Metabolism of metals. Iron and copper

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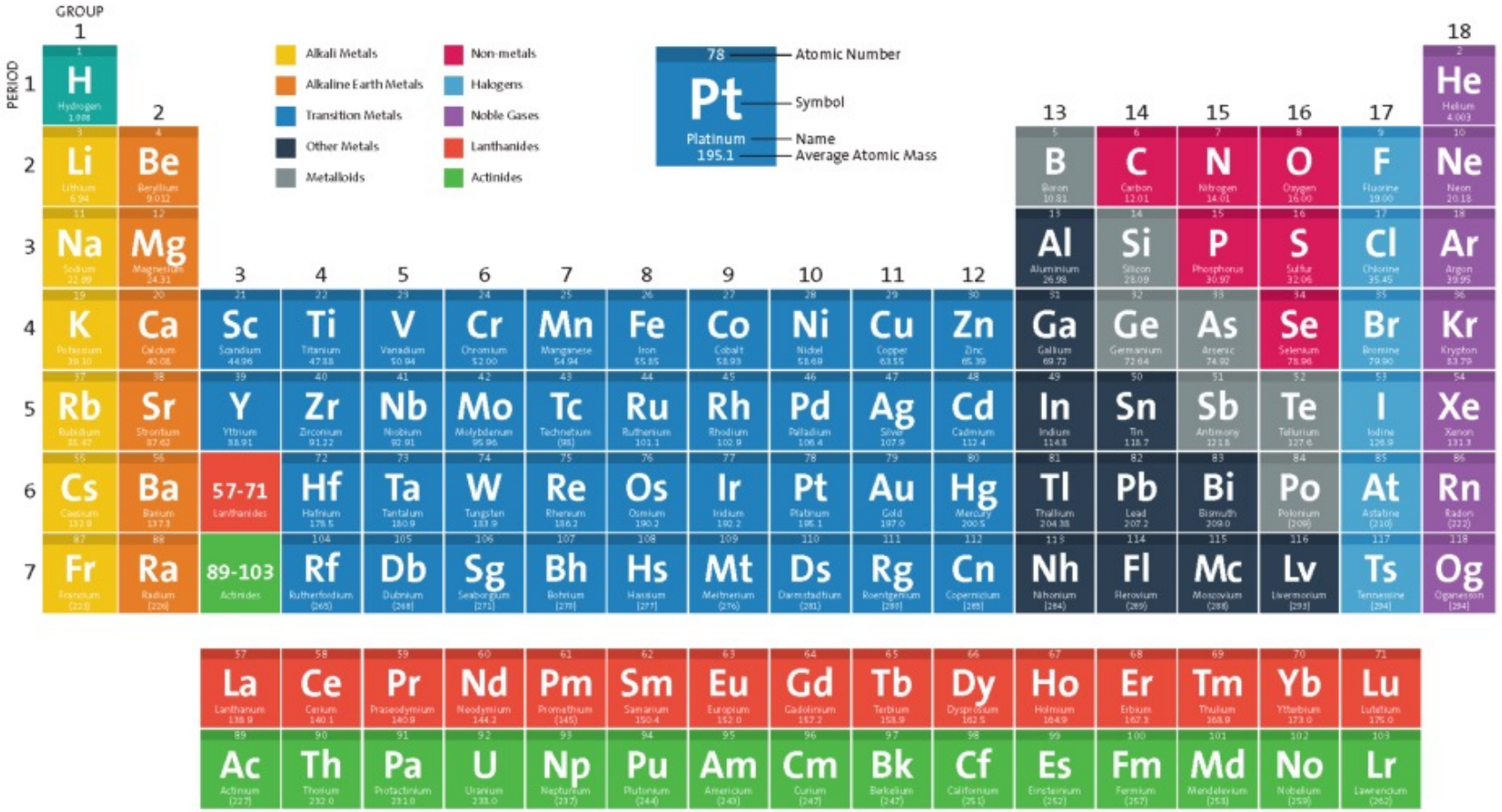
PERIODIC TABLE OF ELEMENTS



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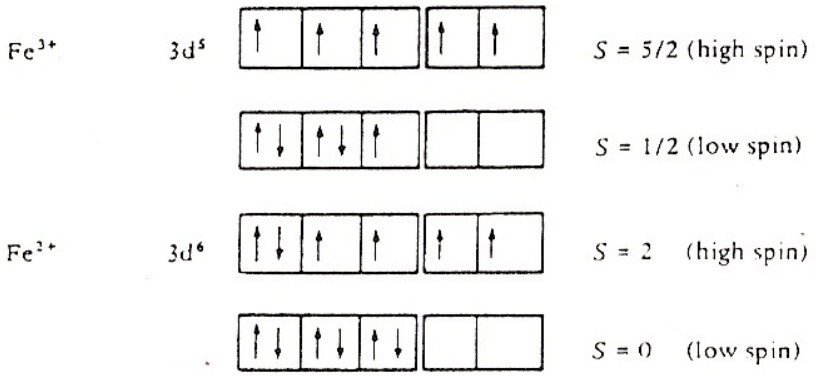
Biological role of iron and copper

Iron and copper are essential metals for all living beings because they are bound to proteins indispensable for life: respiration, photosynthesis, nitrogen fixation, the Krebs cycle, oxygen transport, DNA synthesis, gene regulation etc.

- Fe
  - cytochrome oxidase – heme proteins (cytochromes, hemoglobin) – Fe-S cluster proteins (electron transport chain) – non-heme iron proteins
- Cu
  - cytochrome oxidase – superoxide dismutase – ceruloplasmin – lysyl oxidase – tyrosinase – dopamine β hydroxylase - metallothionein

Iron

- In physiological conditions iron is found in two oxidation states:
  - Fe^{3+} d^5
  - Fe^{2+} d^6

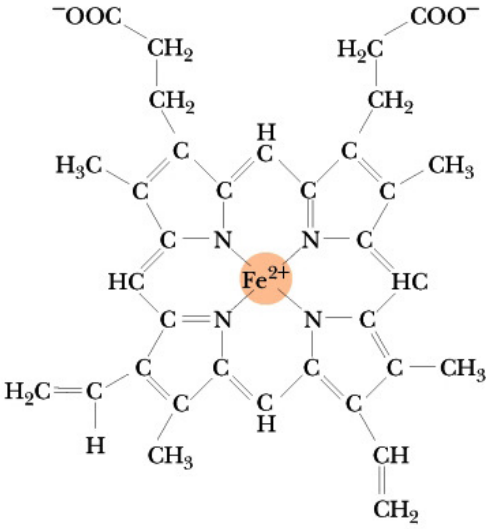


The redox potential of iron in proteins varies from -300 mV to +700 mV

Fe^{2+} is unstable in aerobiosis and is rapidly oxidized at pH 7

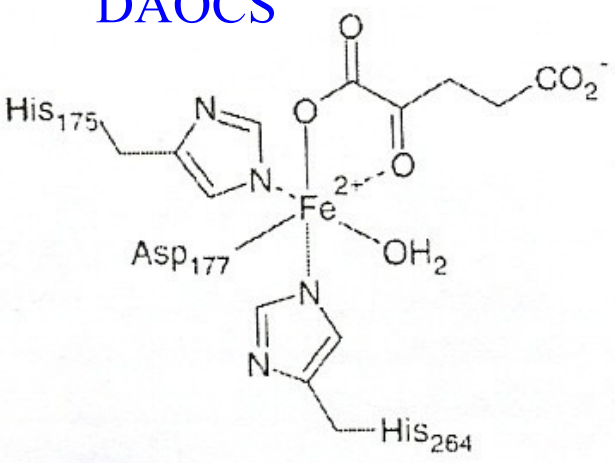
Fe^{3+} is insoluble at pH 7 (10^{-18} M)

Structures of iron-binding sites in proteins

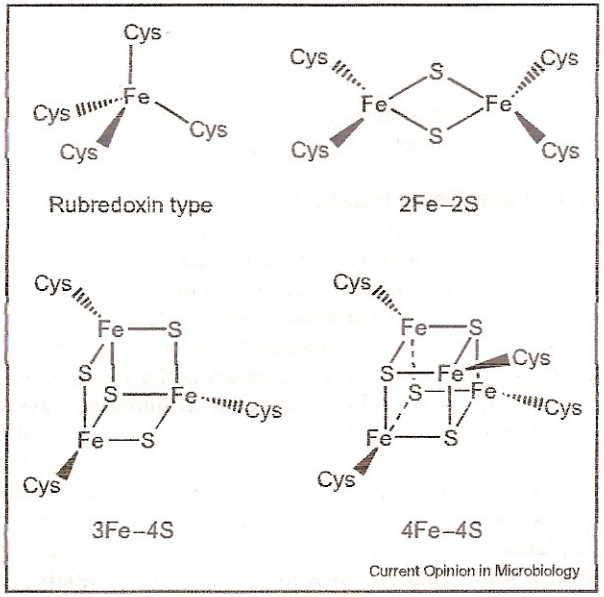


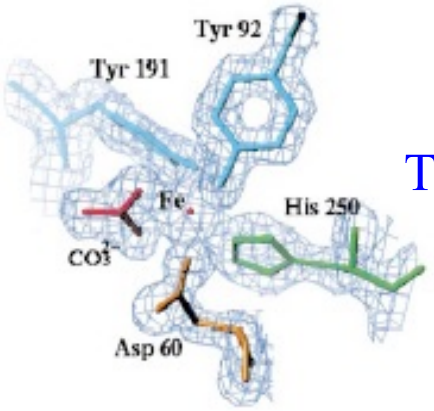
Eme
(Fe-protoporfirina IX)

DAOCS



Fe-S cluster

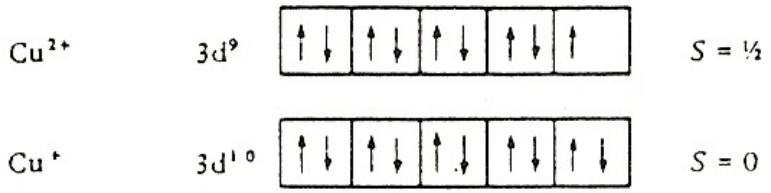




Transferrin

Copper

- In physiological conditions copper is found in two oxidation states:
  - Cu^{2+} d^9
  - Cu^{+} d^{10}



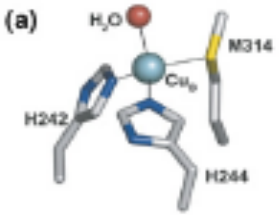
The redox potential of copper in proteins varies from +200 mV to +750 mV

Cu^{+} is unstable in aerobiosis and is rapidly oxidized at pH 7

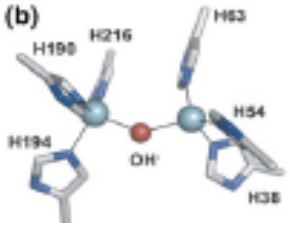
Both Cu^{2+} and Cu^{+} are soluble at pH 7

