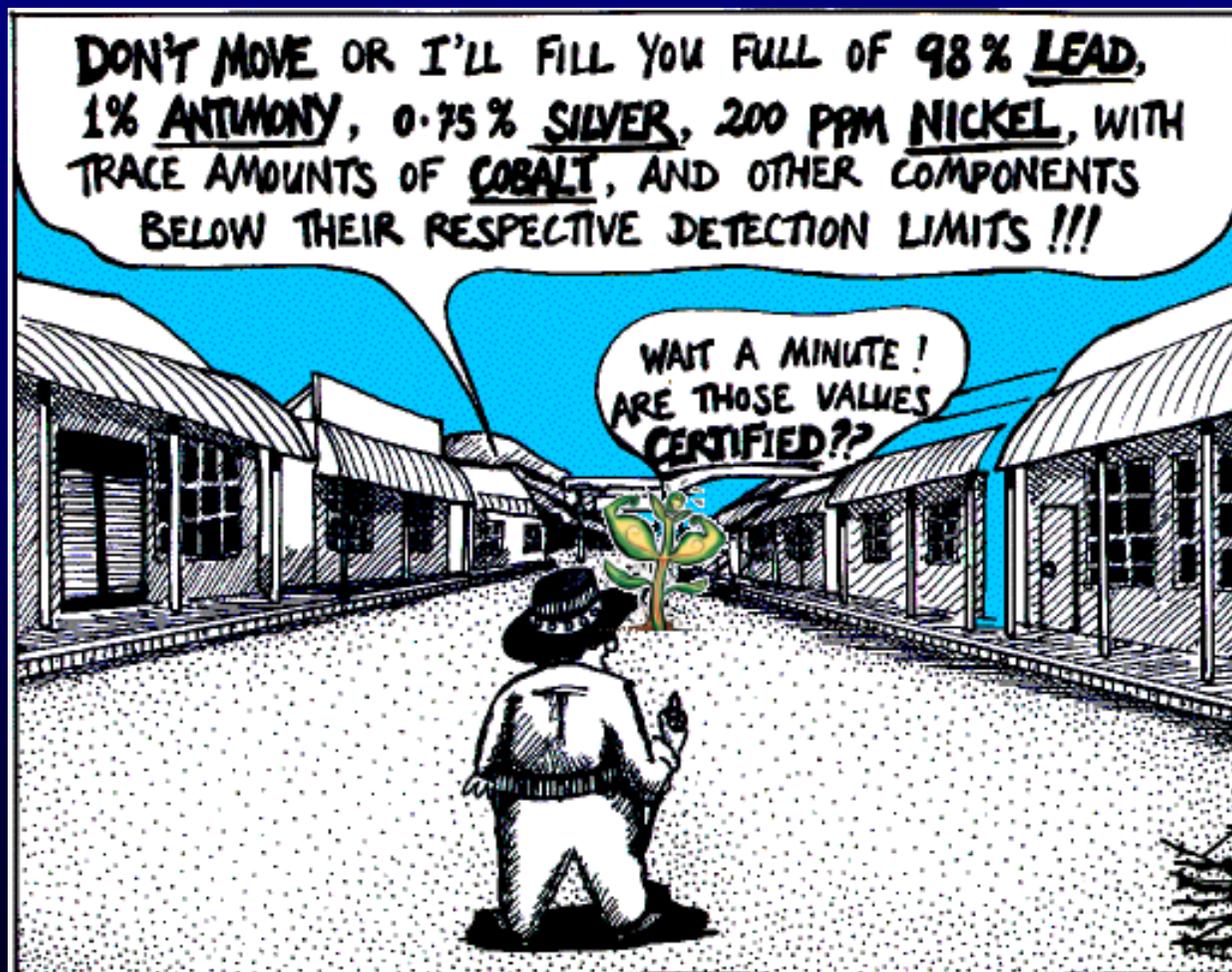


# Trace metals as micronutrients

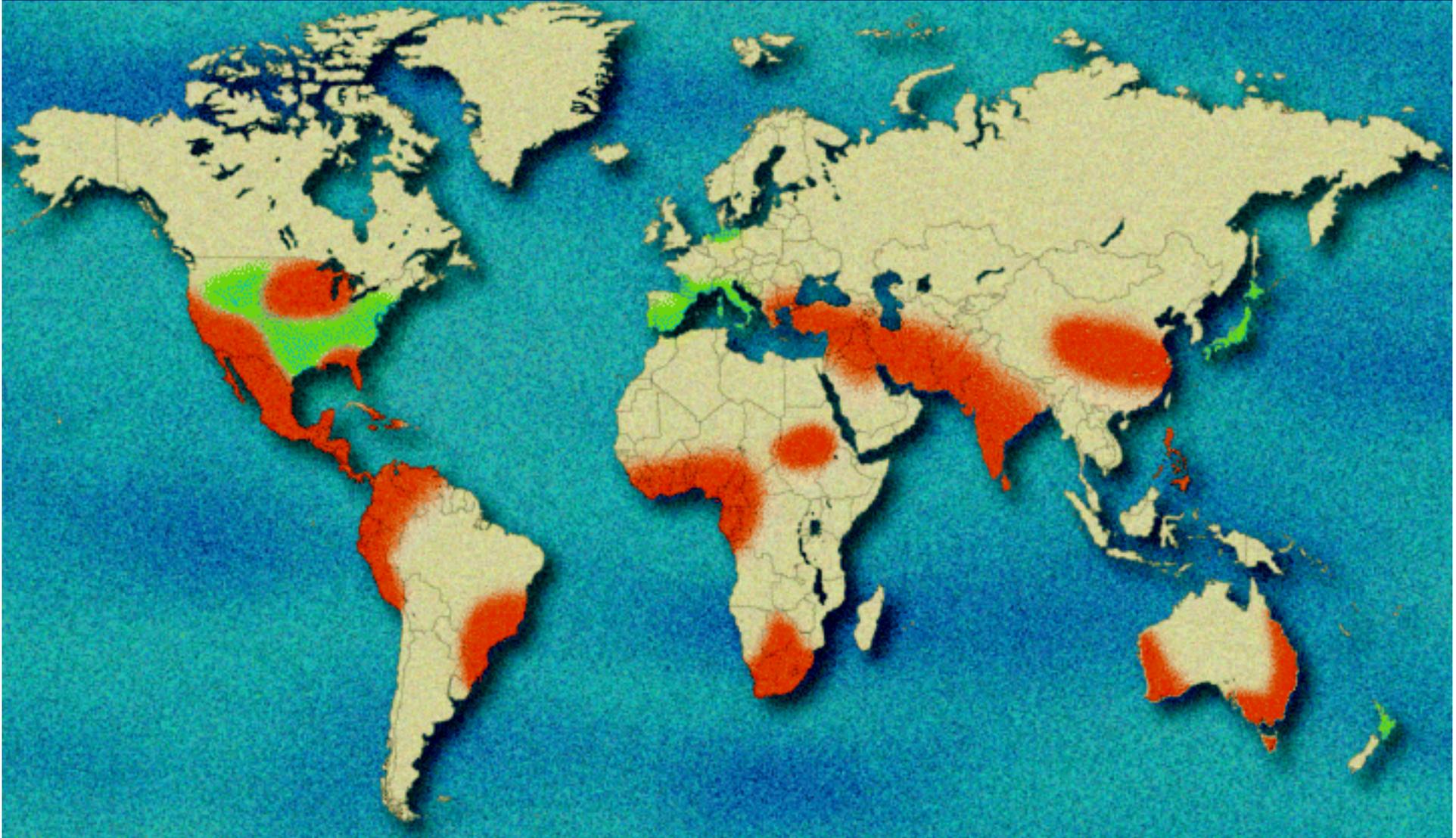


Metal uptake in the Wild West

modified from: <http://strangematter.sci.waikato.ac.nz/>

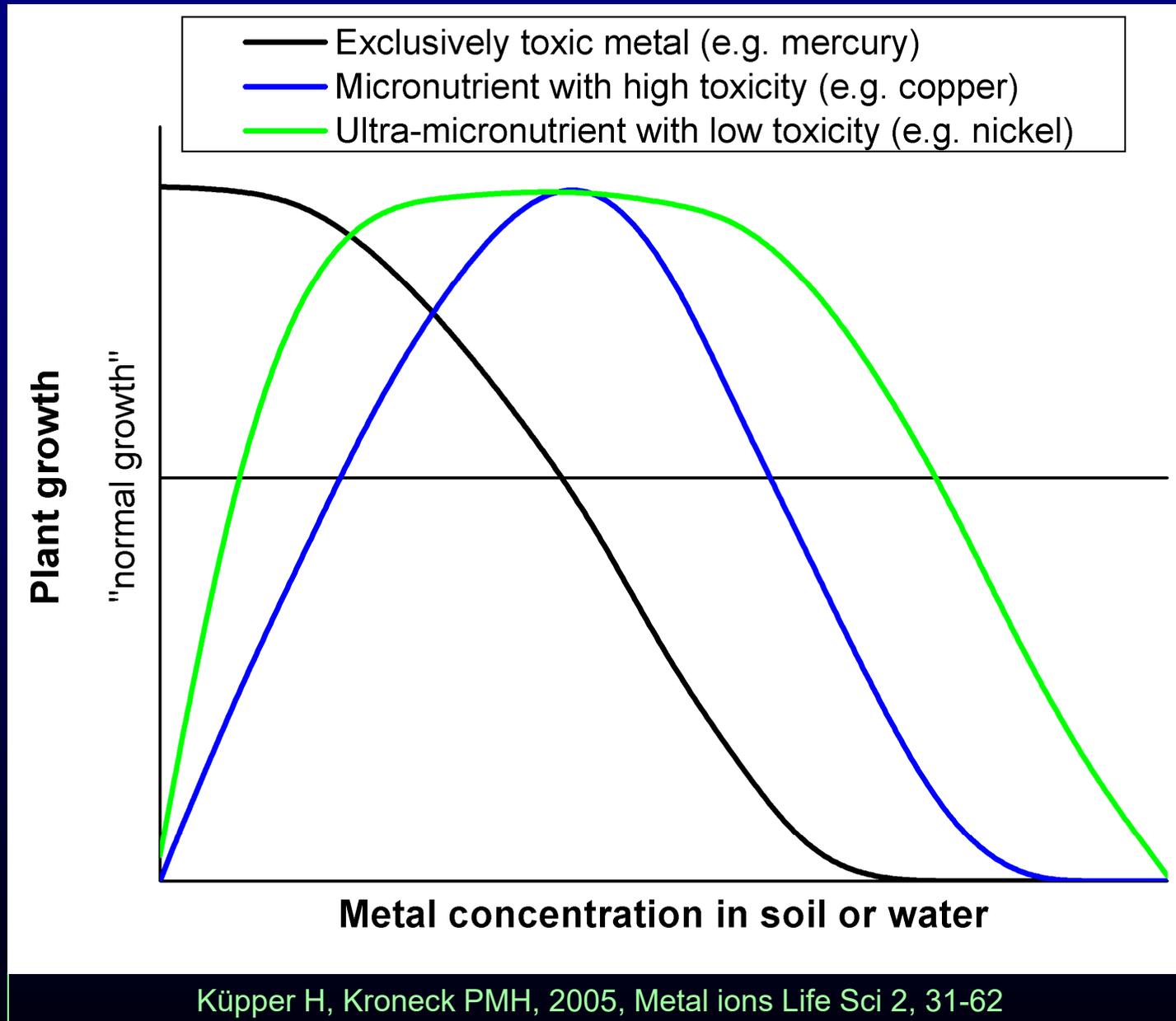
# Heavy metal deficiency as a global problem of agriculture

