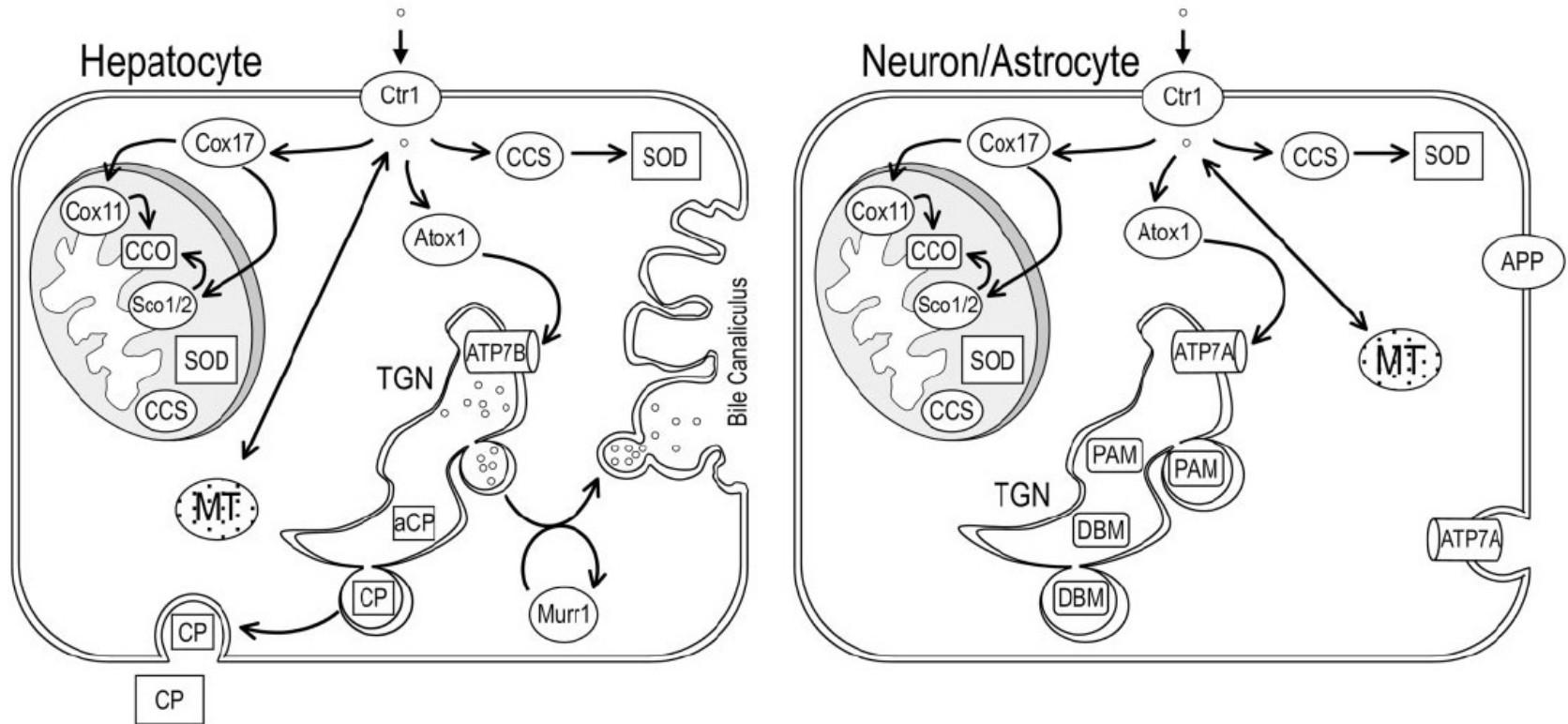


FIGURE 1 Copper transport in hepatocytes, neurons, and astrocytes. Cuproenzymes (rectangles) are dependent on copper chaperones (ellipses) and copper-transporting ATPases (ATP7A and ATP7B) for the delivery of imported copper to biosynthesis and metal-transfer sites. Chaperones and ATPases, which normally 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).

Enzimi che ricevono rame nel 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

Chaperoni del rame

Gli chaperoni del rame sono proteine di piccole dimensioni (circa 70-80 amminoacidi) conservate dai batteri all'uomo. Il rame viene legato come Cu^+ da due residui di cisteina con una coordinazione atipica.