Structures of copper-binding sites in proteins

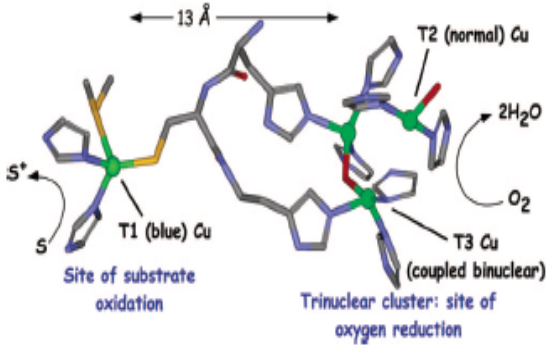
Mononuclear

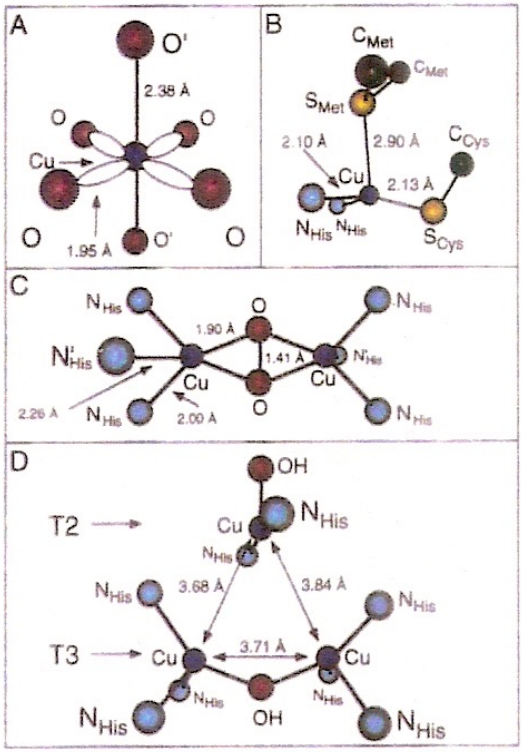


Binuclear

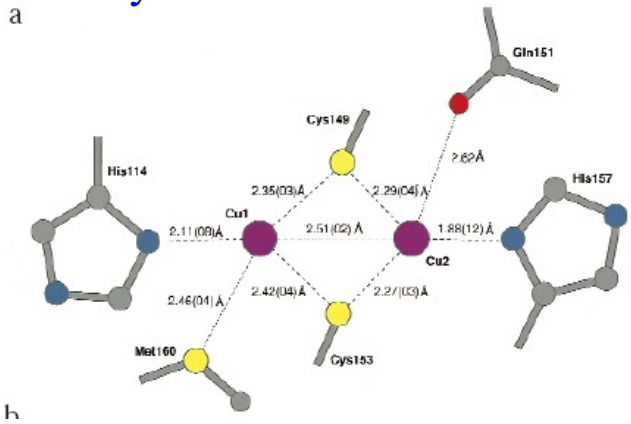


Trinuclear

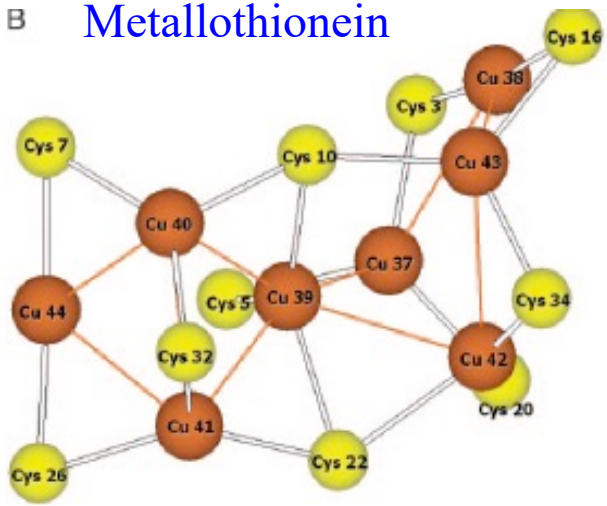




CuA cytochrome oxidase



B Metallothionein



Biological role of iron and copper

Iron and copper are toxic if present at high levels and if they are not complexed to proteins because they react with O_2 generating reactive oxygen species (ROS) that damage proteins, DNA and lipids (membranes)

Metal-catalyzed Haber-Weiss reaction

$$O_2 + Fe^{2+} \rightarrow O_2^{-} + Fe^{3+}$$

$$2\ O_2^{-} + 2\ H^{+} \rightarrow O_2 + H_2O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + OH\cdot$$

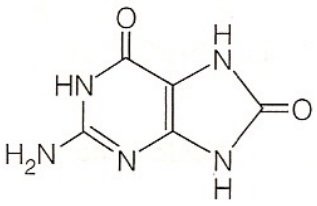
Metal-catalyzed oxidation reactions

- Proteins: modifications of amino acids

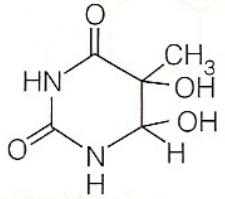
Amino acid	Oxidation product
Cysteine	Disulfide, cysteic acid
Methionine	Methionine sulfoxide, sulfone
Tryptophan	Hydroxytryptophan, nitrotryptophan, kynurenine
Phenylalanine	2,3-dihydroxyphenylalanine, hydroxyphenylalanine
Tyrosine	3,4 dihydroxyphenylalanine, tyrosine dimers, dinitrotyrosine
Histidine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

Metal-catalyzed oxidation reactions

- DNA: oxidation of bases

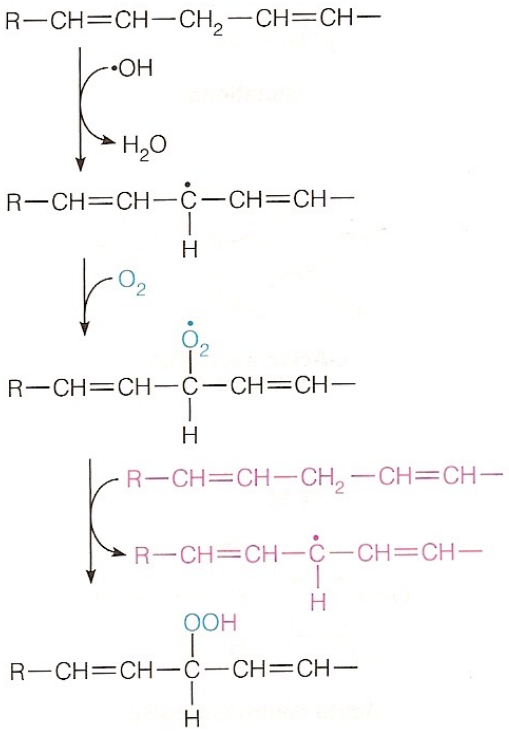


8-Ossoguanina



Glicole della timina

- Lipids: peroxidation

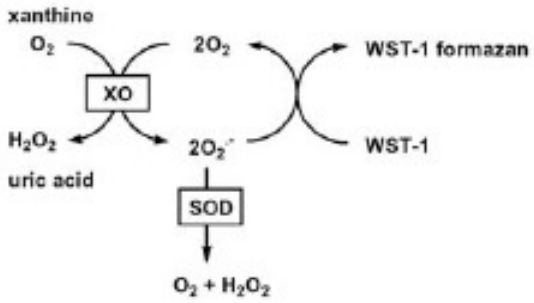


Perossidazione dei lipidi

How is oxidative stress measured?

- Indicators of oxidative stress
  - Antioxidant enzymes (SOD, catalase, peroxidase): measure of enzyme activity or expression levels
  - Non-enzymatic antioxidants (glutathione, vitamin E)

- SOD
  - Spectrophotometric measure at 440 nm of inhibition of formazan production in the presence of superoxide



- Catalase
  - Spectrophotometric measure of H_2O_2 at 240 nm
- Glutathione peroxidase
  - Coupled assay measures NADPH consumption at 340 nm

$$\text{R-OOH} + 2\,\text{GSH} \xrightarrow{\text{GPx}} \text{R-OH} + \text{GSSG} + H_2O$$

$$\text{GSSG} + \text{NADPH} + H^+ \xrightarrow{\text{GR}} 2\,\text{GSH} + \text{NADP}^+$$

How is oxidative stress measured?

- Assay of reactive oxygen and nitrogen species with fluorescent probes as DCFDA (dichlorofluorescein diacetate) or DAF (diaminofluorescein) or chromogens as NBT (nitroblue tetrazolium)

- Assay of damaged macromolecules due to oxidative stress
  - Malondialdehyde (MDA), oxidized proteins

Control of iron and copper toxicity

- Reactive oxygen species (ROS) detoxification systems
  - Enzymatic: superoxide dismutase, catalase and glutathione peroxidase
  - Non enzymatic: vitamin E and C, glutathione, uric acid, bilirubin ecc.
- Repair systems and storage proteins
  - Ferritin
  - Metallothionein

- Cellular mechanisms for aquisition of iron and copper are tightly regulated to ensure adequate intracellular levels of these metals

Iron transport in bacteria

- Bacteria produce siderophores, low molecular weight molecules that chelate Fe^{3+} with high affinity ($K_a > 10^{30}$ M).
- Siderophores form octahedral hexadentate complexes with iron, via hydroxammate, α-hydroxy-carboxylate or catecholate groups.
- Most siderophores are produced by NRPS.
- Siderophores are internalized by a receptor-mediated mechanism.
- Siderophores are virulence factors of pathogenic bacteria.