green = moderate zinc deficiency; red = severe zinc deficiency

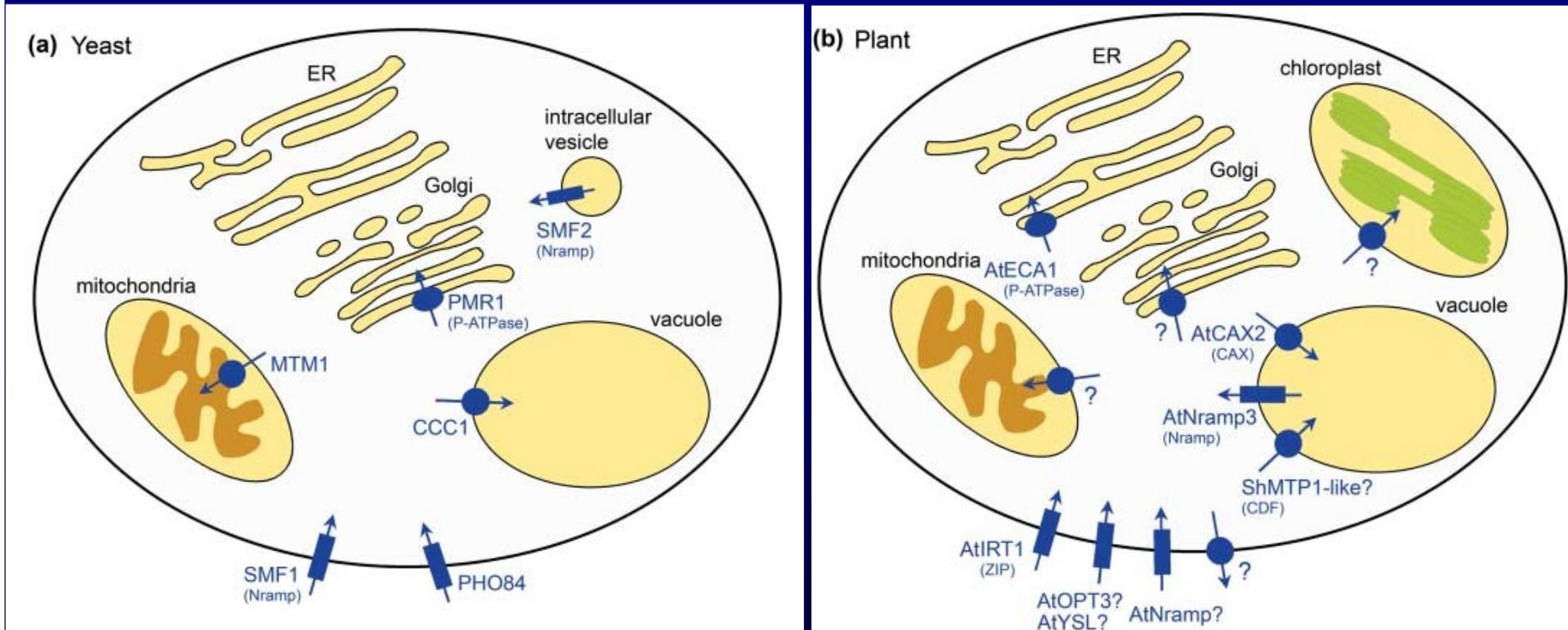


From: Alloway BJ. 2001. Zinc the vital micronutrient for healthy, high-value crops. Brussels, Belgium: International Zinc Association.

## Dose-response principle for heavy metals



# Mechanisms of metal uptake in Eucaryotes: Main families of metal transport proteins example: manganese transport in yeast and plants

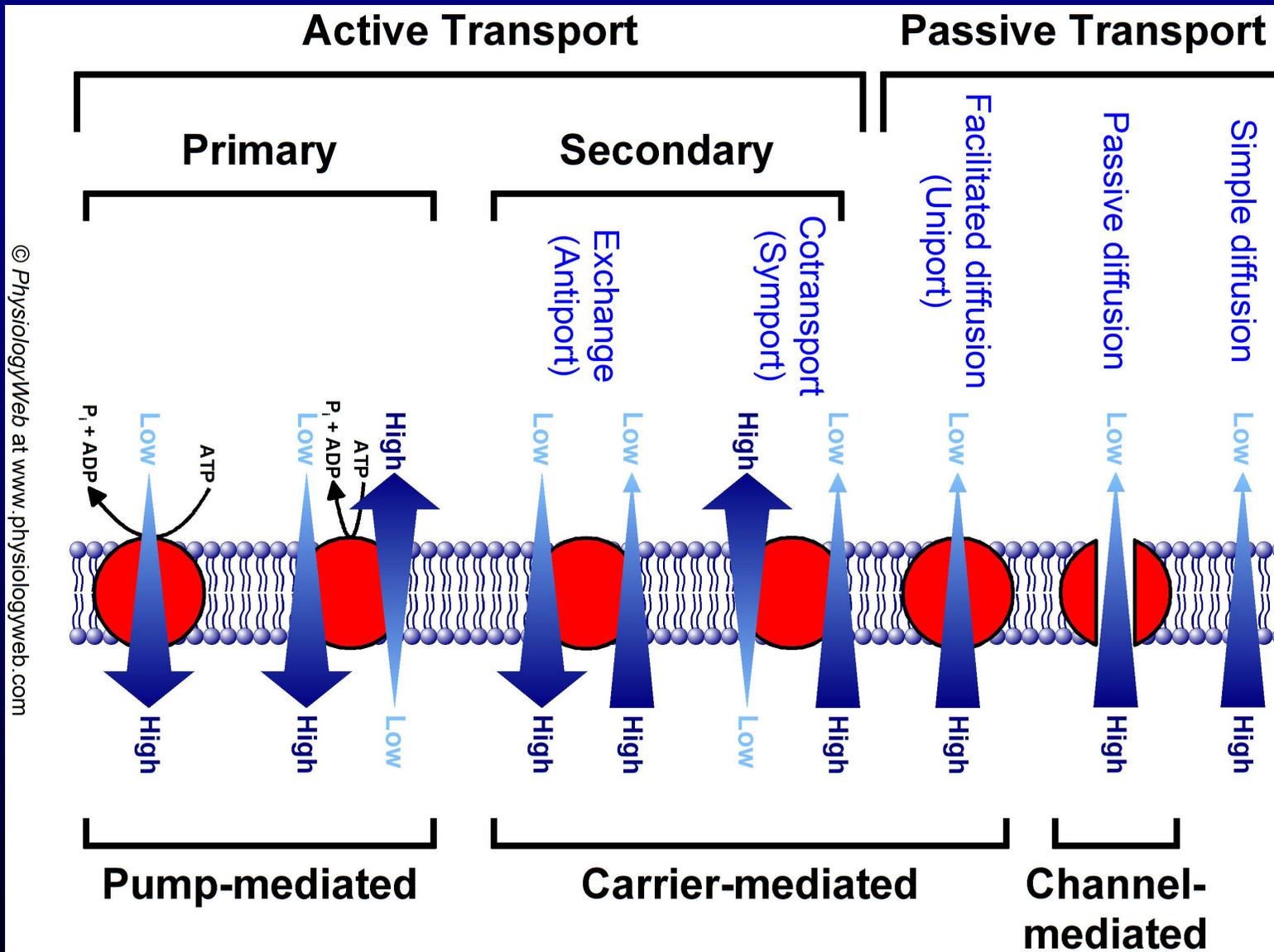


From: Pittman JK, 2005, *NewPhytol*167, 733-742

## 4 main families of transmembrane metal transport proteins

- P-type **ATPases**
- cation diffusion facilitators (**CDF**-transporters)
- ZRT-/IRT-like proteins (**ZIP**-transporters)
- Natural resistance associated Macrophage proteins (**Nramp**-transporters)

# Energetics and variants of metal transport



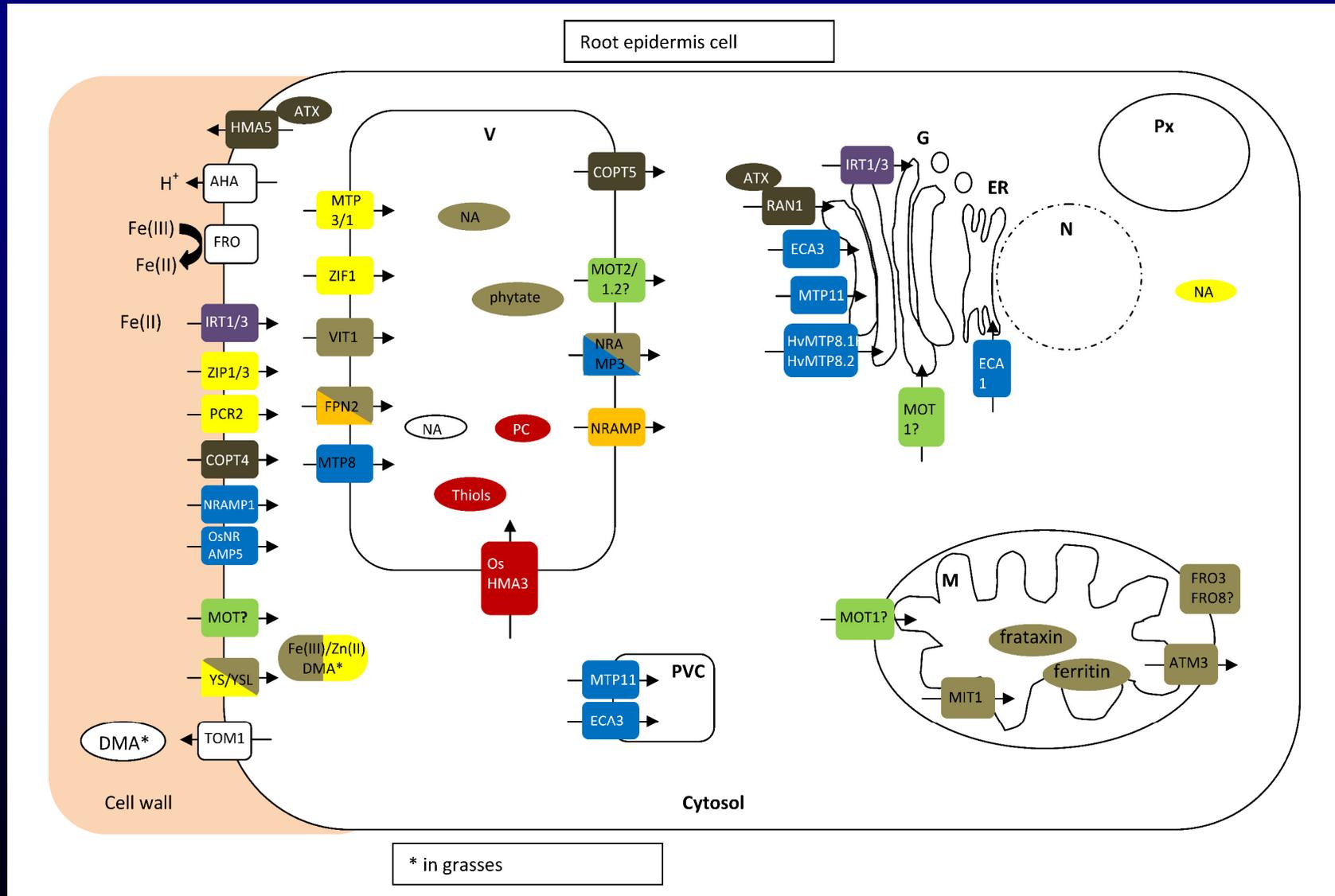
$$\Delta G = n_{\text{Ionen}} * R * T * \ln (c_{\text{inside}} / c_{\text{outside}}) + 3F (\varphi_{\text{outside}} - \varphi_{\text{inside}})$$

(R = gas constant, T = temperature, F = Faraday constant,  $\varphi$  = electrochemical potential)

# Mechanisms of metal uptake in plants:

## Different transport steps require different transporters

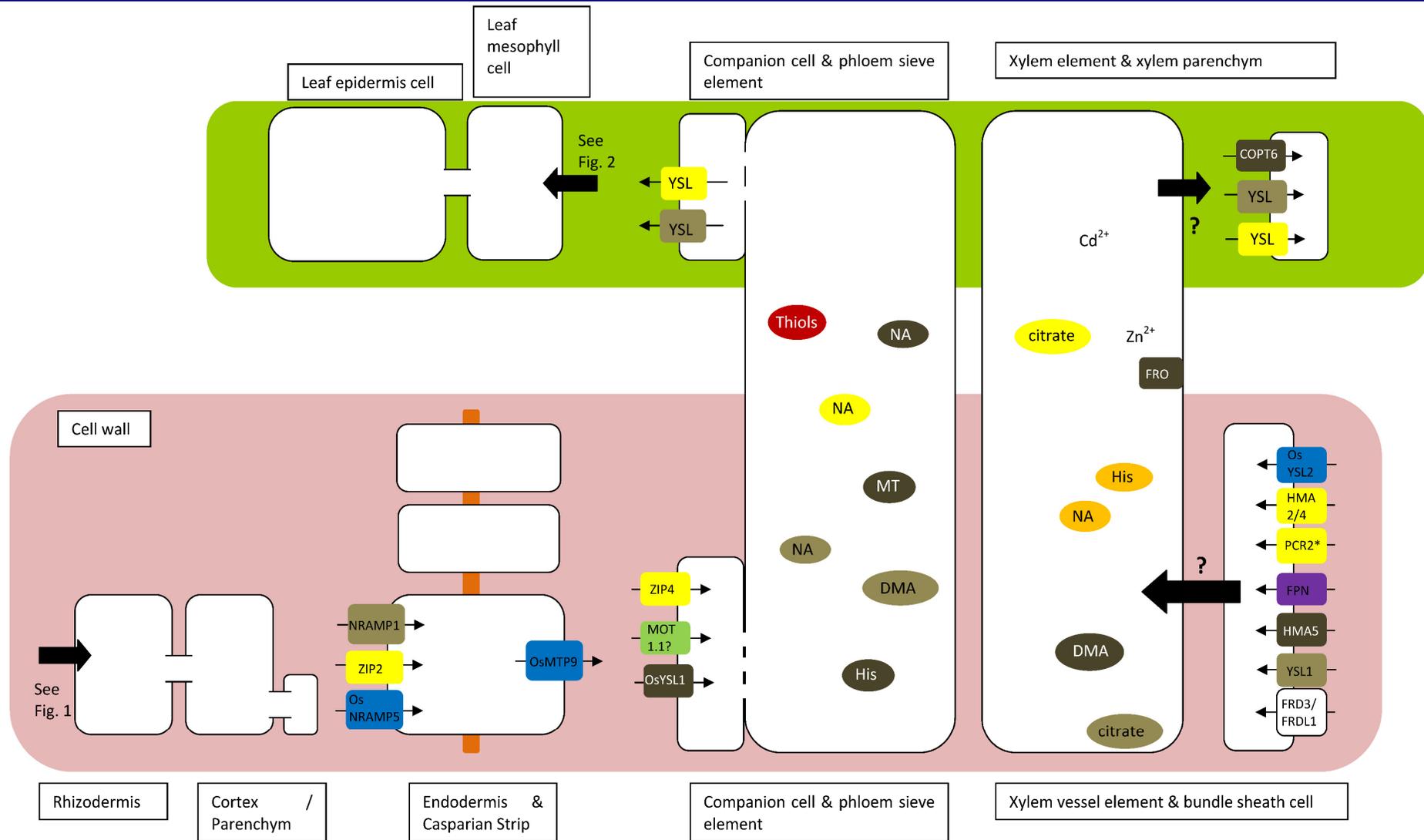
### 1) Root uptake and intracellular distribution