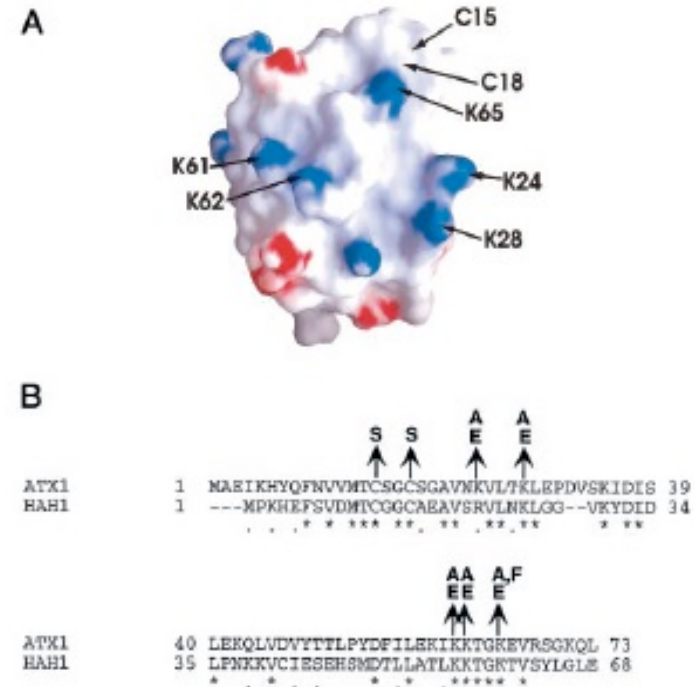
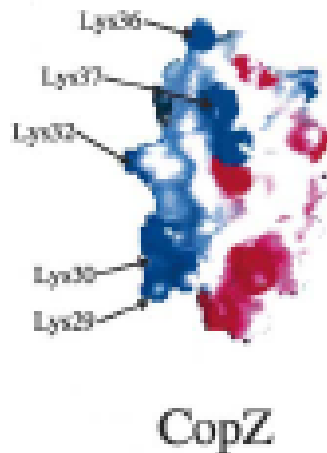


FIG. 1. X-ray crystal structure and sequence alignment of Atx1p. **A**, An electrostatic surface representation of the Hg(II) form of Atx1p with residues selected for mutagenesis indicated. The positively, negatively charged and neutral amino acids are represented in blue, red and white, respectively.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

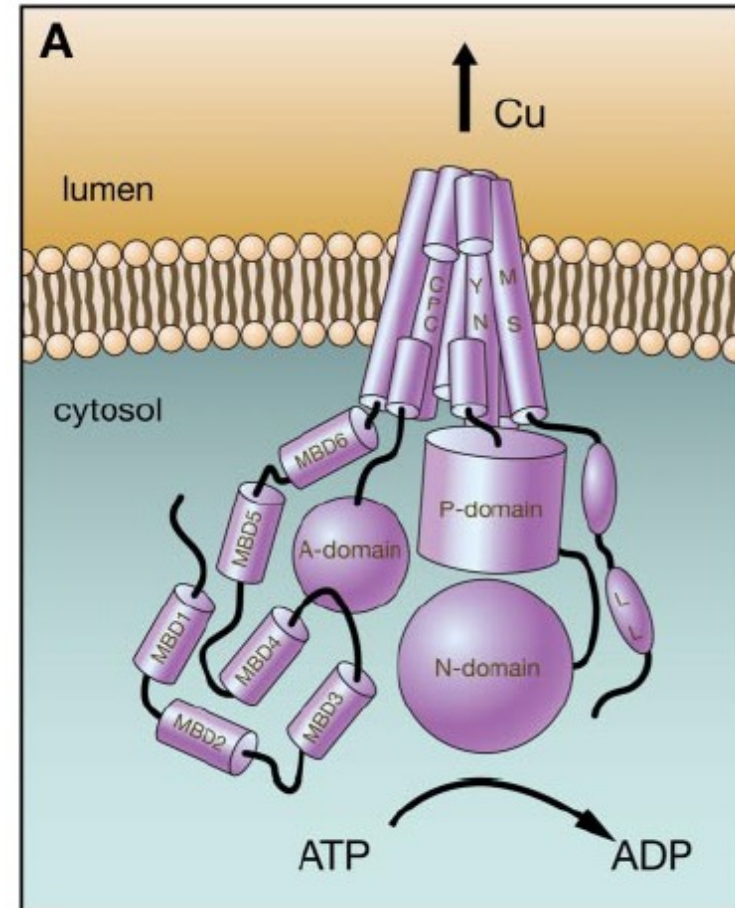
ATPasi di trasporto del rame

Le pompe per il trasporto del rame appartengono alla famiglia delle ATPasi di tipo P o CPx e si trovano in batteri, lievito ed eucarioti superiori. Accoppiano il trasporto del metallo all'idrolisi dell'ATP con formazione di un intermedio acil-fosfato su un residuo di aspartato.

Presentano 8 regioni transmembrana e una regione N-terminale di legame del rame in domini che contengono sequenze conservate MXCXXC.

Nel dominio P e nel dominio N avvengono la fosforilazione dell'aspartato e il legame del nucleotide. Nel dominio A avviene la defosforilazione dell'aspartato.