Siderophore structures

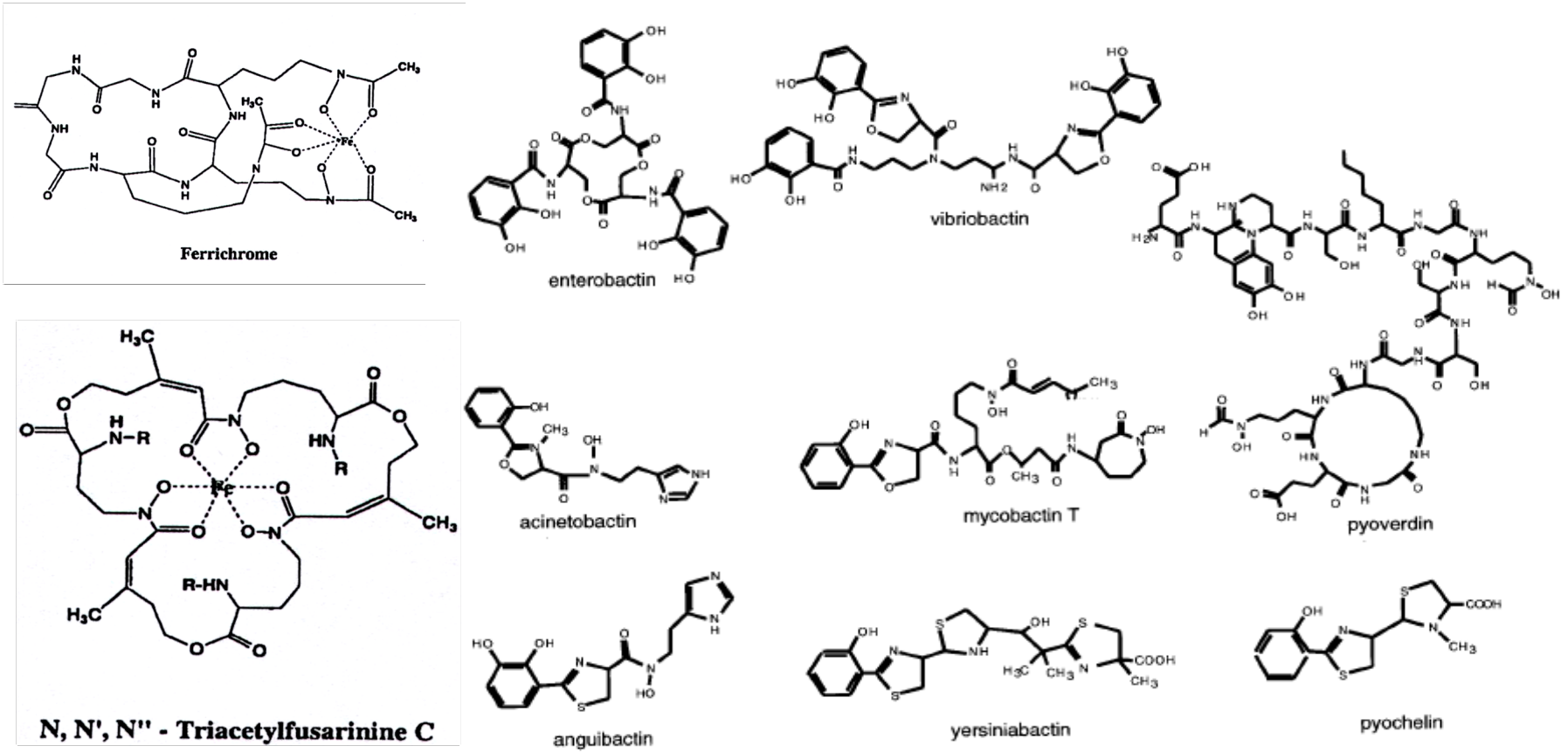
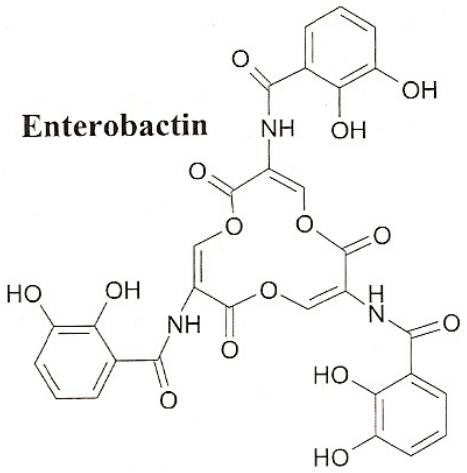
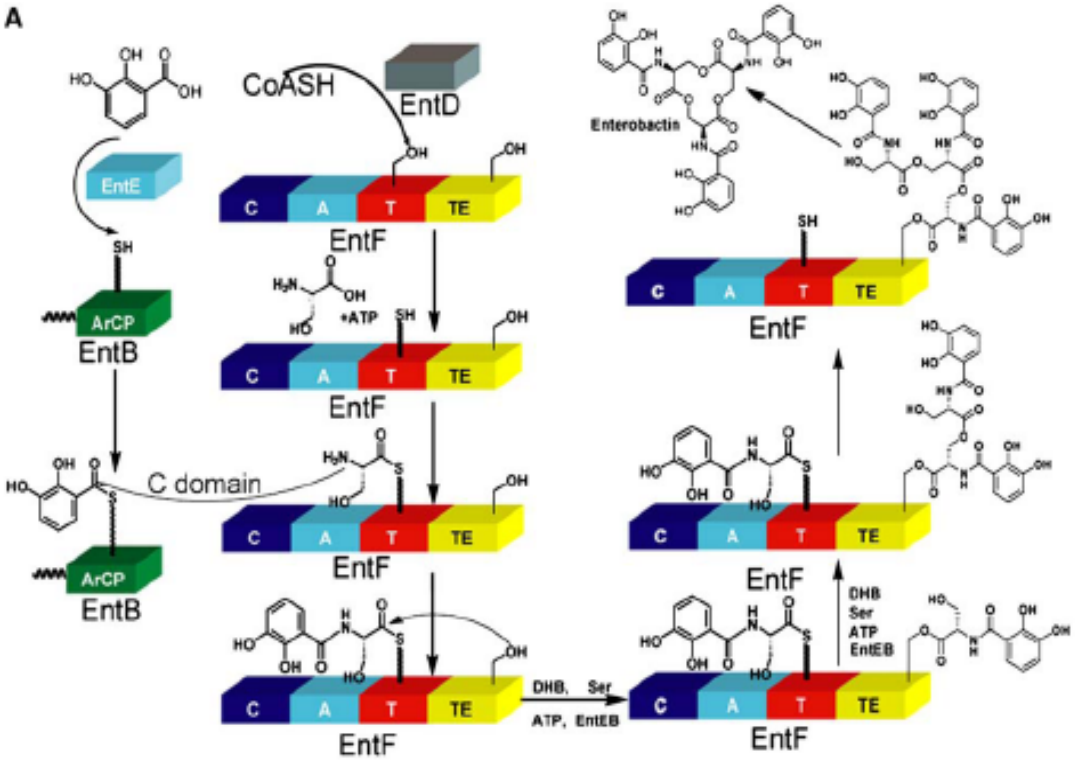


FIG. 1. Structures of various bacterial siderophores: enterobactin from *E. coli*; vibriobactin from *V. cholerae*; acinetobactin from *Acinetobacter calcoaceticus*; mycobactin T *from M. tuberculosis*; pyoverdin and pyochelin from *P. aeruginosa*; anguibactin from *V. anguillarum*; and yersiniabactin from *Y. pestis*.

Enterobactin synthesis in *E. coli*

Enterobactin is produced by an NRPS from 2,3-dihydroxybenzoic acid (DHB) and serine. The EntE and EntB genes synthetize DHB and translocate it on the NRPS encoded by the EntF gene where condensation with serine and siderophore assembly take place.





Mechanisms of siderophore transport in bacteria

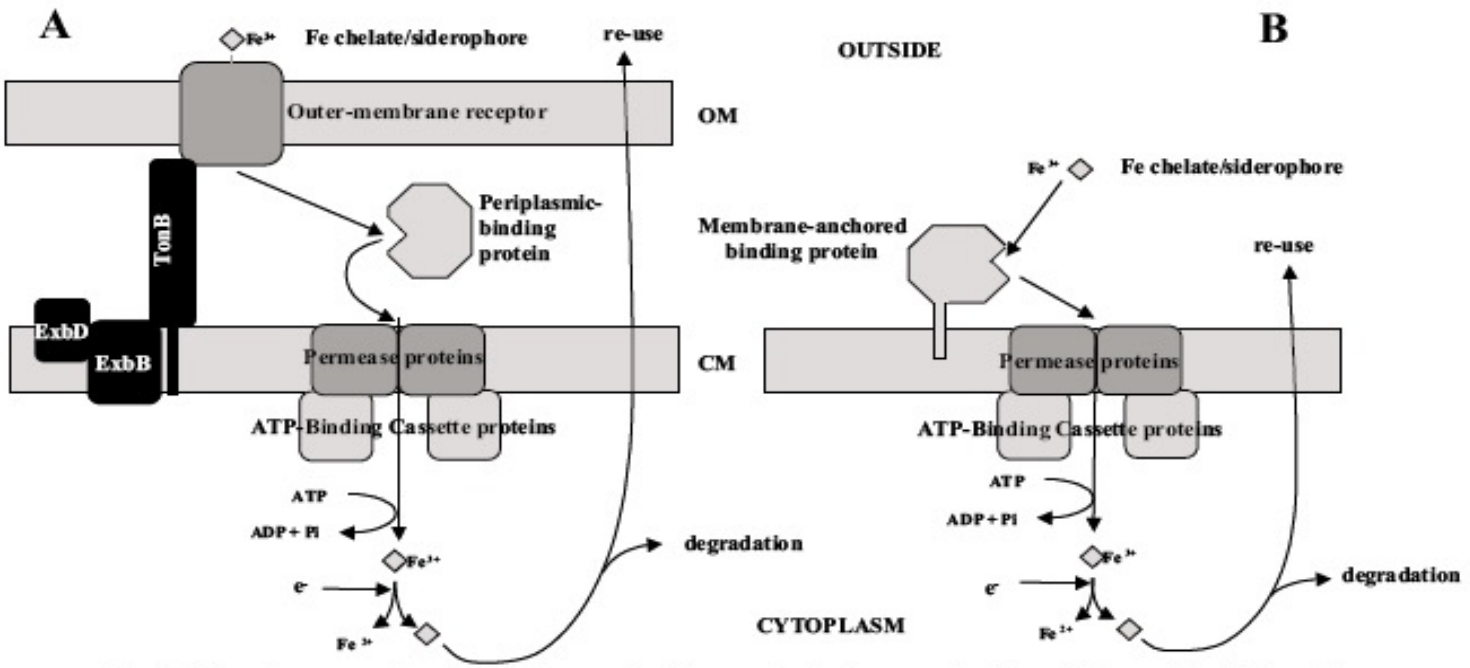


Fig. 1. Schematic representation of siderophore-mediated iron uptake in Gram-negative (A) and Gram-positive (B) bacteria.

Mechanisms of siderophore transport in *E. coli*

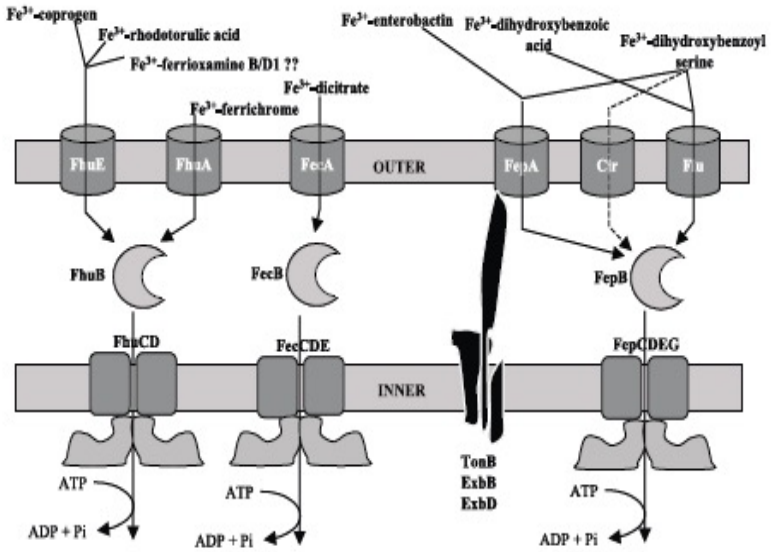


Fig. 4. Schematic representation of siderophore-mediated iron uptake systems in *E. coli* K-12. Note that the TonB-ExbB-ExbD interacts with all the OM receptors shown (not just FepA).