# Mechanisms of metal uptake in plants:

## Different transport steps require different transporters

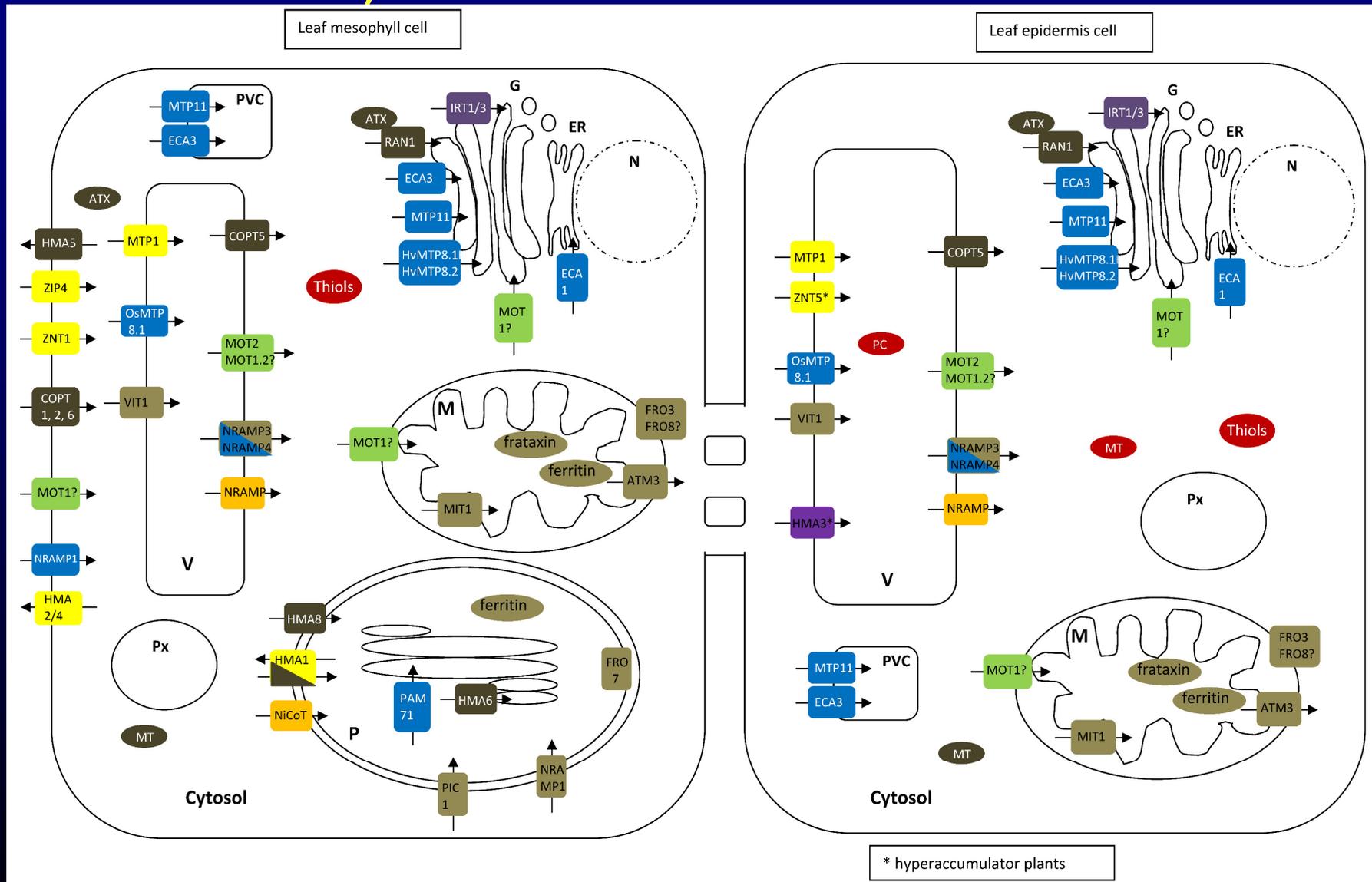
### 2) Translocation. Root-to-shoot: Xylem, shoot-to-root: phloem



# Mechanisms of metal uptake in plants:

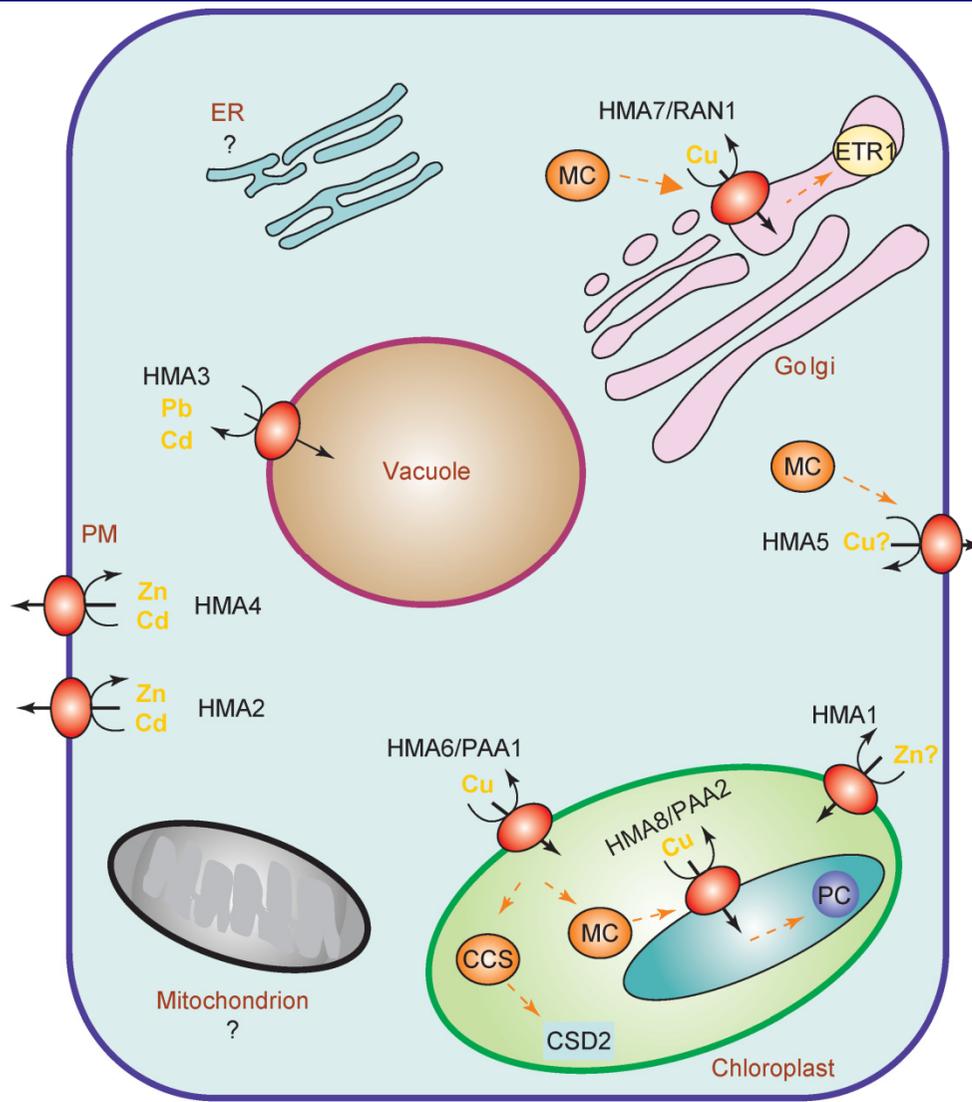
## Different transport steps require different transporters

### 2) Distribution in shoot cells



# Mechanisms of metal uptake+compartmentation in plants (I)

## CPx-type (=P<sub>1B</sub>-type) ATPases



- Functions (concluded mostly from differential expression studies)**
- translocation **into** the root xylem, that means **out** of root cells (→ e.g. HMA4)
  - xylem unloading in shoots
  - intracellular metal sequestration e.g. in the vacuole
  - transport into the chloroplast, inside the chloroplast into the thylakoids
  - transport into the Golgi apparatus

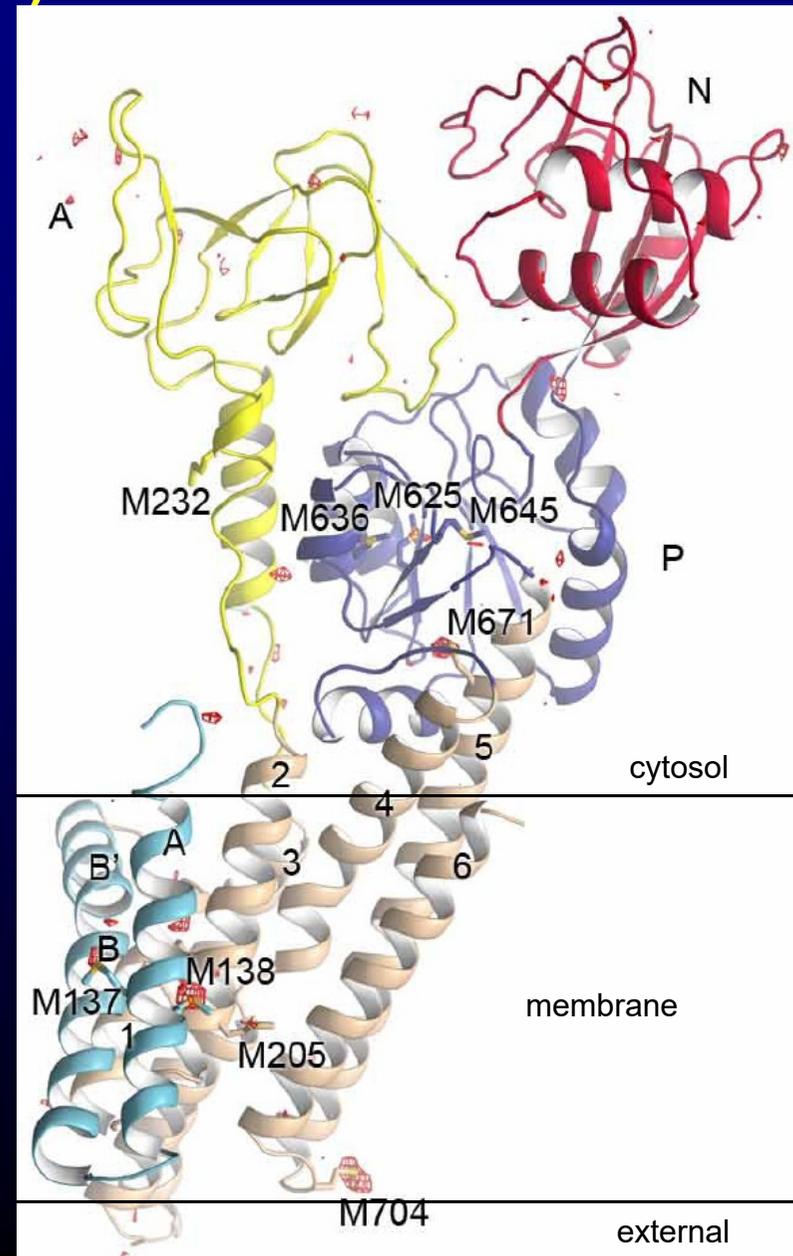


# Mechanisms of metal uptake+compartmentation in plants (I)

## CPx-type (=P<sub>1B</sub>-type) ATPases

### Structural characteristics, from an X-ray structure of a bacterial protein

- large cytosolic domain
- electronegative funnel connects membrane surface to high-affinity Zn-binding site
- the size and structure of the channel suggests that it interacts with aqueous Zn<sup>2+</sup>, not a larger complex
- high affinity Zn-binding site contains two cysteins