Batteri	CopA, copB
Lievito	Ccc2
Mammiferi	ATP7A, ATP7B



Meccanismo delle ATPasi di trasporto del rame

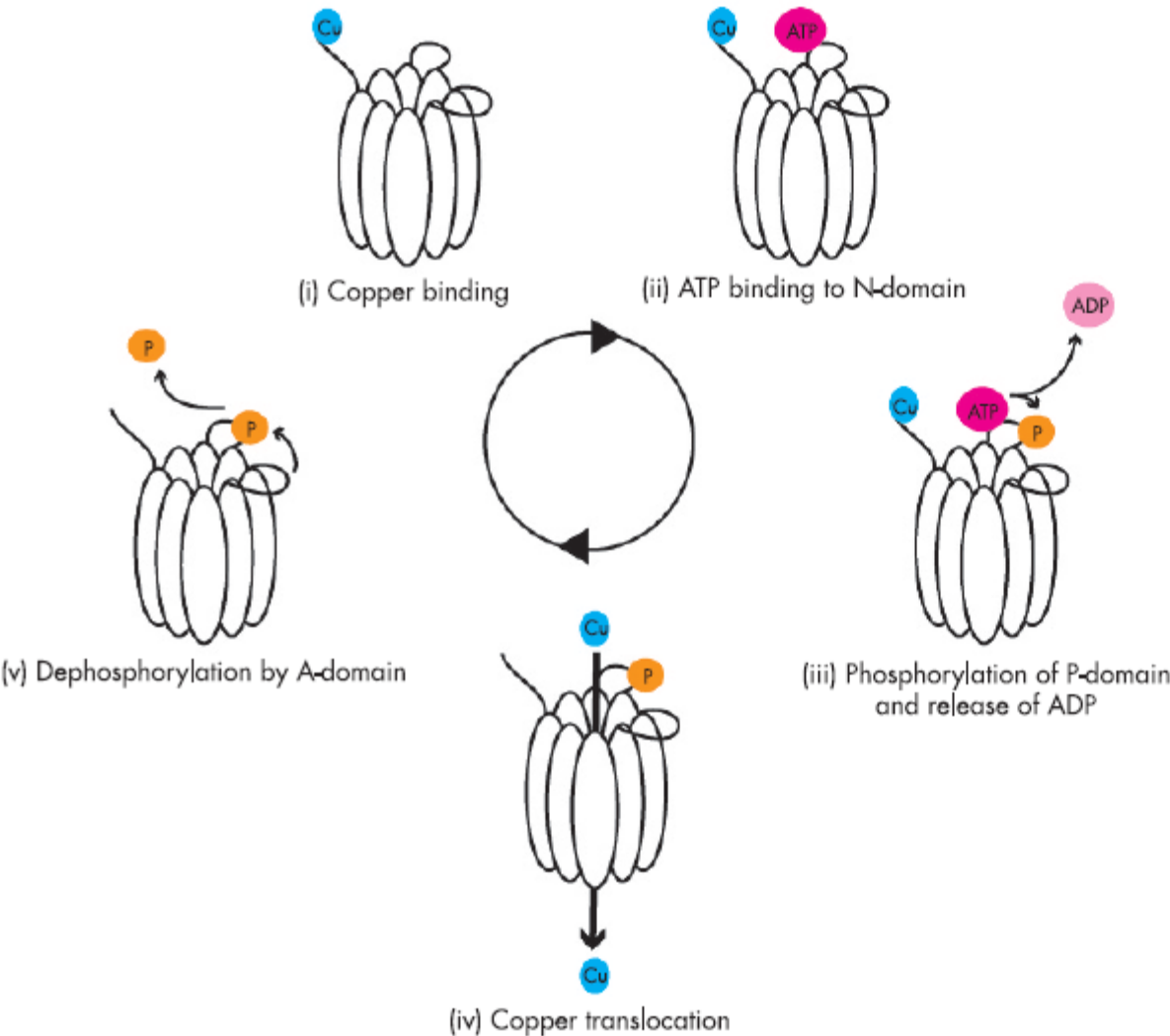


Figure 1 Schematic representation of the general ATPase catalytic cycle. Copper translocation by ATP7A and ATP7B is believed to occur through a general cycling model involving several discrete stages. These stages include (i) binding of the target ion, (ii) binding of ATP to the N-domain, (iii) ATP hydrolysis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

Efflusso del rame dalle cellule: Proteine di Menkes (ATP7A) e Wilson (ATP7B)

- ATP7A possiede sei domini di legame del rame
- È localizzata nel trans-Golgi ed è espressa in enterociti, nell'endotelio della barriera emato-encefalica e numerosi altri tessuti.
- ATP7B possiede sei domini di legame del rame
- È localizzata nel trans-Golgi ed è espressa nel fegato e a bassi livelli nel rene, placenta, cervello e cuore.