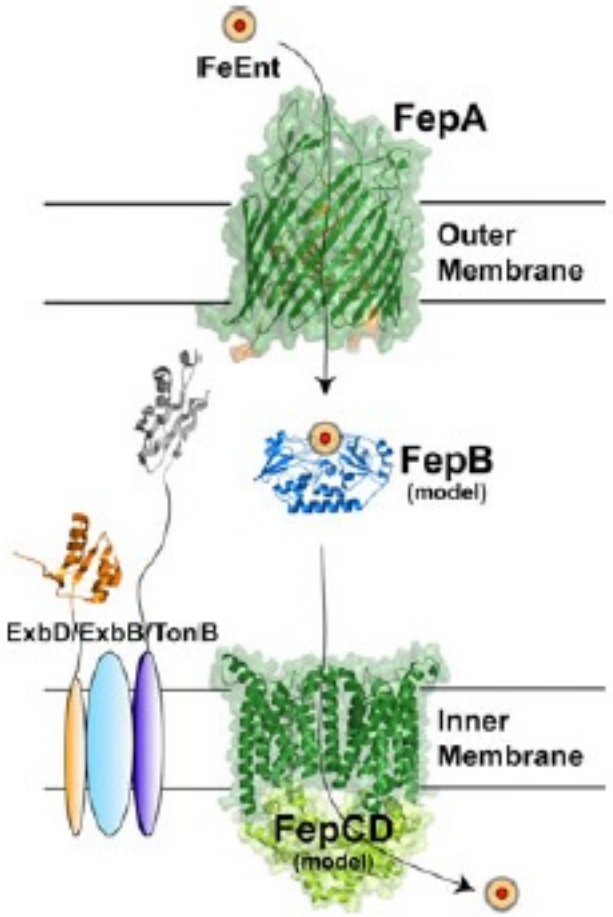


Fig. 3 A representation of the siderophore uptake pathway in *E. coli* using ferric-enterobactin (FeEnt) transport as an example (see text for details)

There are specific systems for each siderophore

Iron transport in bacteria.
Structural model of the FhuA-TonB-FhuD complex for ferrichrome internalization

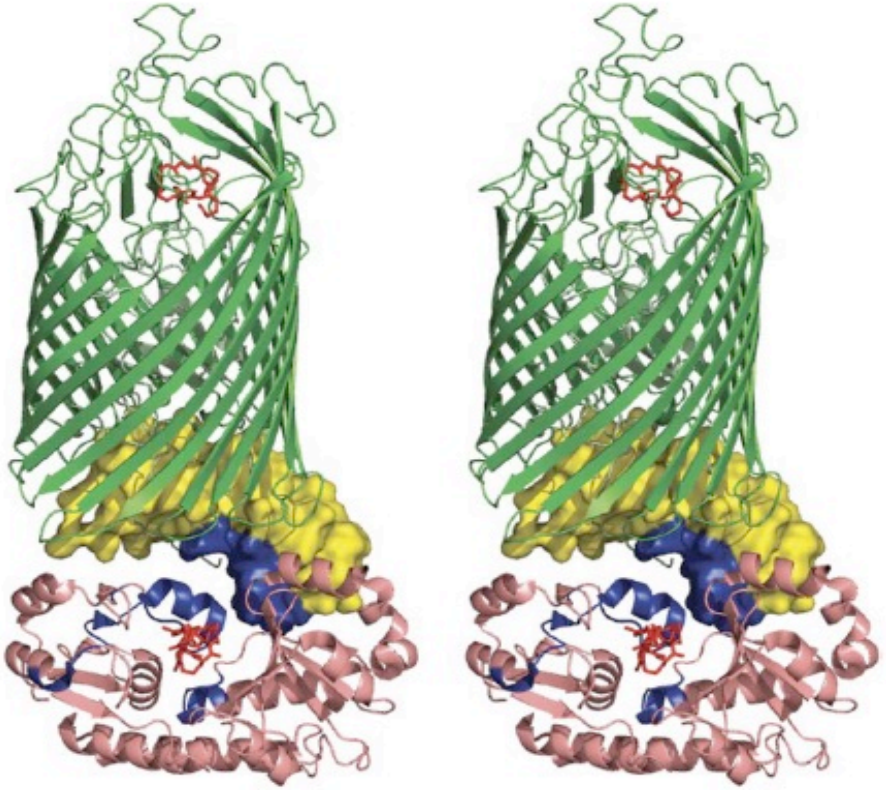


FIGURE 9. **Model of a FhuA-TonB-FhuD ternary complex.** Stereo image depicting a possible ternary complex between FhuA, TonB, and FhuD. FhuD (PDB code 1EFD) was manually docked under the TonB-FhuA crystal structure (PDB code 2GRX) using phage display-identified protein-protein interaction surfaces as docking constraints. Complementary phage display-identified surfaces are colored *blue* on both TonB (*yellow*, surface representation) and FhuD (*salmon*, ribbon representation). The orientation localizes the FhuD siderophore-binding site beneath the lumen of FhuA (*green*, ribbon representation). For clarity, a molecular surface is projected on TonB.

Mechanisms of iron transport in bacteria.

- Transport of Fe^{2+}: feoAB system
  - Induced in anaerobiosis
- Pathogenic bacteria: aquisition also through receptors for host iron-proteins
  - Receptors for transferrin and lactoferrin
  - Receptors for heme

These receptors are analogous to those for siderophore transport: they are TonB-dependent and require an ABC-permease system

Proteins of iron storage bacterioferritin and Dps

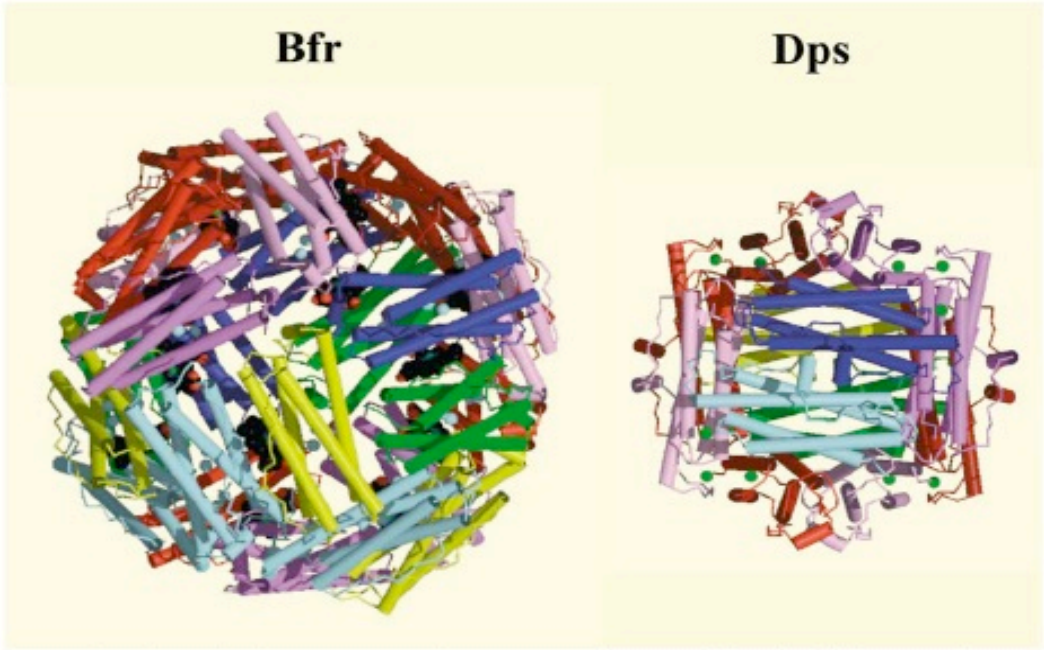
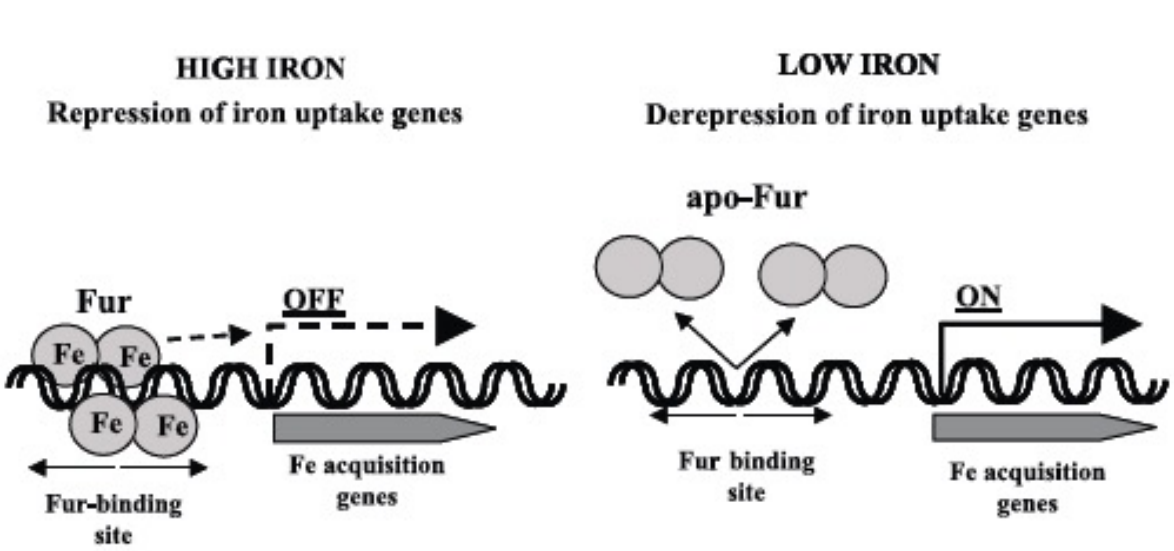


Fig. 6. Structures of the 24-meric and 12-meric iron storage proteins. Structures shown are of bacterioferritin [77] and Dps [78] from *E. coli*, and are approximately to scale. The haem groups and Mn atoms associated with Bfr are shown as black/red and blue space-filled molecules; sodium ions in the Dps structure are in green. Pictures were obtained from the Protein Data Bank [23].