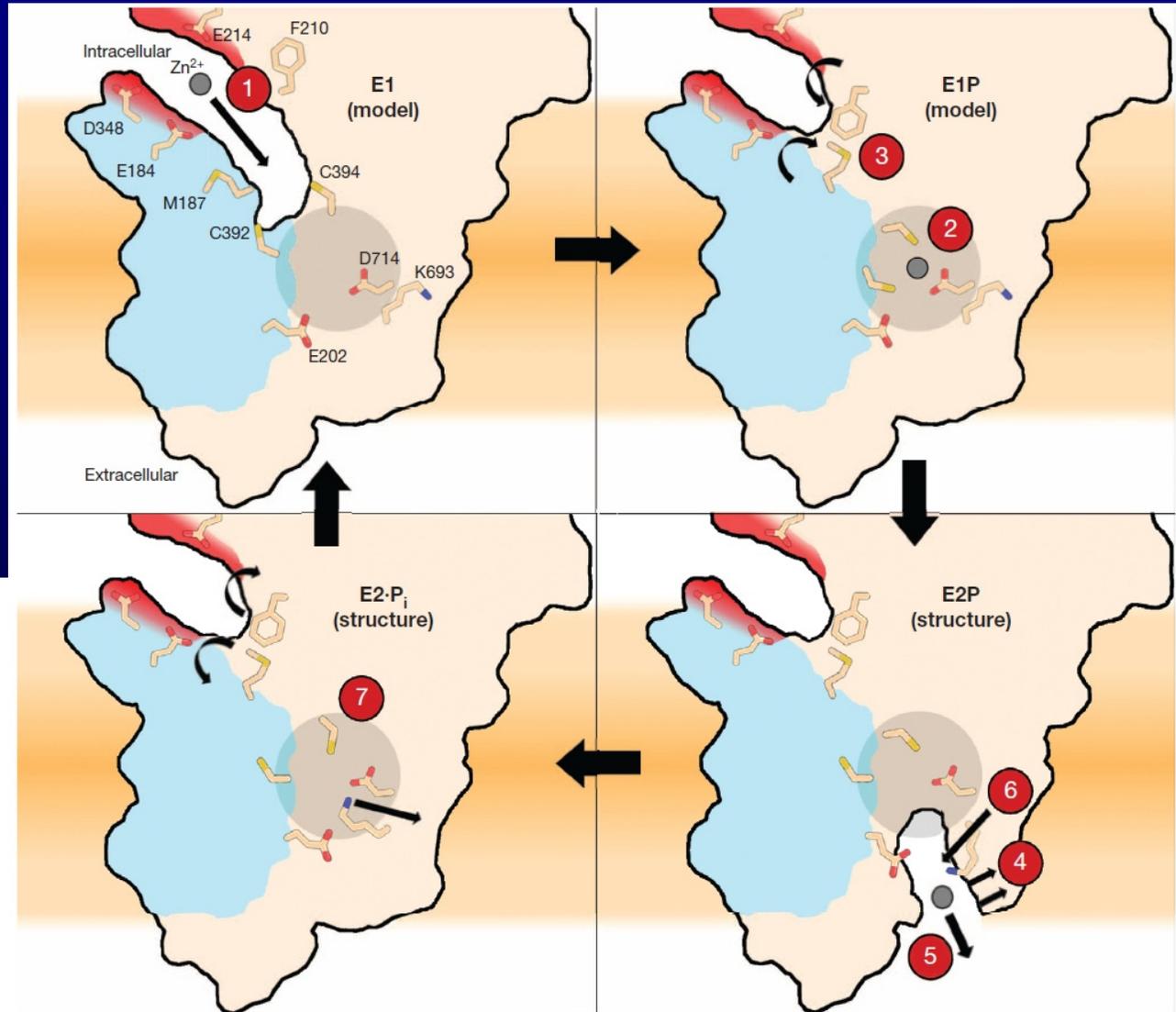
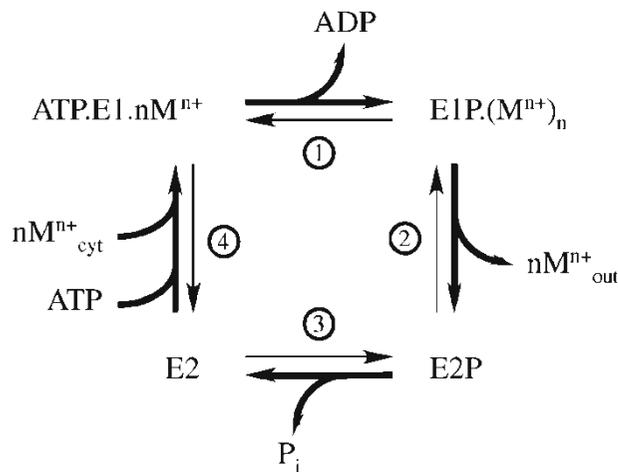
From: Wang K et al, 2014, Structure and mechanism of Zn-transporting P-type ATPases. Nature 514, 518-

# Mechanisms of metal uptake+compartmentation in plants (I)

## CPx-type (=P<sub>1B</sub>-type) ATPases

### Mechanism

- Zn is guided into binding pocket by negatively charged residues
- binding pocket closes after ATP binding
- pore opens on other side of protein, release of Zn<sup>2+</sup>
- pore closes after ATP hydrolysis

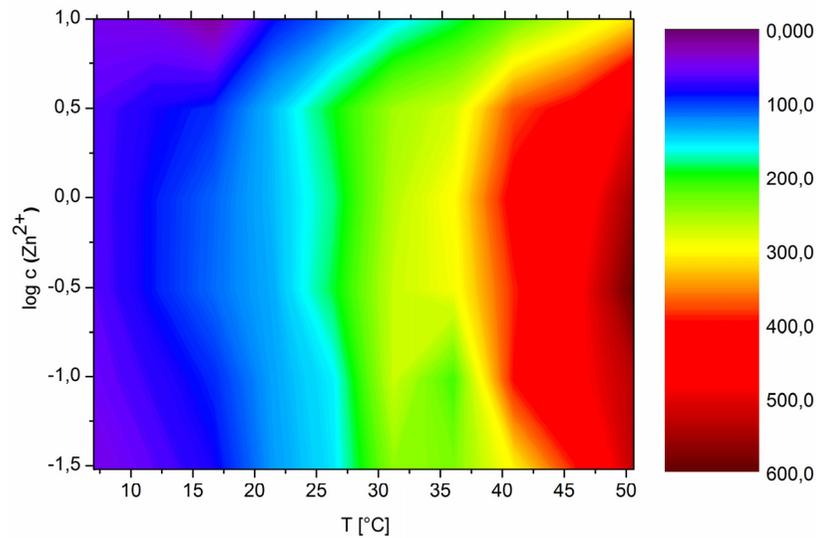


From: Argüello JM et al., 2007, Biometals, DOI 10.1007/s10534-006-9055-6

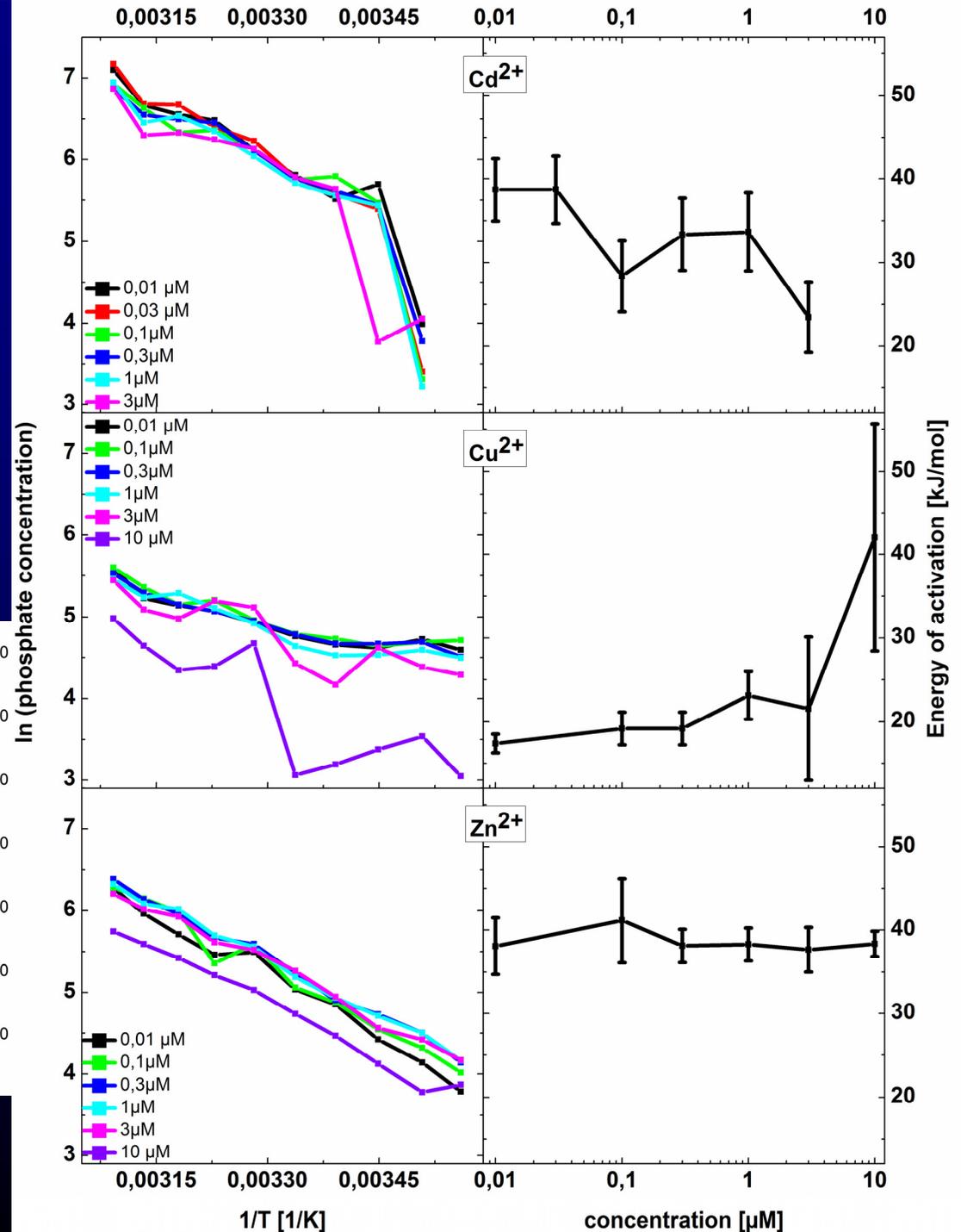
From: Wang K et al, 2014, Structure and mechanism of Zn-transporting P-type ATPases. Nature 514, 518-

# Activation and substrate inhibition of TcHMA4

- Activation energies for TcHMA4 (CPx = P<sub>1B</sub> ATPase) are similar to other metal ATPases.
- Activation energy changes with the concentration and type of the metal to be pumped.



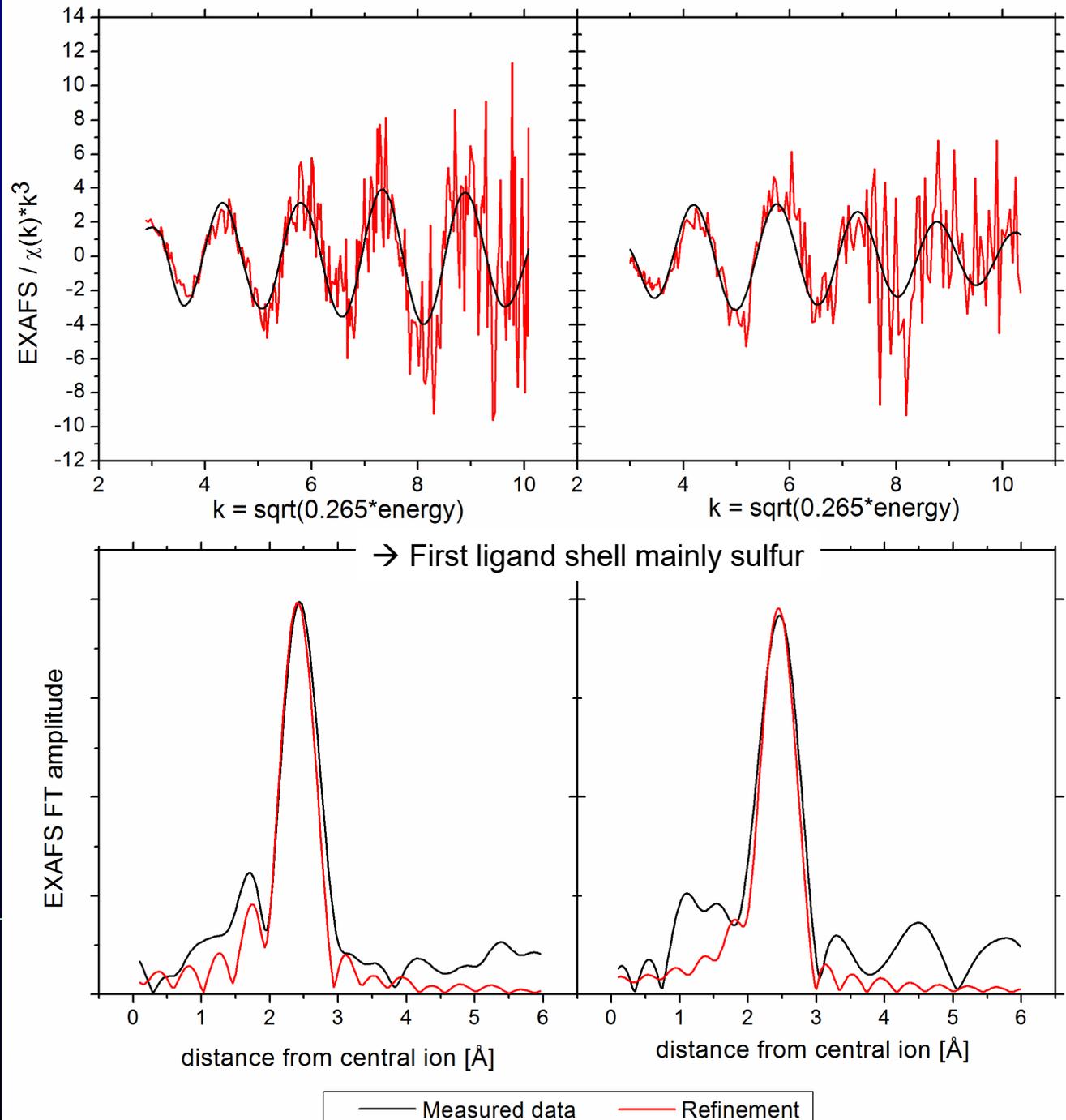
Leitenmaier B, Witt A, Witzke A, Stemke A, Meyer-Klaucke W, Kroneck PMH, Küpper H (2011)  
 Biochimica et Biophysica Acta (Biomembranes) 1808, 2591-2599