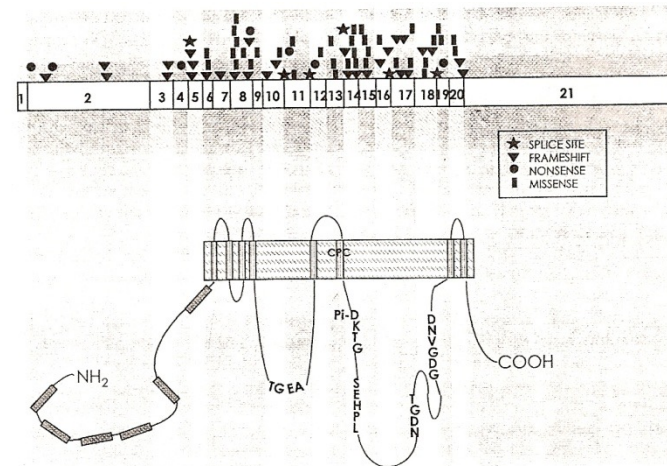
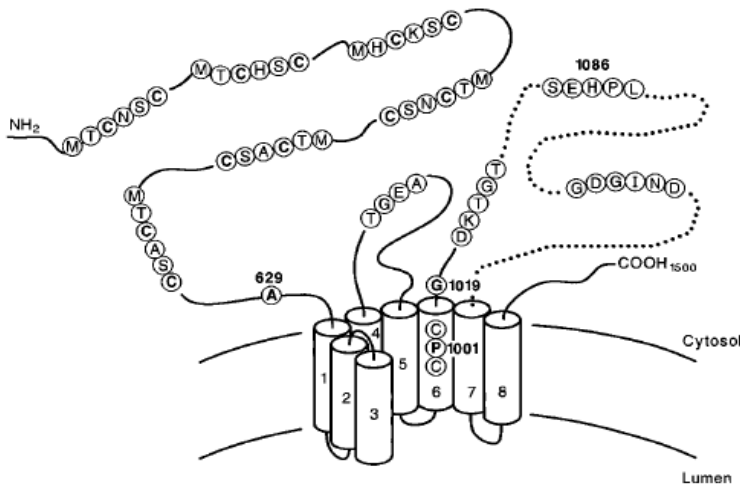
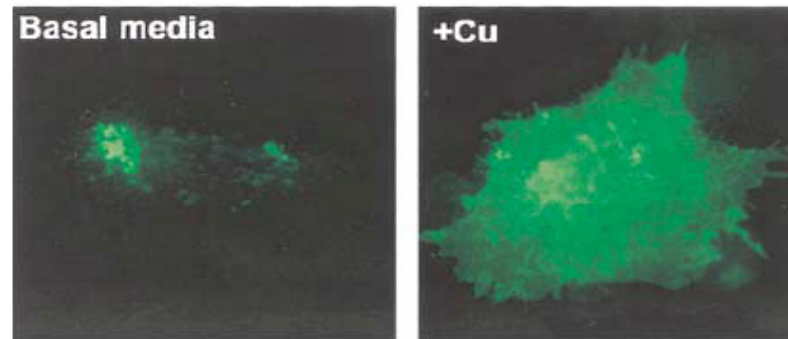


Figure 3 Summary of all published WD mutations in exonic and splice-site-junction sequences (Bull et al. 1993; Tanzi et al. 1993; Figus et al. 1995; Thomas et al. 1995; Loudianos et al. 1996; Waldenström et al. 1996; Kempainen et al. 1997).

La localizzazione subcellulare di ATP7A e ATP7B cambia in funzione della concentrazione di rame