Regulation of iron transport in bacteria

The repressor Fur is a homodimer that binds between -35 and -10 sequences of promoters of regulated genes, to a consensus sequence

NAT(A/T)AT NAT(A/T)AT N AT(A/T)ATN



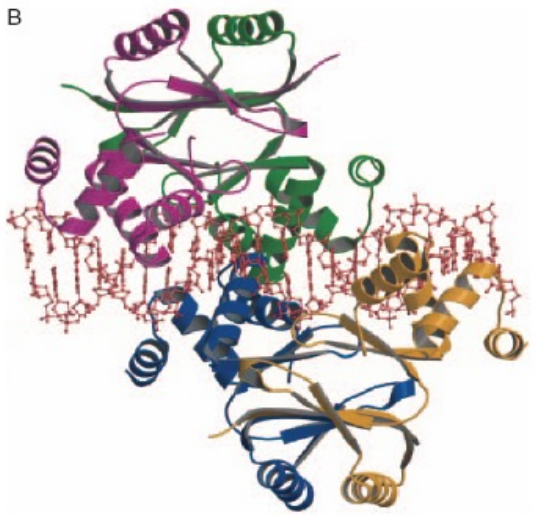
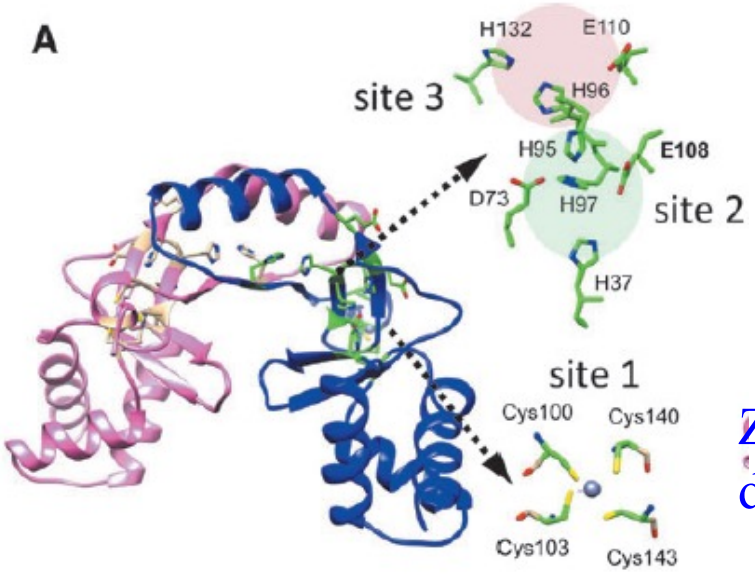


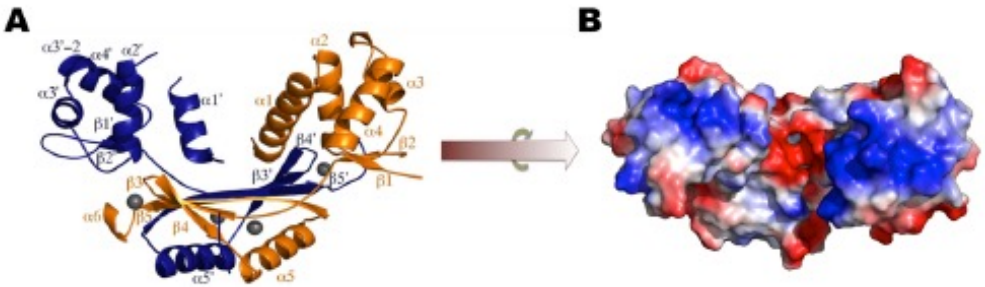
Fig. 8. Schematic representation of Fur-mediated gene repression.

Fur possesses three metal-binding sites

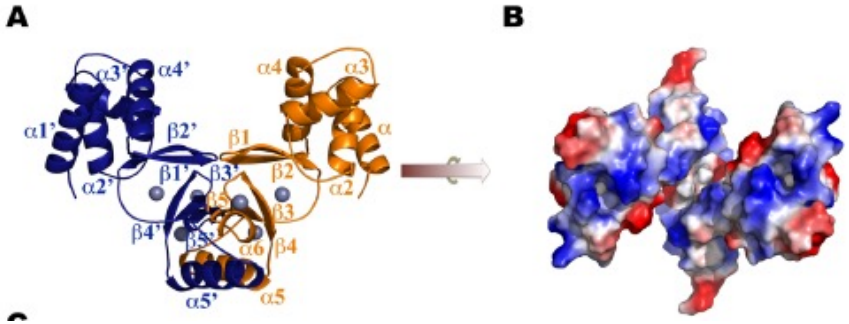


Zn is necessary for dimerization

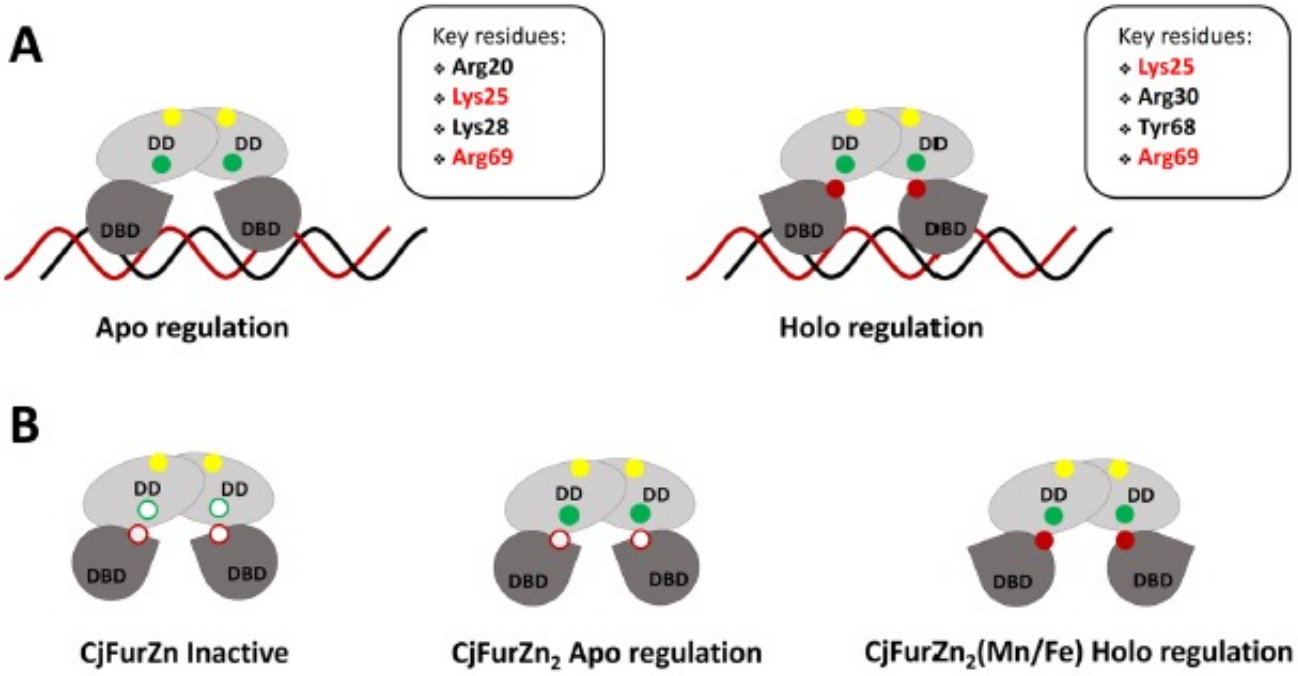
Structure of apo-Fur



Structure of holo-Fur



Model for Fur-mediated regulation



Fur-regulated genes in *E. coli*

Table 1
Fur- and iron-regulated genes in *E. coli* K-12

Gene	Function	+/−
acnA	Aconitase, [Fe–S] protein	+
bfd	Release of iron from Bfr?	−
bfr	Iron storage	+
cir	Ferric dihydroxybenzoate uptake	−
cyoA	Terminal respiratory oxidase subunit	−
entABCDEF	Enterobactin biosynthesis	−
entS	Export of enterobactin	−
exbBC	Siderophore and vitamin B_{12} transport	−
fecABCDE	Ferric dicitrate transport	−
fepA	Ferri-enterobactin transport	−
fepBCDEG	Ferri-enterobactin transport	−
fes	Ferri-enterobactin utilisation	−
fhuABCD	Ferric hydroxamate uptake	−
fhuE	Ferric coprogen rhodotorulate uptake	−
fhuF	Ferrioxamine utilisation	−
fiu	Dihydroxybenzoyl serine uptake	−
flbB	Motility	−
ftnA	Iron storage	+
fumA	Aerobic fumarase, [Fe–S] protein	+
fumB	Anaerobic fumarase, [Fe–S] protein	+
fumC	Non-[Fe–S] fumarase	+
fur	Ferric uptake regulation	−
nohA	Phage recombinase	−
nrdHIEF	Deoxyribonucleotide reductase 2	−
orf78	Unknown	−
gpmA	Glycolysis	−
metH	Methionine biosynthesis	−
nohB	Phage function	−
purR	Purine regulon regulation	−
ryhB	Small regulatory RNA	−
sdhCDAB	TCA cycle	+
sodA	Mn-superoxide dismutase	−
sodB	Fe-superoxide dismutase	+
tonB	Siderophore and vitamin B_{12} transport	−
ygaC	Unknown	−
yhhY	Unknown	+

'+/−' indicates induction (+) or repression (−) by the Fe^{2+}–Fur complex.