# EXAFS-analysis of TcHMA4

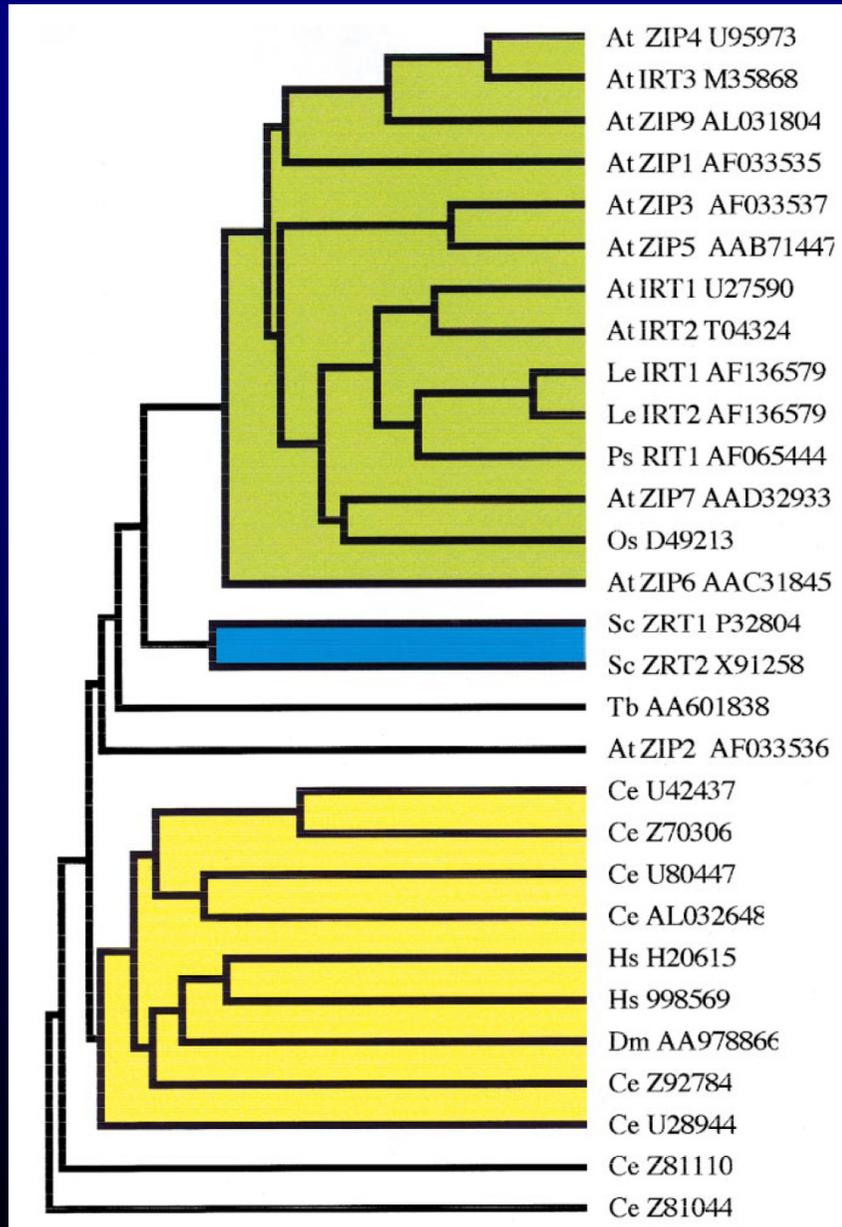
- at low Cd concentrations, the first ligand shell in this ATPase consists mainly of S (thiol groups from some of the 58 cysteines in the sequence)

Barbara Leitenmaier, Annelie Witt,  
Annabell Witzke, Anastasia Stemke,  
Wolfram Meyer-Klaucke,  
Peter M.H. Kroneck, Hendrik Küpper  
(2011) *Biochimica et Biophysica Acta*  
(Biomembranes) – 1808, 2591-2599



# Mechanisms of metal uptake in plants (II)

## ZIP-transporters



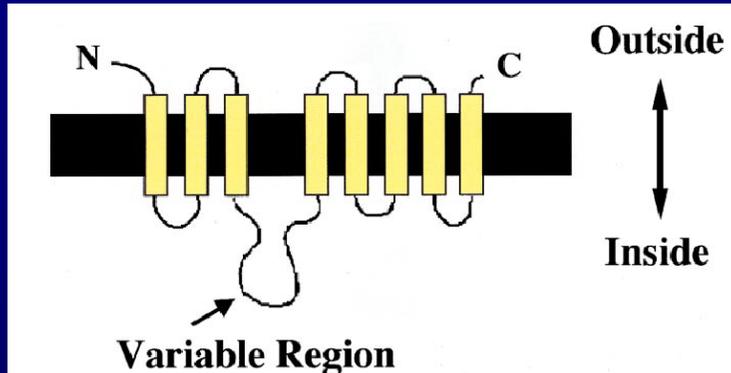
### Likely Functions (deduced from expression studies)

- uptake of metals into cells over the cytoplasmic membrane
- abundant in all eucaryotes, incl. humans, plants and fungi

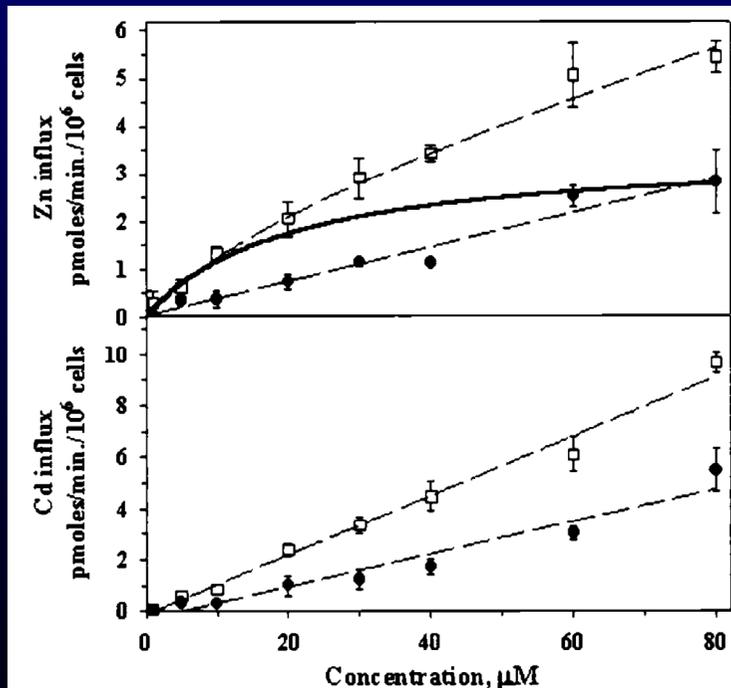
From: Guerinot ML, 2000, BBA 1465, 190-8

# Mechanisms of metal uptake+compartmentation in plants (II)

## ZIP-transporters



From:Guerinot ML, 2000, BBA 1465, 190-8



From:Pence NS et al., 2000, PNAS 97, 4956-60

### Functions suggested by expression studies)

- uptake of metals into cells over the cytoplasmic membrane
- abundant in all eucaryotes, incl. humans, plants and fungi

### Structure predicted by sequence

- usually 8 transmembrane helices, one long variable region, predicted to be in the cytoplasm
- 309-476 amino acids
- still no complete 3D structure available

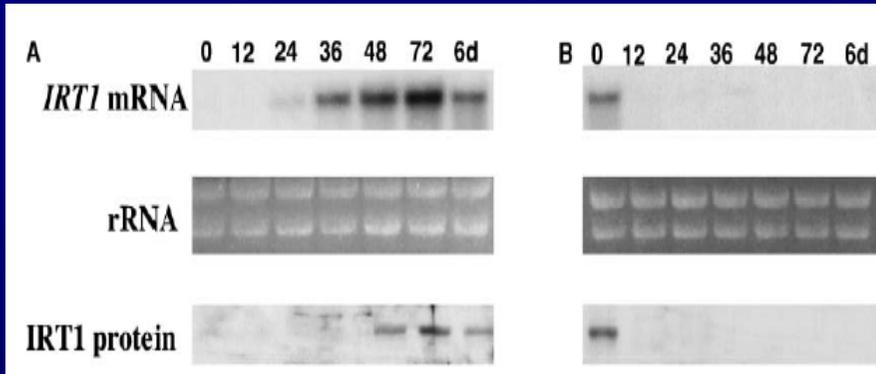
### Characteristics revealed by yeast expression studies

- High affinity and saturable kinetics for selected metal (e.g. Zn in ZNT1)
- Lower affinity uptake for related metals (e.g. Cd in ZNT1)

# Mechanisms of metal uptake+compartmentation in plants (II)

## Transcriptional regulation of ZIP transporters

Fe deficiency (left) and iron replete conditions (right)