Menkes protein



Wilson protein

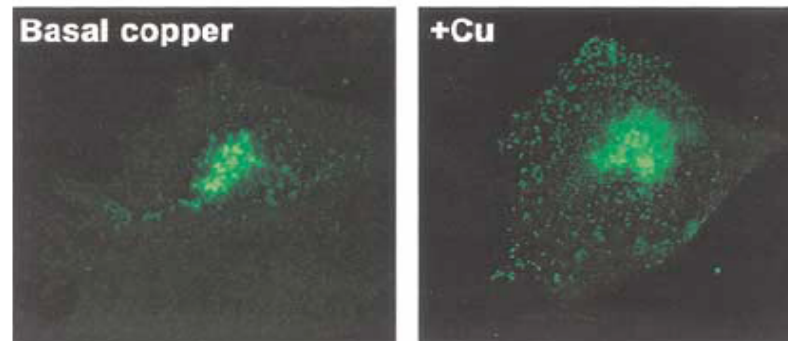
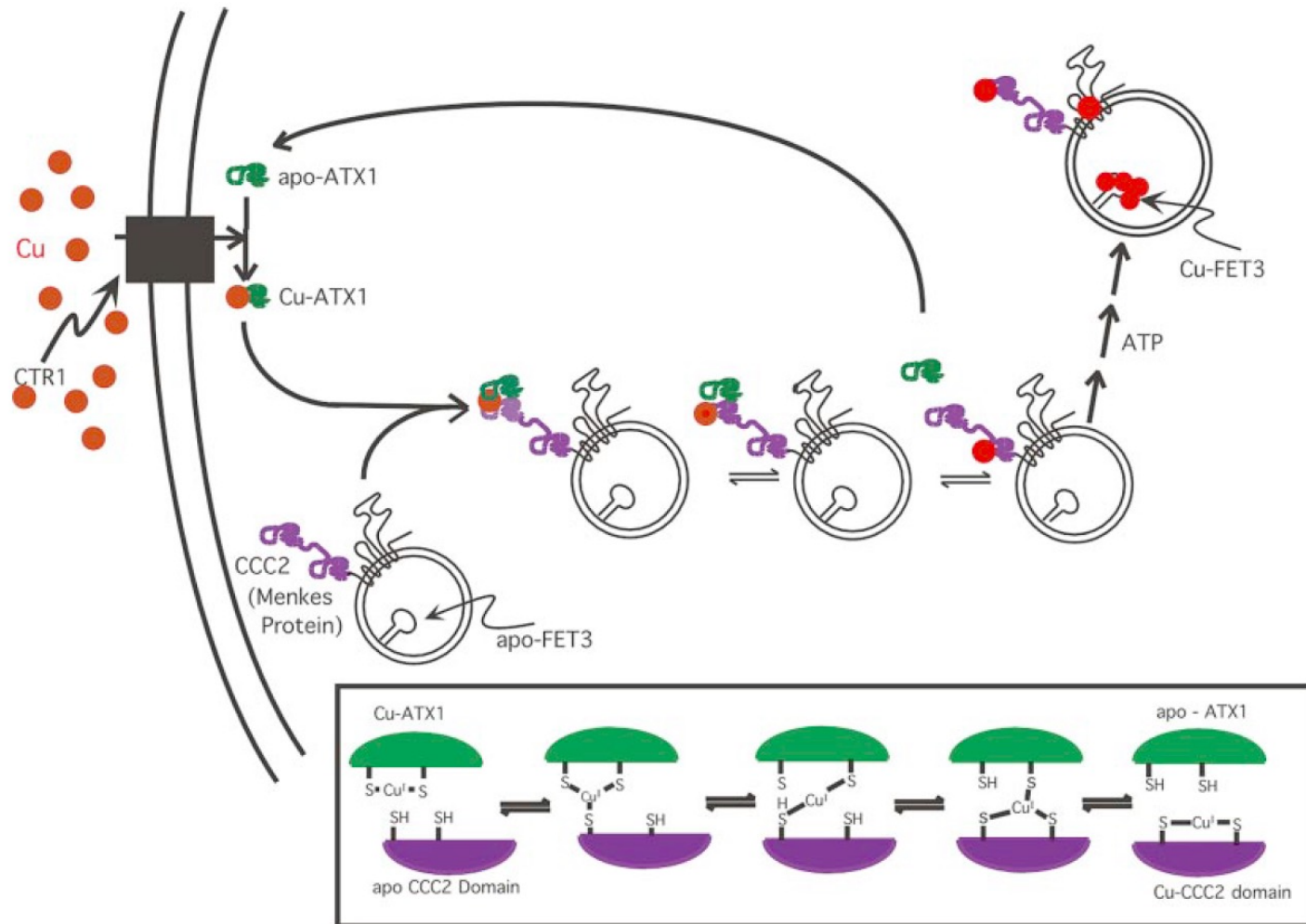


Fig. 5. Copper-dependent localization of the MNKP and WNDP. Immunostaining with specific antibody and fluorescence microscopy demonstrates typical perinuclear localization of MNKP and WNDP in the trans-Golgi network of CHO cells cultured in basal minimal media (*left photos*). Under elevated copper conditions,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

Gli chaperoni e le pompe del rame.