Fe-S cluster biosynthesis in *E. coli*

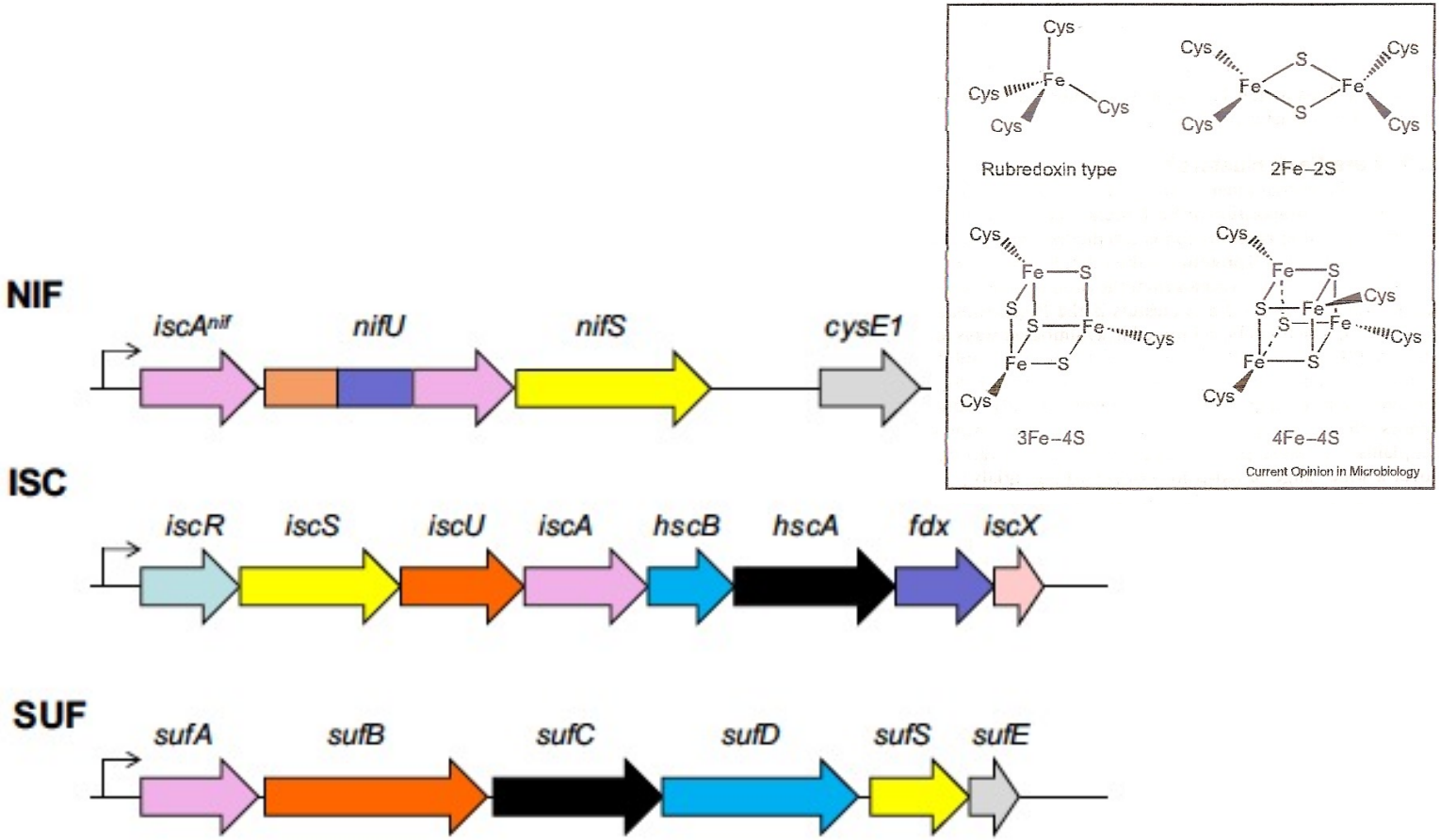
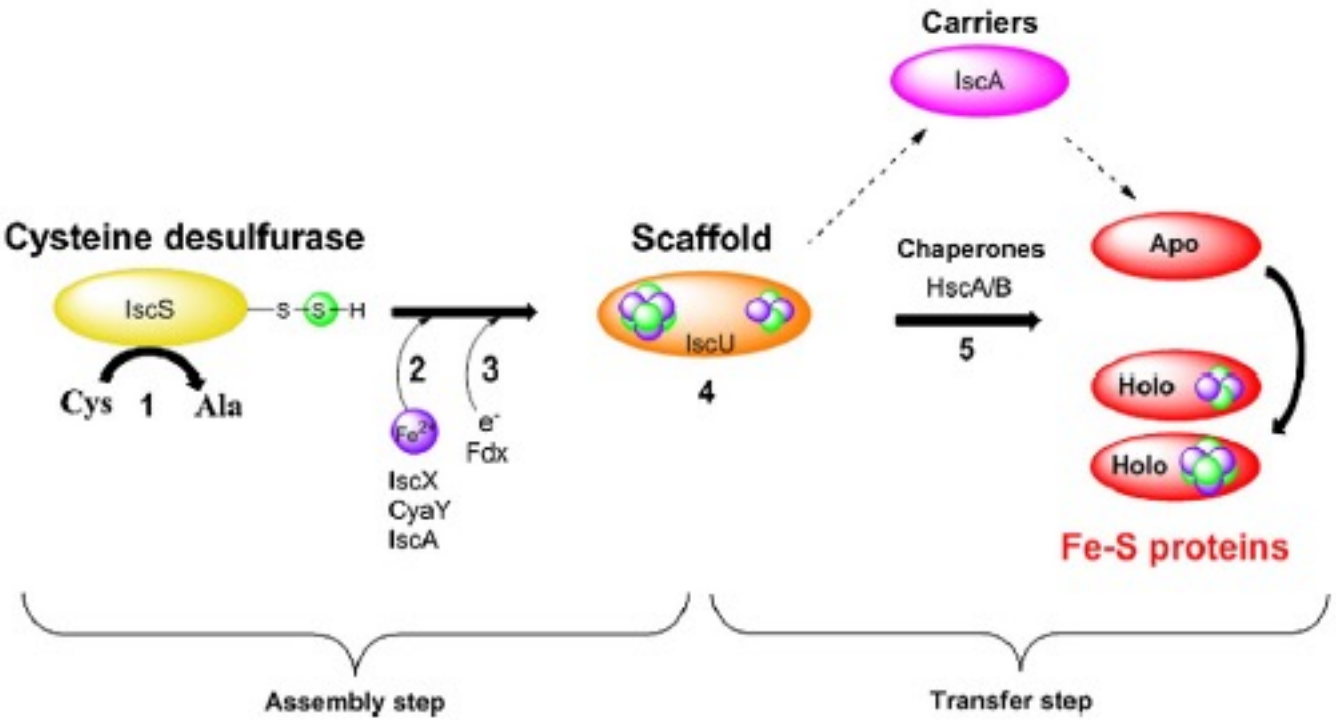


Fig. 1. Various systems involved in Fe/S assembly in bacteria and comparison of their genetic organization in operons: NIF, ISC and SUF (5,7,8). Genes or regions having homologous sequences or similar functions between the three systems are color-coded. Different colors within *nifU* indicate different domains within this modular protein.

Fe-S cluster biosynthesis in *E. coli*



Iron transport in yeast

- In the yeast *Saccharomyces cerevisiae* iron transport in the cell requires different mechanisms:

- Reductase-independent transport
  - Siderophore receptors/transporters (Arn1-4)
- Reductase-dependent transport
  - NADPH-dependent metalloreductase Fre1 and Fre2
  - Low affinity system (Km 30 μM) Fet4
  - High affinity system (Km 0.15 μM) Fet3-Ftr1

Iron transport in yeast.
Low affinity system

- Fet4 is a divalent metal transporter Fe^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}.
- It is regulated by iron levels and it is induced in anaerobiosis.

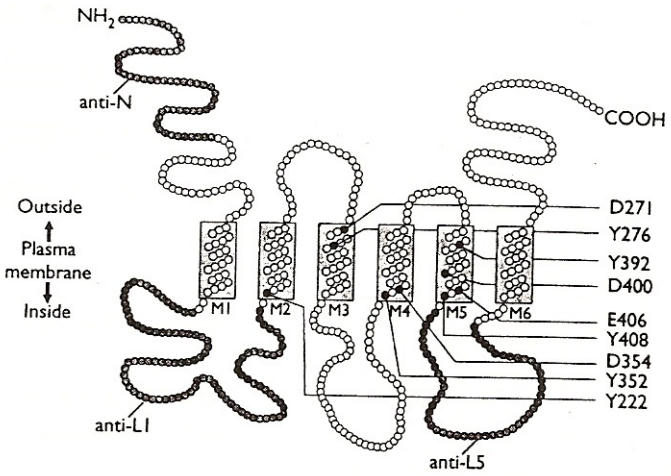


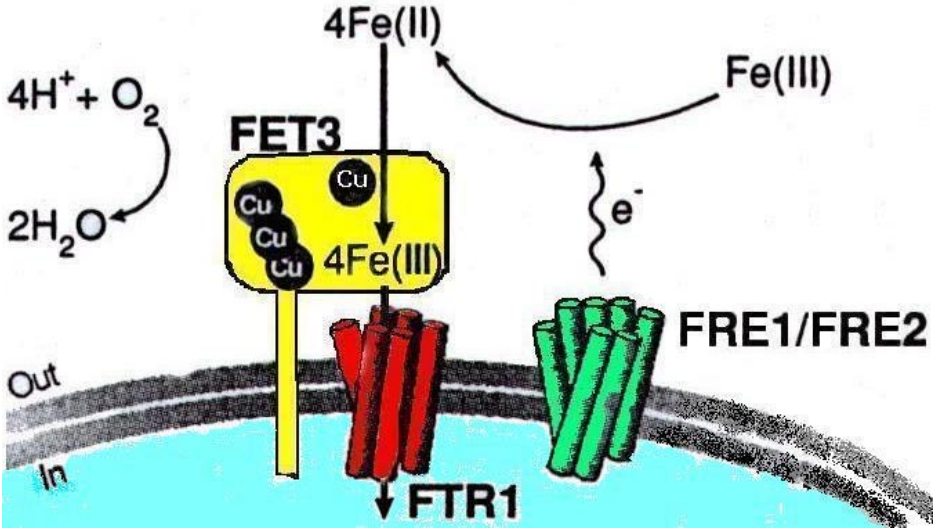
FIG. 1. **A model of FET4 membrane topology.** Transmembrane domains M1 through M6 are depicted as *rectangles*, and individual amino acid residues are indicated by the *circles*. Segments of the protein used in antibody preparation are shaded in *gray*. Mutated residues are *filled* and labeled using the single-letter amino acid code followed by the number of their position in the primary sequence.

Iron transport in yeast.
High affinity system

- The ferroxidase Fet3 catalyzes the reaction

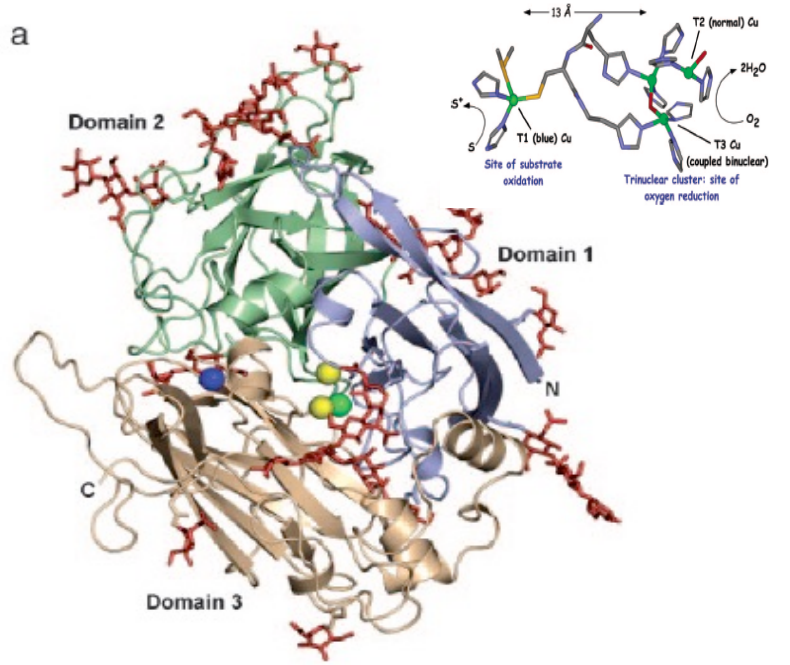
$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O$$

- The permease Ftr1 transports Fe^{3+} inside the cell
- The Fet3-Ftr1 transport system is conserved in many different yeasts such as *Schizosaccharomyces pombe, Candida albicans* and *Pichia pastoris.*

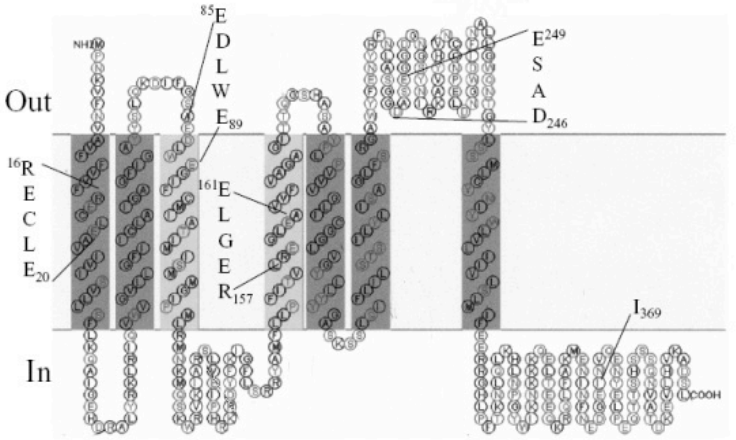


The ferroxidase-permease Fet3-Ftr1 complex

- Fet3 belongs to the family of blue multicopper oxidases (MCO), enzymes that bind multiple Cu atoms and couple monoelectronic oxidation of substrates to reduction of oxygen to water.