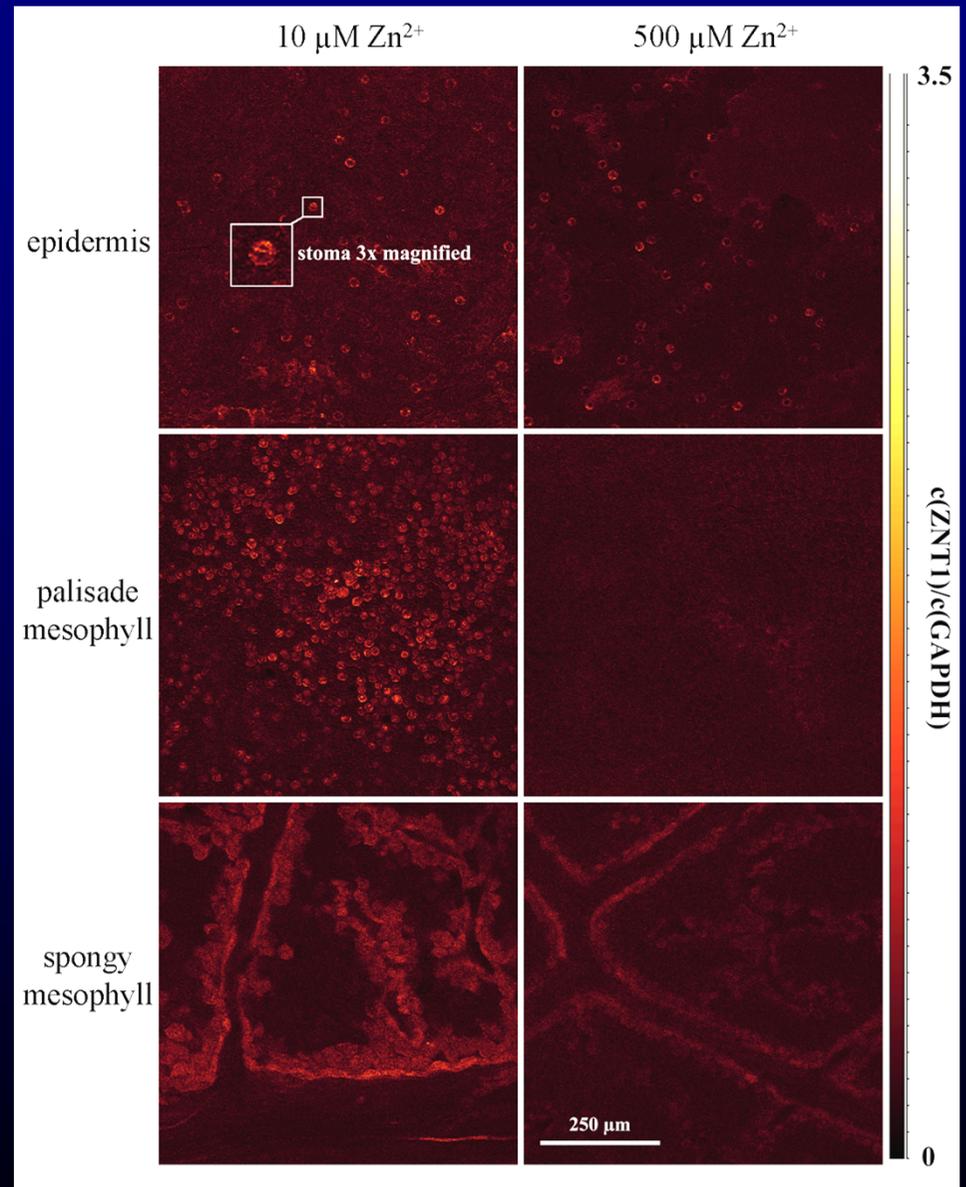


From: Connolly et al., 2002, PlantCell 14, 1347-57

### Expression pattern

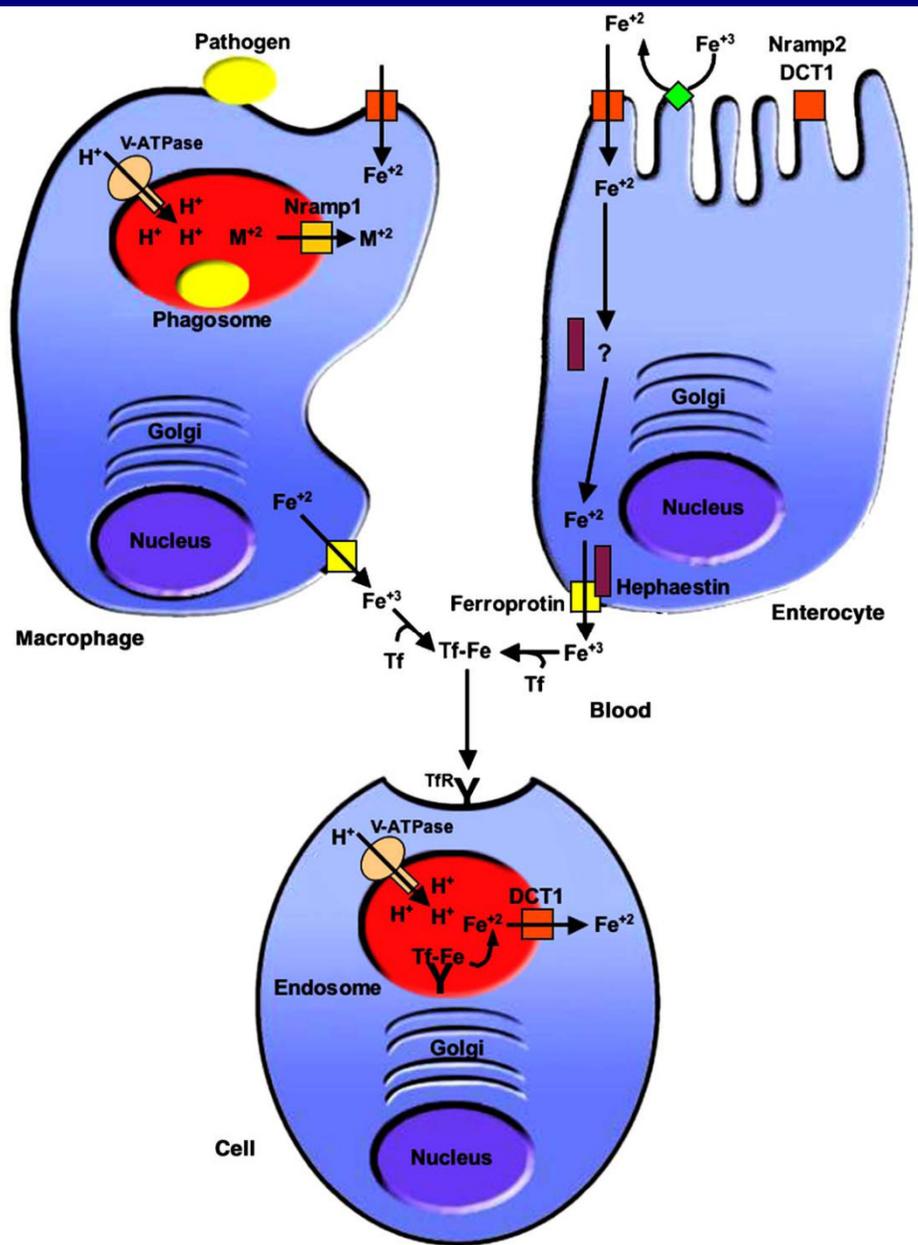
- expressed in most plants mainly under metal deficient conditions
- in metal hyperaccumulators rather strong expression at all metal levels
- expression mainly in metabolically active cells, not metal storage cells

From: Küpper H, Seib LO, Sivaguru M, Hoekenga OA, Kochian LV, 2007 The Plant Journal 50(1), 159-187



# Mechanisms of metal uptake in plants (III)

## Natural resistance associated macrophage proteins (Nramps)

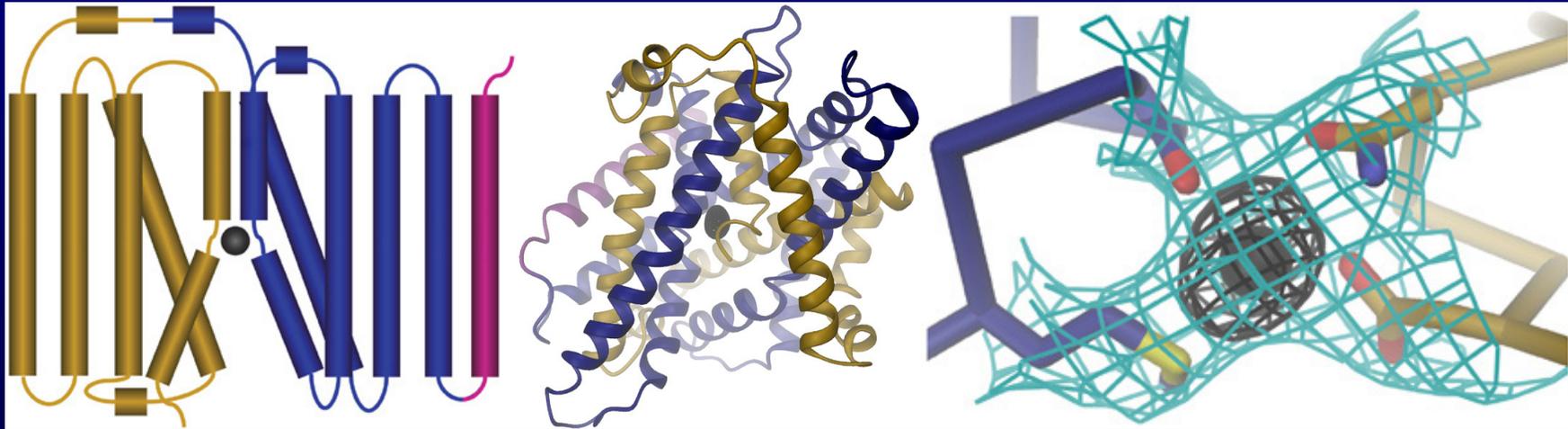


### Likely Functions (deduced from expression studies)

- Discovered to have a role in the immune response of animals (→ name!)
- abundant in all eucaryotes, incl. humans, plants and fungi
- predicted to play a role in uptake into the cell as well as trafficking inside the cell

# Mechanisms of metal uptake in plants (III)

## Natural resistance associated macrophage proteins (Nramps)



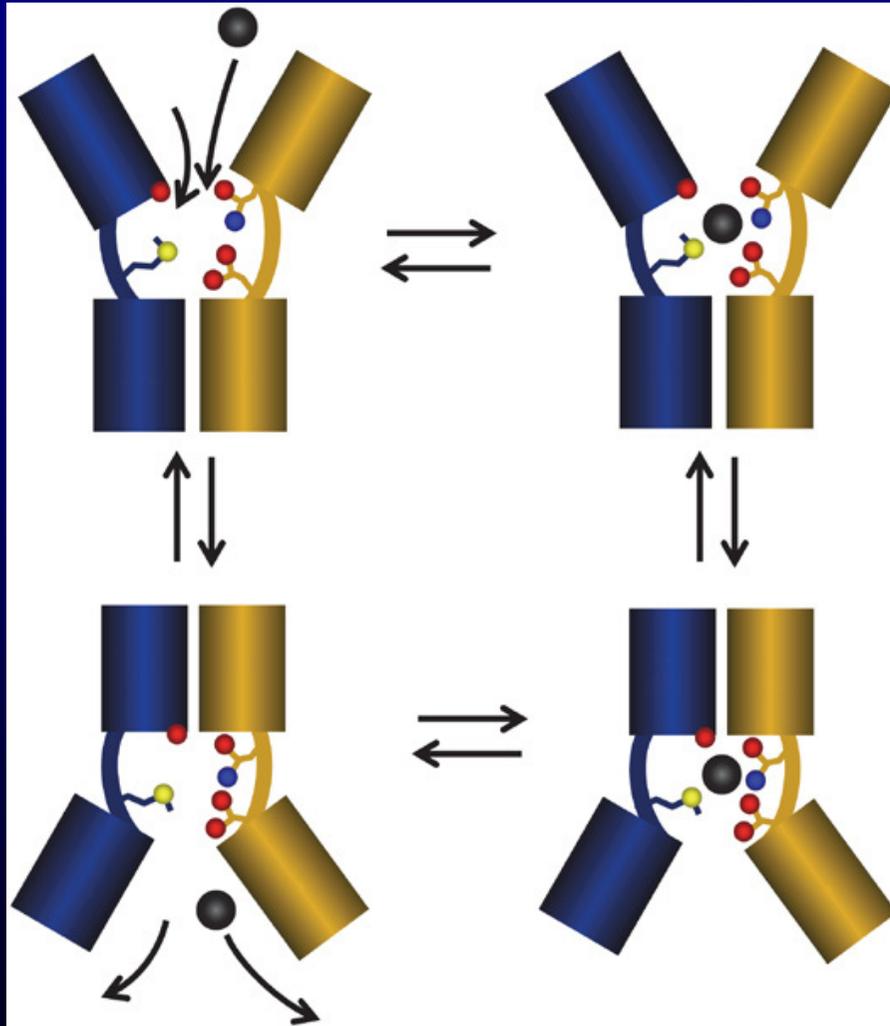
From: Ehrnstorfer IA, Geertsma ER, Pardon E, Steyaert J, Dutzler R. Nat Struct Mol Biol. 21, 990-6

### Structure of a bacterial protein with high similarity to eukaryotic proteins

- 11 transmembrane helices, 10 of them with conserved sequence
- long loops on both sides of the membrane
- main metal binding site in the middle of transmembrane helices 1 and 6
- metal (in this case  $Mn^{2+}$ ) coordination in main binding site by one methionine-S and oxygens from alanine, aspartate and asparagine

# Mechanisms of metal uptake in plants (III)

## Natural resistance associated macrophage proteins (NRAMPs)

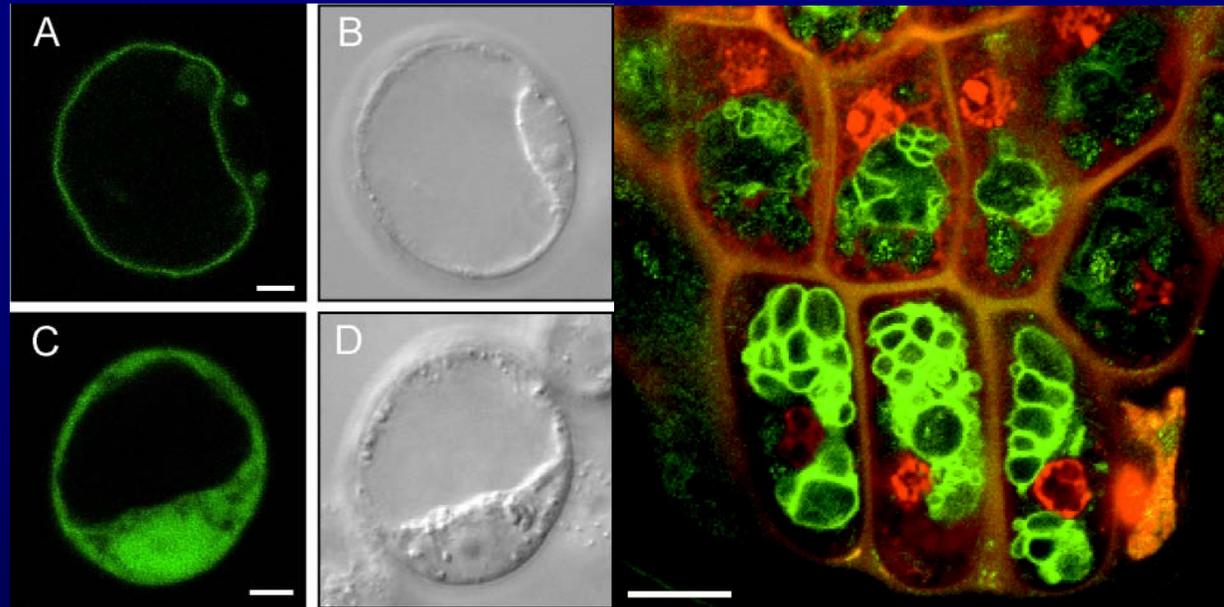


Mechanism deduced from crystal structure and enzyme kinetic studies

- proton symport with the electrochemical gradient drives metal translocation against the gradient
- binding of metal and proton induces a conformational change of the two halves of the helices 1 and 6 around a hinge in the metal binding site
- the conformational change closes the pore on the outer side and opens a pore on the inner side
- the opening of the intracellular pore releases metal and proton.