Meccanismo di trasferimento del metallo



Chaperoni e pompe hanno strutture simili

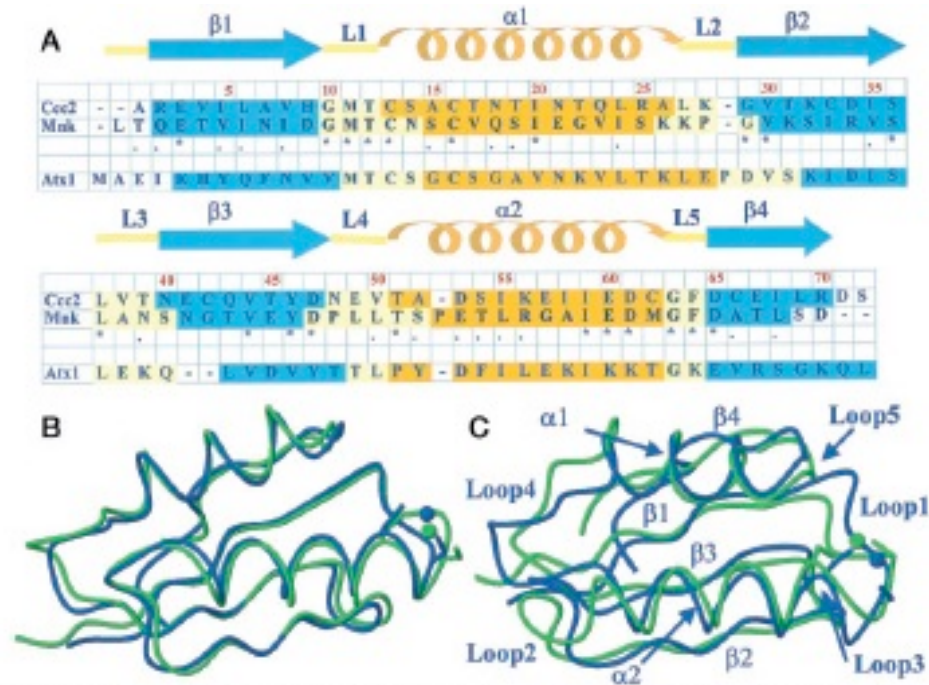
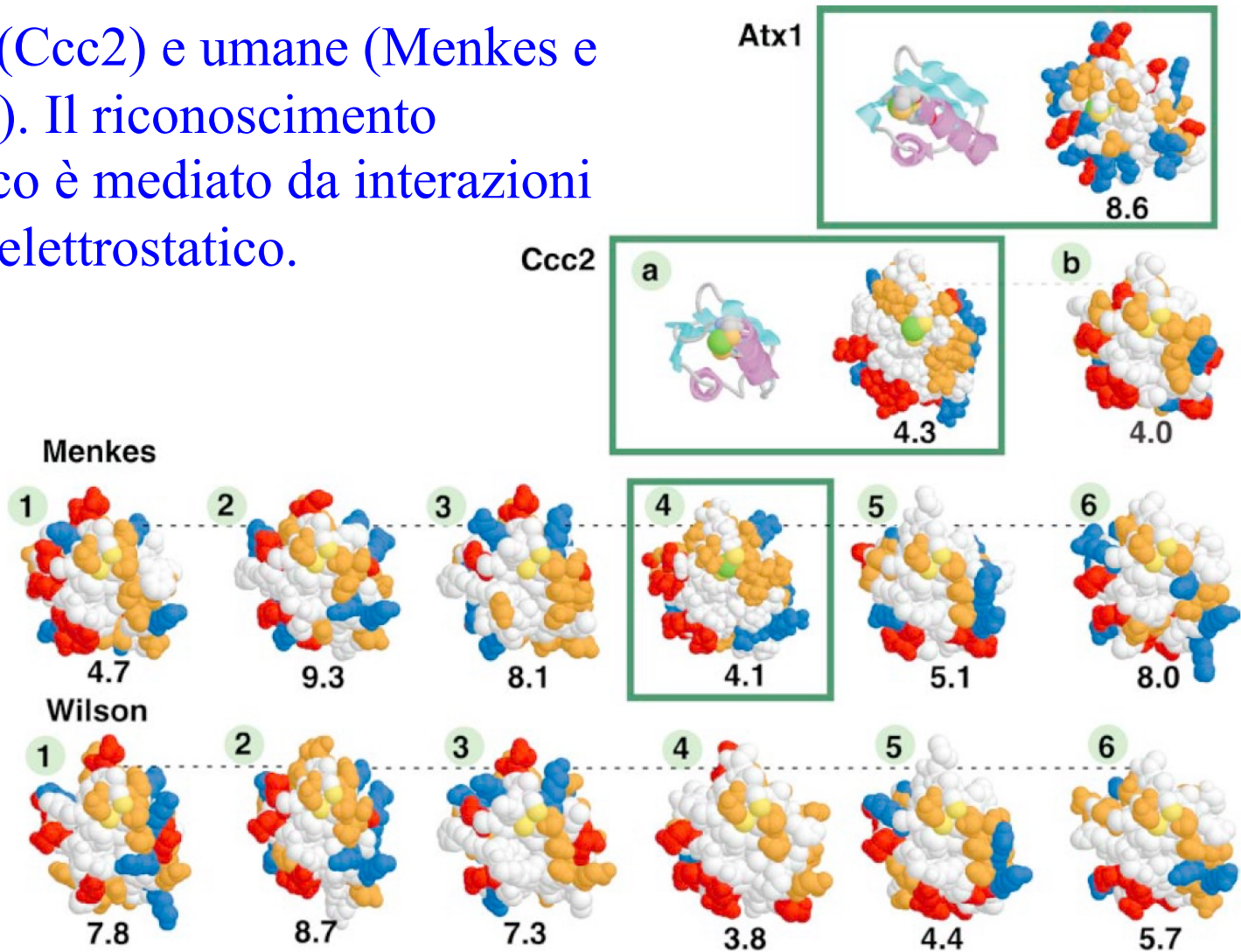