- Ftr1 is a permease that is predicted to present 7 TM segments. A REGLE sequence motif in TM4 is necessary for activity of the protein.



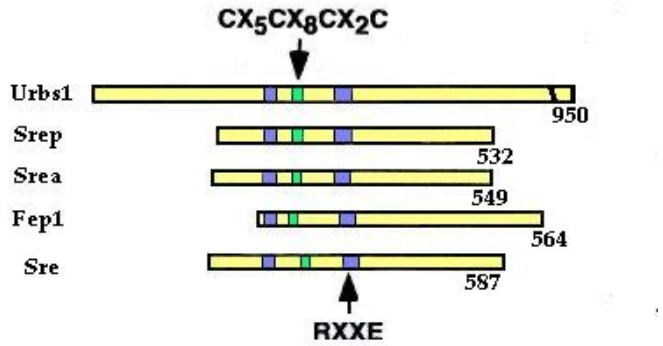
Vacuolar and mitochondrial iron metabolism

- The vacuole is the site of iron storage. Ccc1 is the iron transporter from cytosol to the interior of the vacuole, a ferroxidase-permease complex formed by Fet5 and Fth1 is necessary for mobilization of the metal.
- In the mitochondrion some steps of heme biosynthesis and Fe-S cluster biosynthesis take place. Proteins Atm1, Mrs3, Mrs4 and Yfh1 are involved in transport of iron in the mitochondrion and/or transport of Fe-S cluster from the mitochondrion to the cytosol.

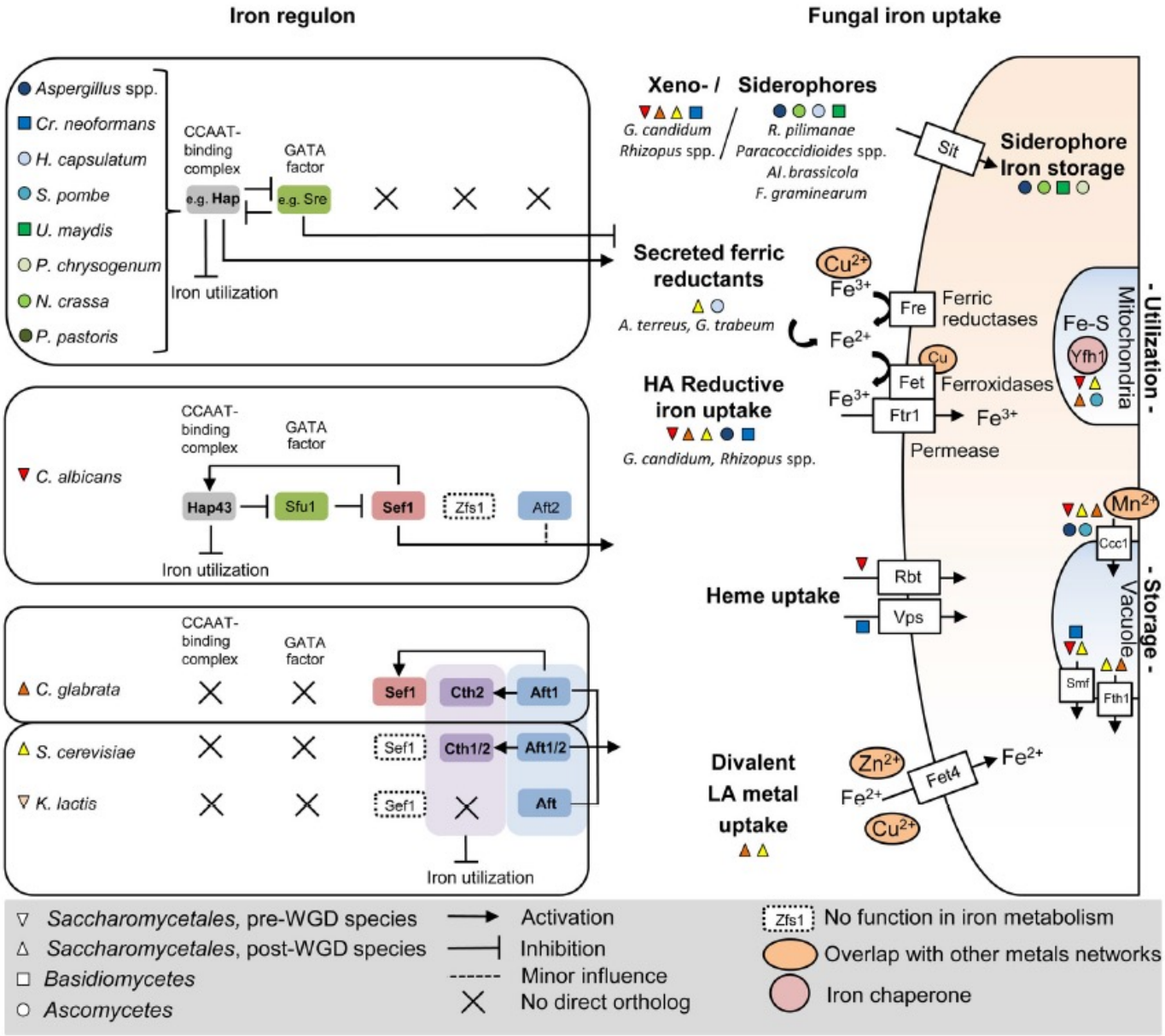
Regulation of iron transport in yeast

- In *S. cerevisiae* Aft1 and Aft2 are transcription factors that regulate many genes in conditions of iron deprivation.
- Aft1 translocates from the cytosol to the nucleus when iron is limiting and it activates transcription of target genes.
- Aft1 and Aft2 contain a Cys-X-Cys motif in the DNA binding domain.

- In *S. pombe, C. albicans, P. pastoris* and other fungi, iron-dependent regulation is mediated by transcriptional repressors belonging to the family of zinc-finger GATA factors.



Regulation of iron transport in yeast and fungi



Factors that regulate Aft1

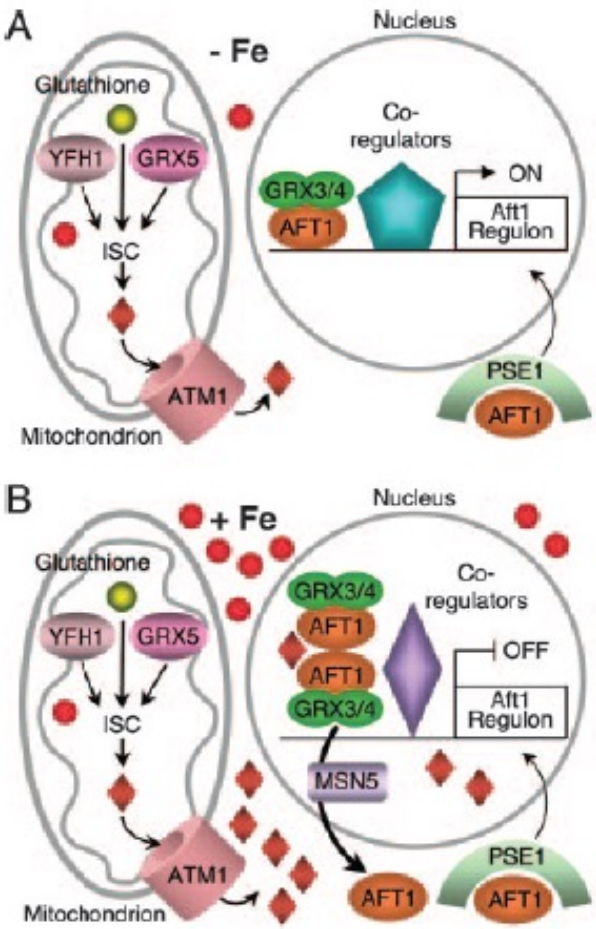


FIG. 1. Iron-dependent transcriptional regulation in *Saccharomyces cerevisiae*. (A) Activation of Aft1p under conditions of iron deprivation. The nuclear importin Pse1p mediates Aft1p translocation into the nucleus. Aft1p forms a complex with Grx3p and Grx4p, binds to DNA, and activates transcription. Although complex formation is not regulated by iron, it is not known whether complex formation occurs exclusively in the nucleus or also in the cytosol. "Coregulators" represent the numerous coactivators and corepressors that contribute to the regulation of the Aft1p regulon. These include the mediator complex, Snf1p/Snf4p, Ssn6, Nhp6p, Tup1, Hda1p, Cti6p, and heme. (B) Regulation of Aft1 activity under iron-replete conditions. Yfh1p, Grx5p, and glutathione are required for the production of ISC and the formation of an unknown compound that is a substrate for Atm1p. This compound is exported from mitochondria and may possibly be targeted to the nucleus. Under iron-replete conditions, Aft1p forms dimers that are recognized by the nuclear exportin Msn5p and lead to the accumulation of Aft1p in the cytosol. In a hypothetical model for the regulation of Aft1p, the production of the substrate for Atm1p is proportional to cellular iron levels. This substrate accumulates in the nucleus and leads to the dimerization of Aft1p, perhaps through the formation of a mixed disulfide bridge, and the complex is exported from the nucleus.