## Mechanisms of metal uptake in plants (IV): Cation diffusion facilitator (CDF)-transporters



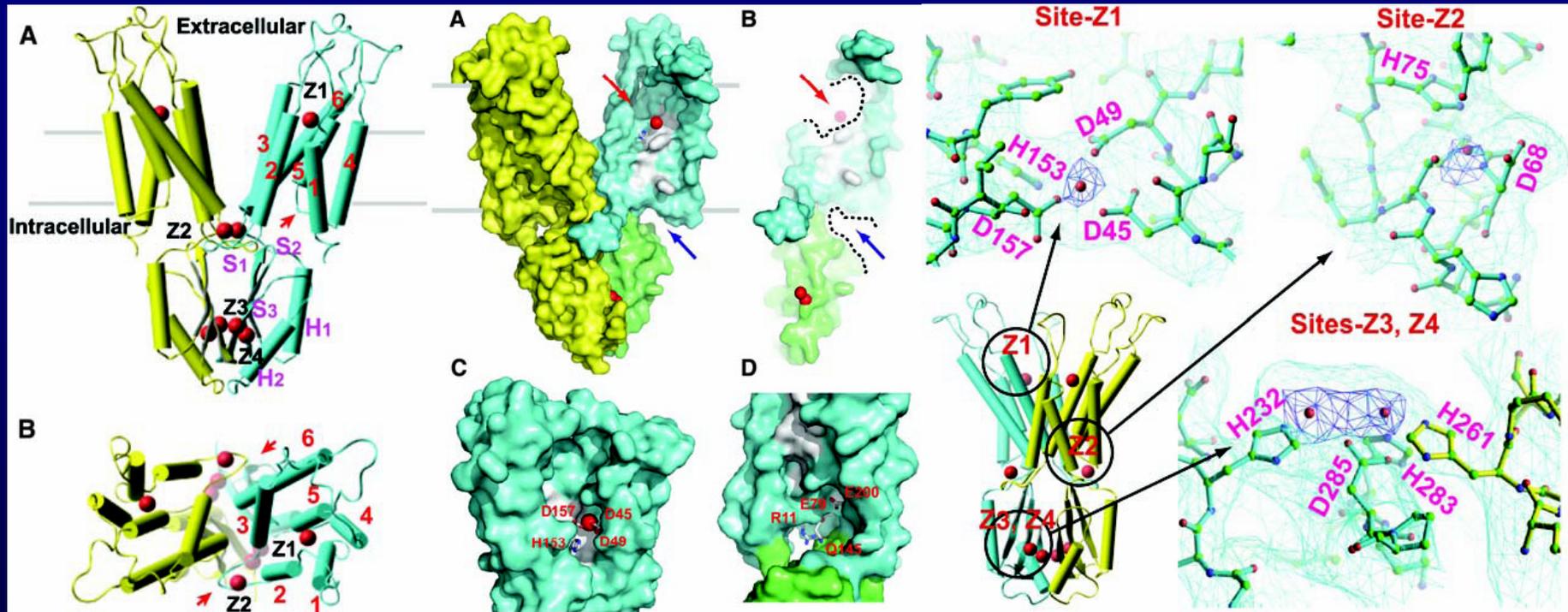
From: Kobae et al., 2004,  
PlantCellPhysiol 45, 1749-58

From: Blaudez D et al., 2003,  
PlantCell 15, 2911-28

### Functions concluded from expression studies (localisation and overexpression/knockout phenotypes)

- Metal detoxification
- Sequester the metals in intracellular compartments (mainly vacuole)

# Mechanisms of metal uptake in plants (IV): Cation diffusion facilitator (CDF)-transporters

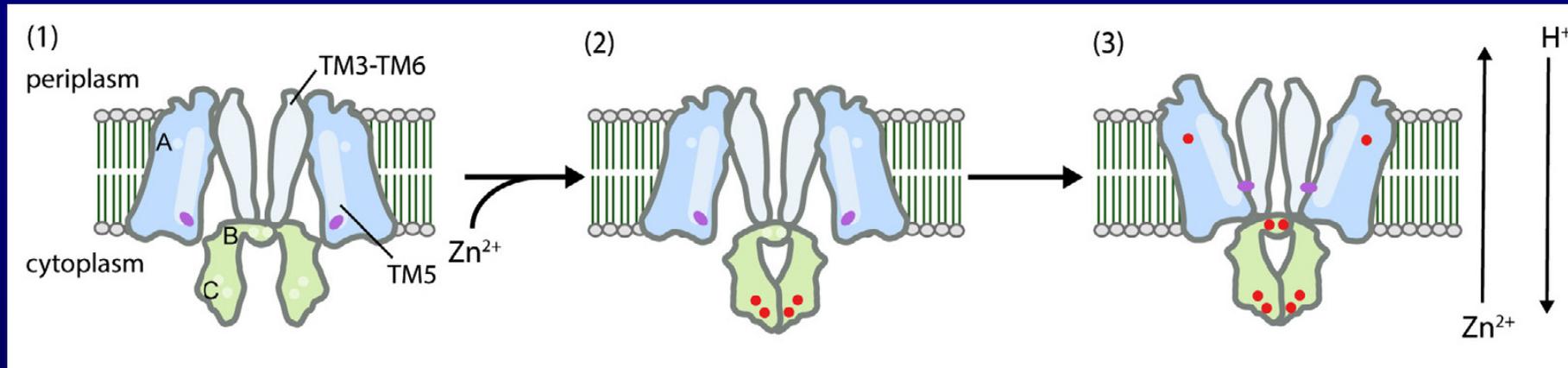


From: Lu M, Fu D. 2007. Structure of the zinc transporter YiiP. Science 317, 1746-8

## Structure of a bacterial Zn-transporting CDF (YiiP) similar to others

- MANY histidines in sequence used for metal binding
- 6 transmembrane helices per protein, active form is dimer held together by four  $Zn^{2+}$  in cytoplasmic domain
- 2 further metal binding domains in the protein at both sides of the membrane

## Mechanisms of metal uptake in plants (IV): Cation diffusion facilitator (CDF)-transporters

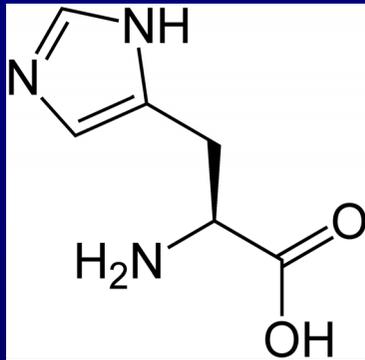


From: Kolaj-Robin O, Russell D, Hayes KA, Pembroke JT, Soulimane T. 2015. FEBS Lett. 589, 1283-95

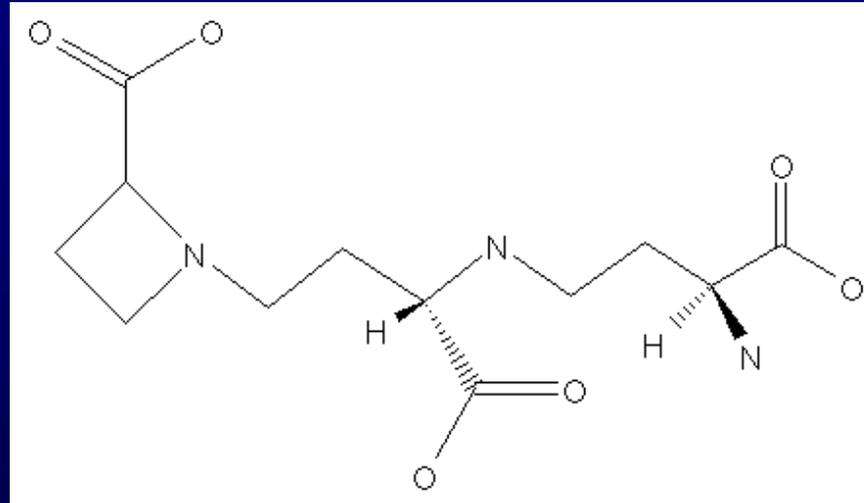
### Mechanism concluded from structure and kinetic studies

- Proton-metal antiport
- The exact movements are still discussed as the only available complete crystal structure is rather low resolution (3.8Å)
- Metal binding causes a conformational change of the cytoplasmic domain
- The conformational change leads to release of the metals on the outside of the cell

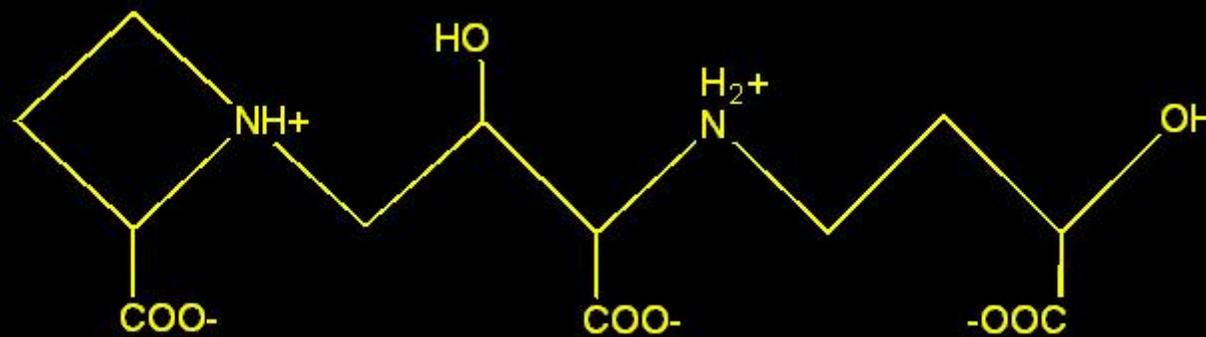
# Mechanisms of metal uptake in plants (V): Long-distance transport ligands