FIG. 11. A, sequence alignment of the Ccc2a amino acid sequence from *S. cerevisiae* with the sequences of the fourth metal-binding domain from Menkes-transporting ATPase (mbd4) (Protein Data Bank accession number 1aw0) (20) and of Atx1. The positions of the Ccc2a secondary structure elements (as found 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 \bullet and * below the sequences. B, comparison of 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.

Struttura di Atx1 e dei domini che legano il rame delle ATPasi di lievito (*Ccc2*) e umane (*Menkes* e *Wilson*). Il riconoscimento specifico è mediato da interazioni di tipo elettrostatico.



Complementarietà di carica tra chaperoni e pompe

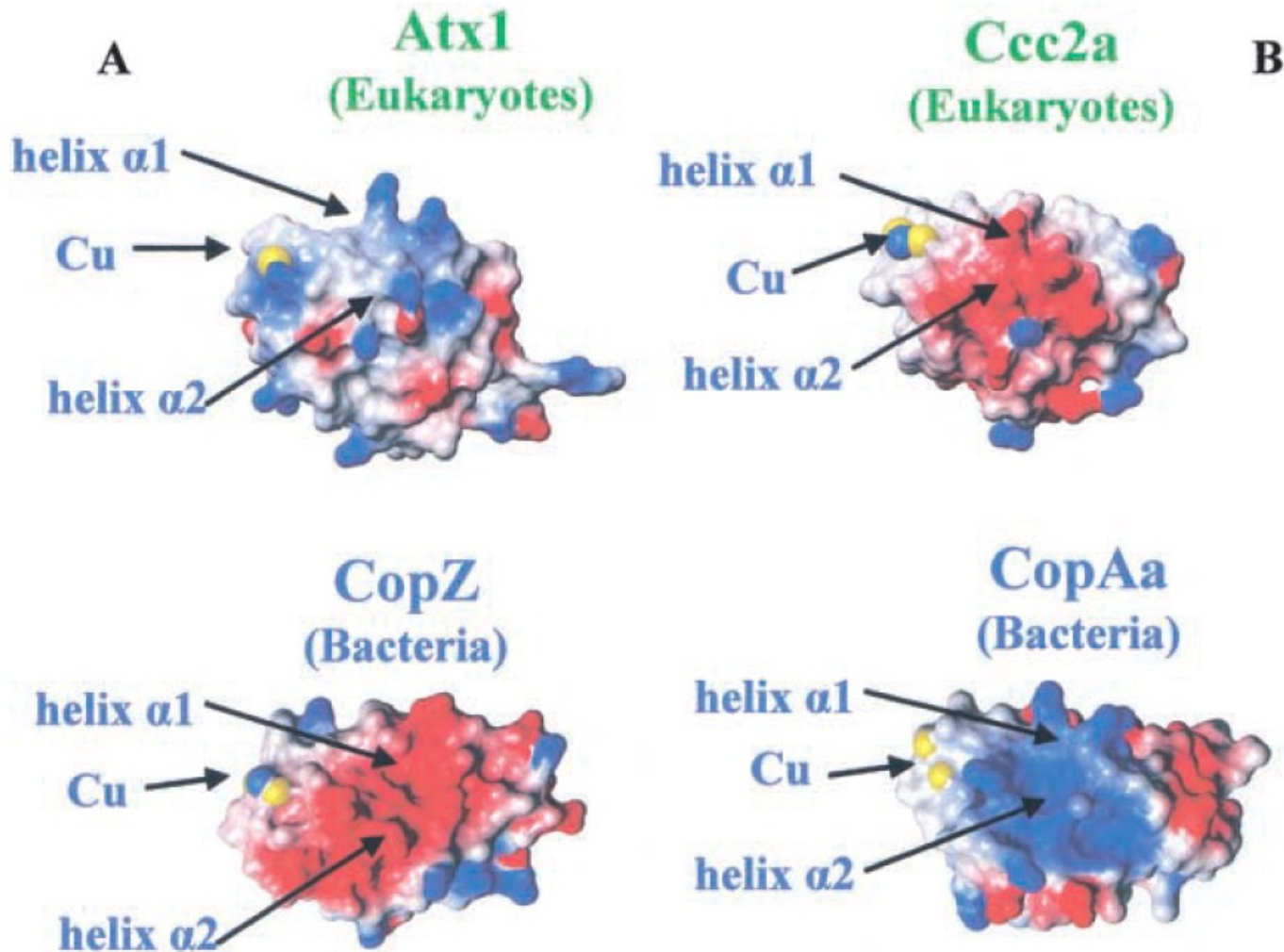