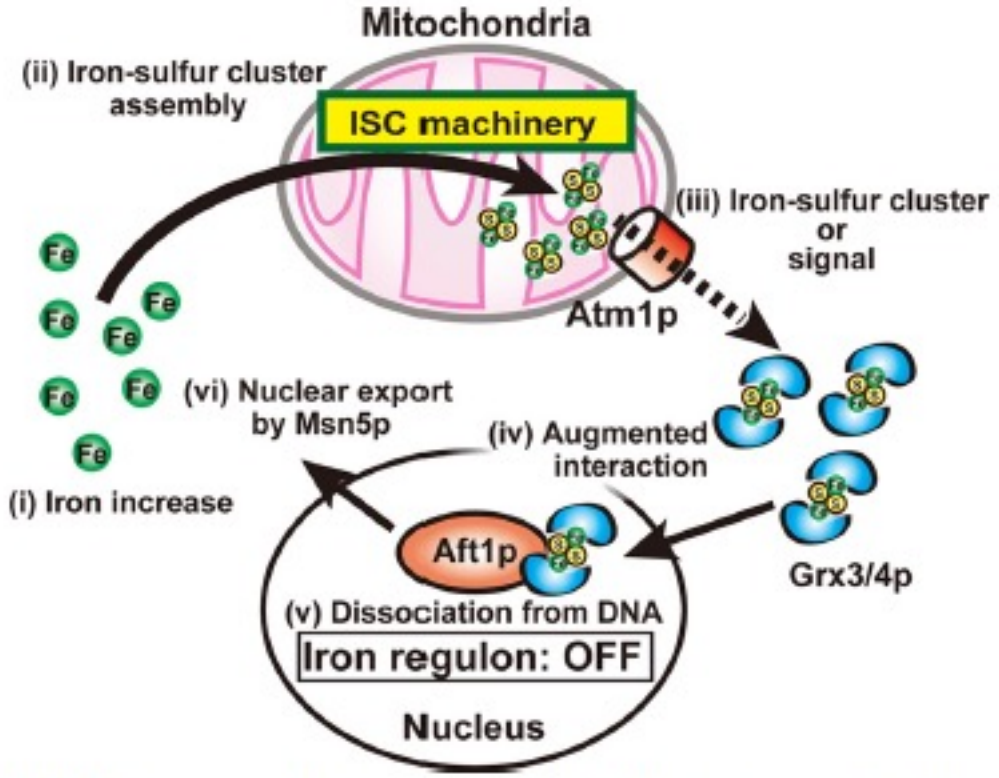


FIG 8 Proposed model for iron sensing by Aft1p. During iron starvation, iron-sulfur assembly in the mitochondria and dimeric Grx3/4p with bound iron-sulfur clusters are minimal. Under these conditions, Grx3/4p binding to Aft1p is attenuated, and Aft1p binds to target promoters to increase the expression of the iron regulon. In response to iron availability (i), iron-sulfur cluster assembly in the mitochondria increases (ii), and the iron-sulfur clusters, or signals that invoke iron-sulfur cluster formation, are delivered to the monothiol glutaredoxins Grx3/4p, which reside in both the nucleus and cytoplasm, via the mitochondrial ABC exporter Atm1p (iii). Grx3/4p with bound iron-sulfur clusters bind to Aft1p (iv), which induces dissociation of Aft1p from its target promoters (v), leaving Aft1p available for nuclear export by Msn5p (vi). The expression of the iron regulon is thereby downregulated.

Genes regulated by iron-dependent transcription factors in yeast

Transcription factor	Description	Gene
Aft1	Transporters	FET4, FTR1, FTH1, SMF3, MRS4, CCC2, COT1
	Cu chaperone	ATX1
	Ferroxidase	FET3, FET5
	Metalloreductase	FRE1, FRE2, FRE3, FRE4, FRE5, FRE6
	Cell wall proteins	FIT1, FIT2, FIT3
	Siderophore transport	ARN1, ARN2, ARN3, ARN4
	Biosynthesis of Fe-S cluster	ISU1, ISU2
	Other	TIS11, HMX1, AKR1, PCL5, ICY2, PRY1
Aft2	Transporters	SMF3, MRS4, FTR1, COT1
	Cu chaperone	ATX1
	Ferroxidase	FET3, FET5
	Metalloreductase	FRE1
	Cell wall proteins	FIT1, FIT2, FIT3
	Biosynthesis of Fe-S cluster	ISU1
	Other	BNA2, ECM4, LAP4, TIS11
Fep1	Transporters	$fip1^{+}$
	Ferroxidase	$fio1^{+}$
	Siderophore transport	$str1^{+}$, $str2^{+}$, $str3^{+}$

Response to iron deprivation in *S. cerevisiae*

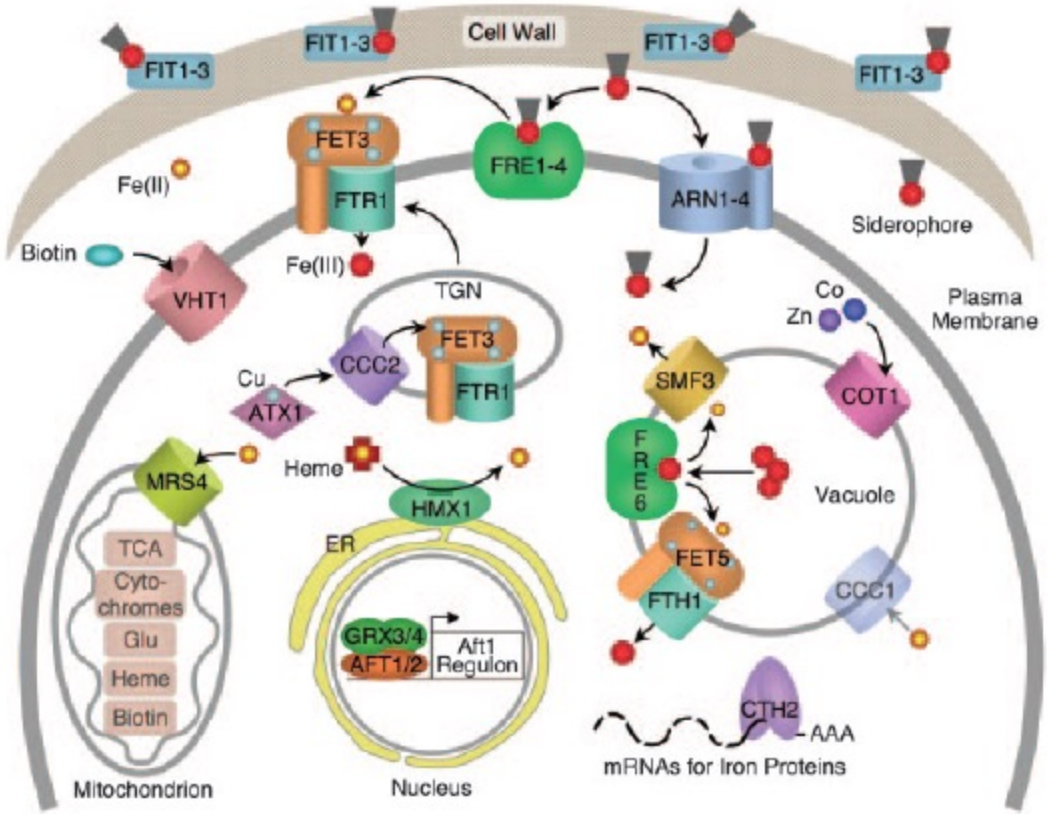


FIG. 2. Response to iron deprivation in *Saccharomyces cerevisiae*. Proteins under the transcriptional control of Aft1p and Aft2p are labeled with black text. Ccc1p, proteins of the tricarboxylic acid cycle, the respiratory cytochromes, and the glutamate, heme, and biotin biosynthetic pathways are down-regulated during iron deficiency and are indicated with gray text.

Metal-regulated expression in yeast

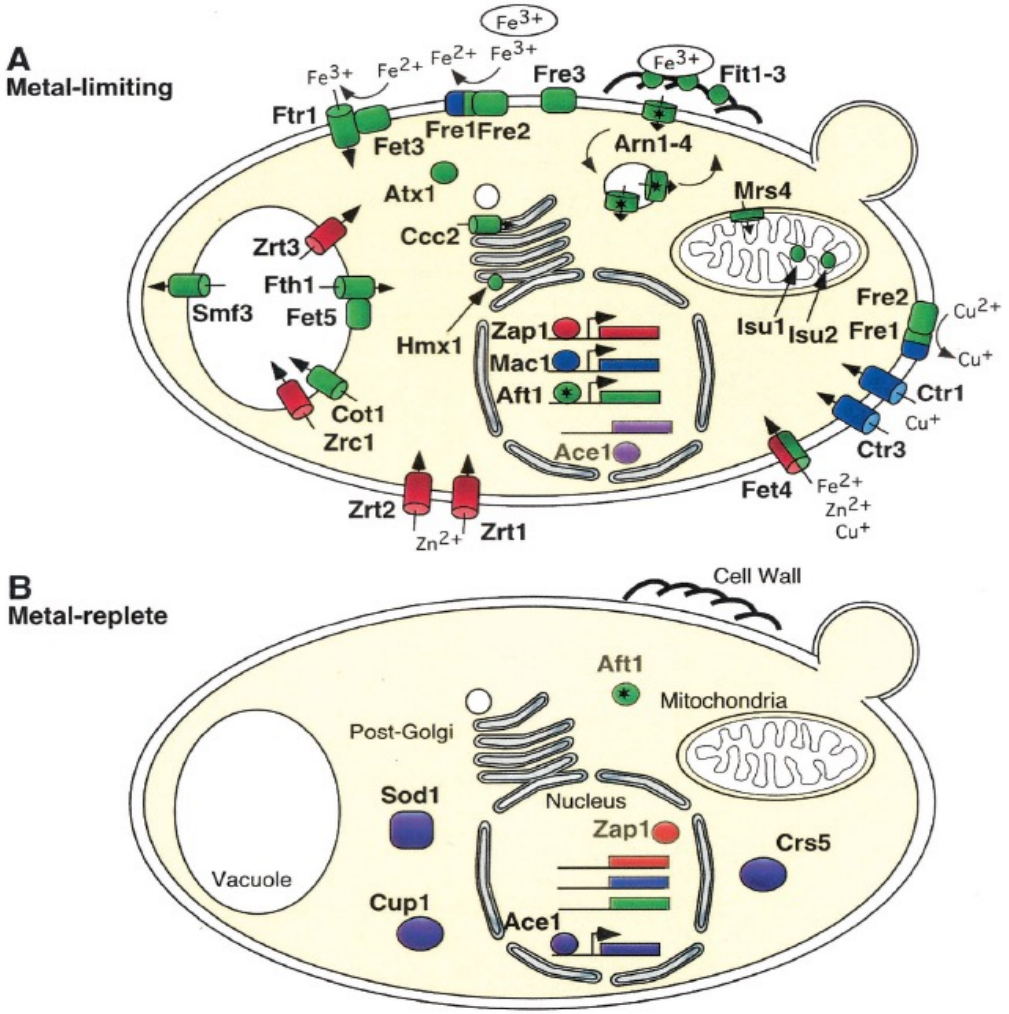


FIG. 1. Protein products of metalloregulated genes involved in metal homeostasis in *S. cerevisiae*. Products of genes that are activated under metal-limiting conditions (A) and metal-replete conditions (B) by Aft1 (green), Mac1 (blue), Zap1 (red), and Ace1 (purple) are shown. Iron that is bound to siderophores has been circled, and stars indicate proteins that undergo iron-dependent cellular trafficking. The metal ion specificities of proteins required for metal uptake are indicated. See the text for further details of the functional roles of each protein.

Iron-dependent transcriptional regulation in *S. pombe*