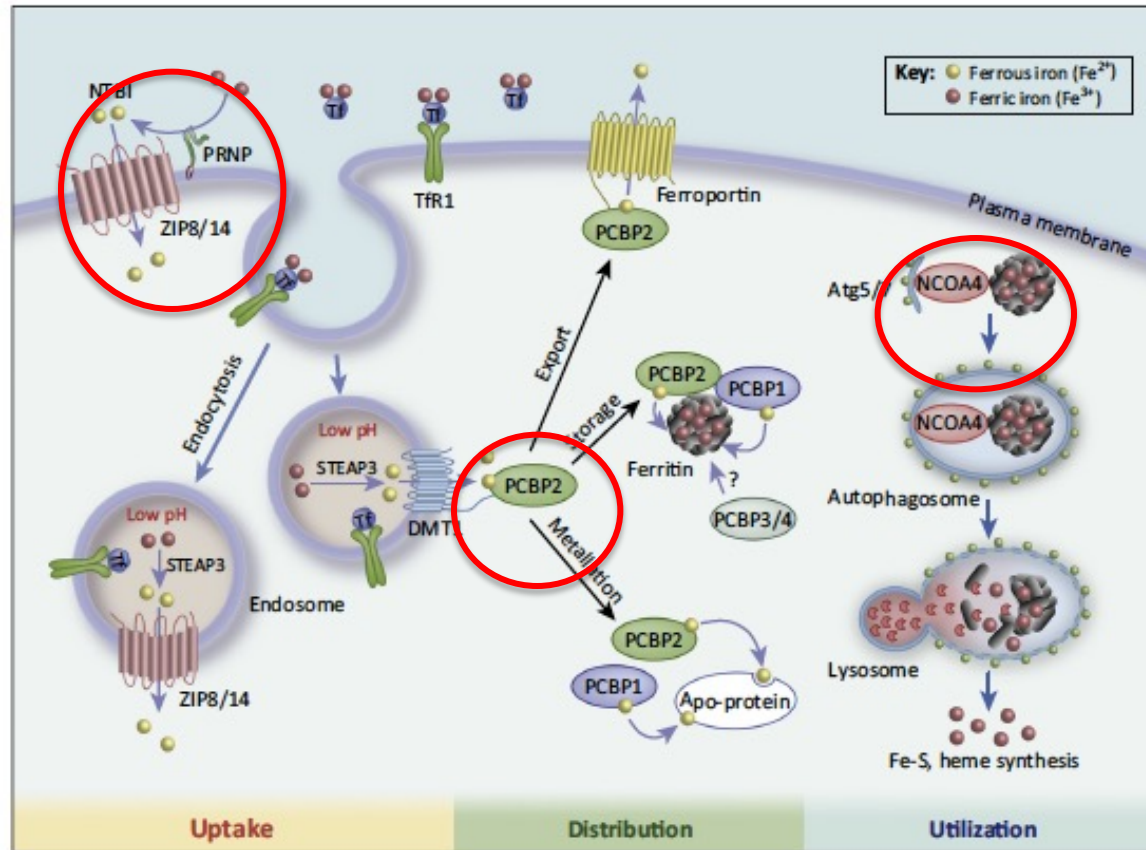


Figure 9 Surface potentials of *S. cerevisiae* Atx1 and *B. subtilis* CopZ structures (A). Surface potentials of *S. cerevisiae* Ccc2a and *B. subtilis* CopAa structures (B).

Nuove proteine coinvolte nel metabolismo intracellulare del ferro



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, Fe³⁺ is released from transferrin (Tf) and free Fe³⁺ is reduced to Fe²⁺ 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 Fe²⁺ to apo-proteins (metallation), such as hypoxia-inducible factor (HIF) prolyl hydroxylases, ferroportin (iron export), and ferritin (oxidation to Fe³⁺ and storage). Nuclear receptor coactivator 4 (NCOA4)-mediated autophagy of iron-loaded ferritin releases iron for utilization in cellular processes (see text).