Histidine



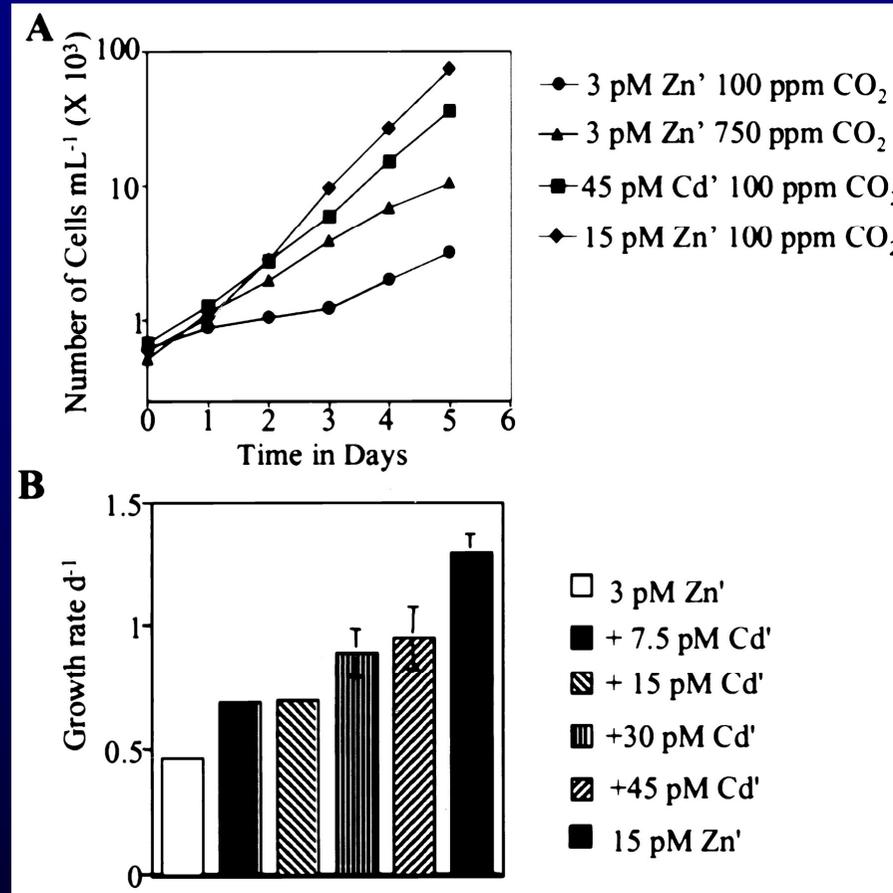
Nicotianamine



Mugineic acid

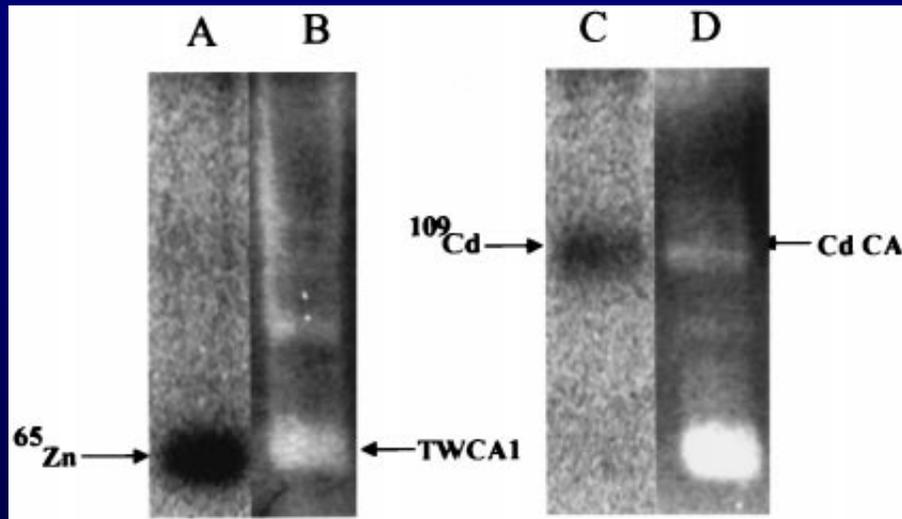
**Now let's have a look at a few plant micronutrients**

# Cadmium as a micronutrient

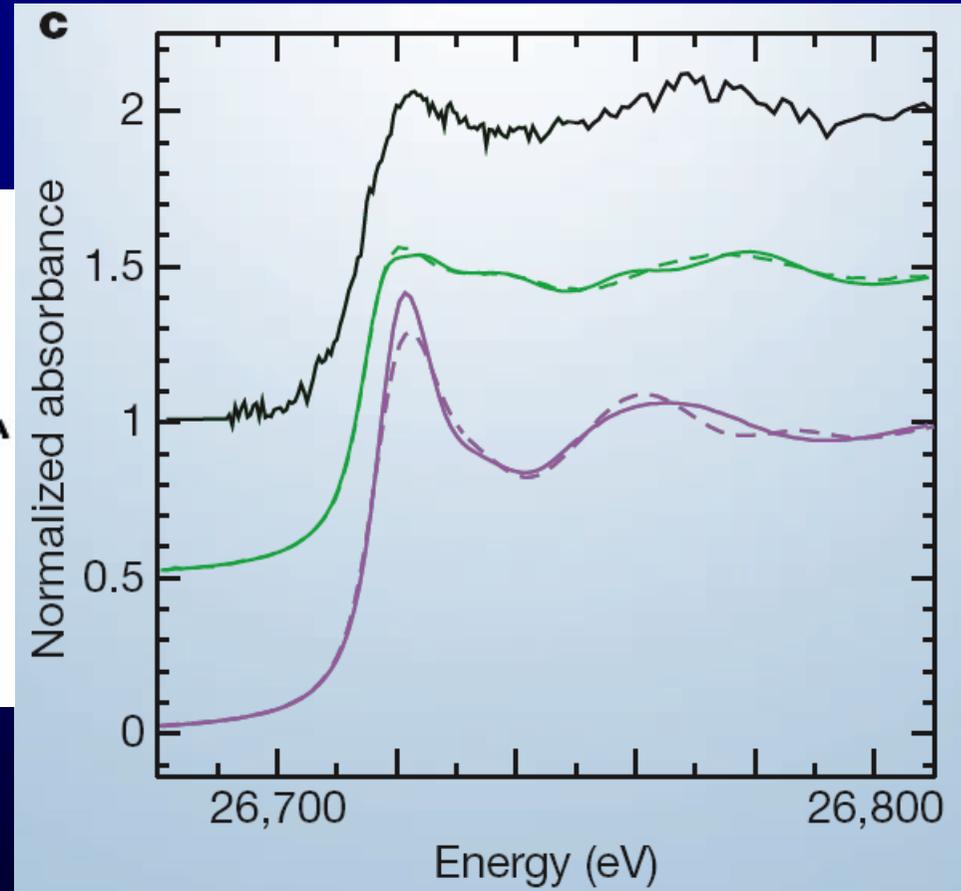


Cadmium as Plant-micronutrient in *Thalassiosira weissflogii*. A, B: growth of the algae. (Lane and Morel, 2000, PNAS97)

# Carboanhydrase from *Thalassioria weissflogii*: An enzyme with cadmium in its active centre



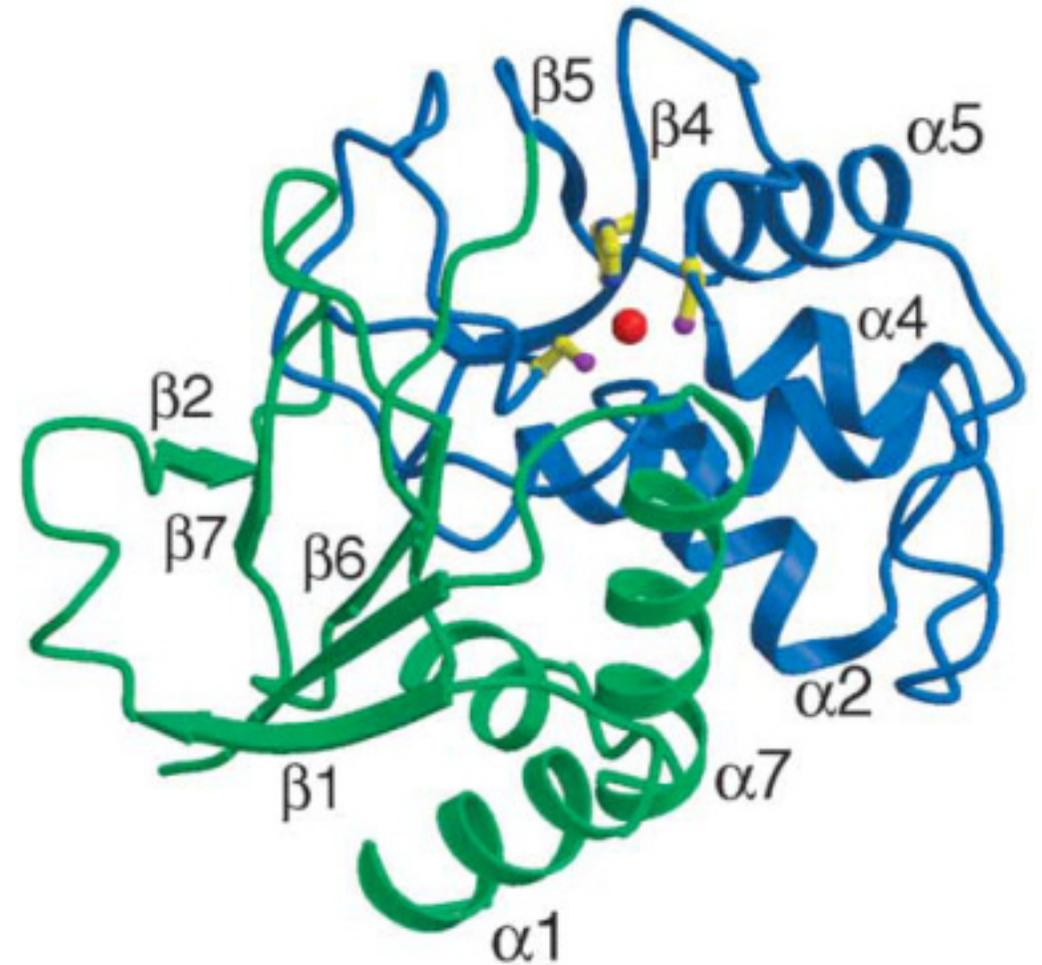
Size of the cadmium-carboanhydrase in comparison to the normal Zn-carboanhydrase (Lane and Morel, 2000, PNAS Vol. 97)



EXAFS-spectrum of the isolated Cd-carboanhydrase (Lane et al., 2005, Nature Vol. 435)

# Properties and structure of the Cd-carboanhydrase

(Xu et al., 2008, Nature 452, pp 56-61)



- Cd-CA can bind both Cd and Zn. Activity with Zn somewhat higher, but activity with Cd much higher than for regular Zn-carboanhydrases.
- Cd-CA has 7  $\alpha$ -helices and 9  $\beta$ -sheets, Cd at the lower end of a funnel-like binding pocket
- $\text{Cd}^{2+}$  is bound via three conserved amino acid residues: 2x cystein and 1x histidin, plus 1x Water ( $\rightarrow$  tetrahedral coordination). Further fixed water molecules nearby

# Cadmium deficiency in the Cd/Zn-hyperaccumulator *Thlaspi caerulescens*

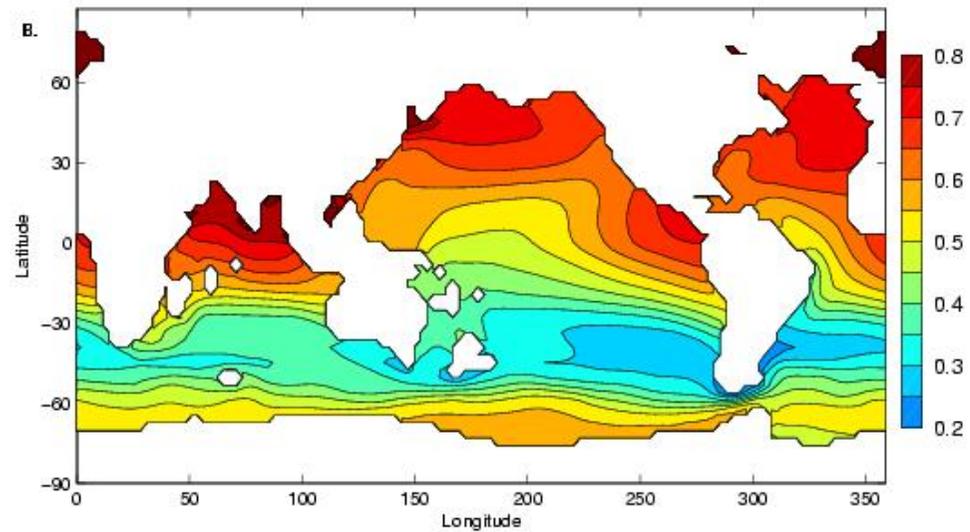
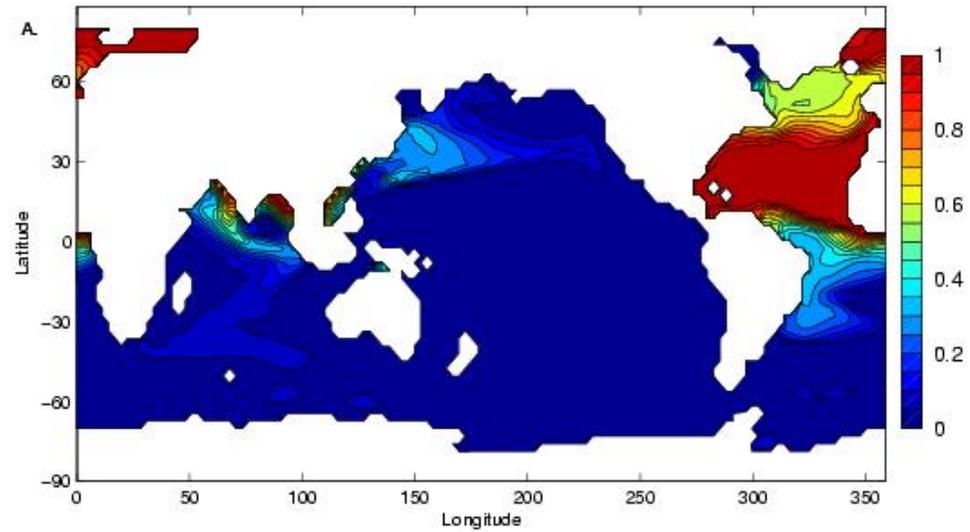


With 10  $\mu\text{M}$  cadmium in the nutrient solution  
--> healthy plants

Without Cd in the nutrient solution  
--> damage by insects

# Iron

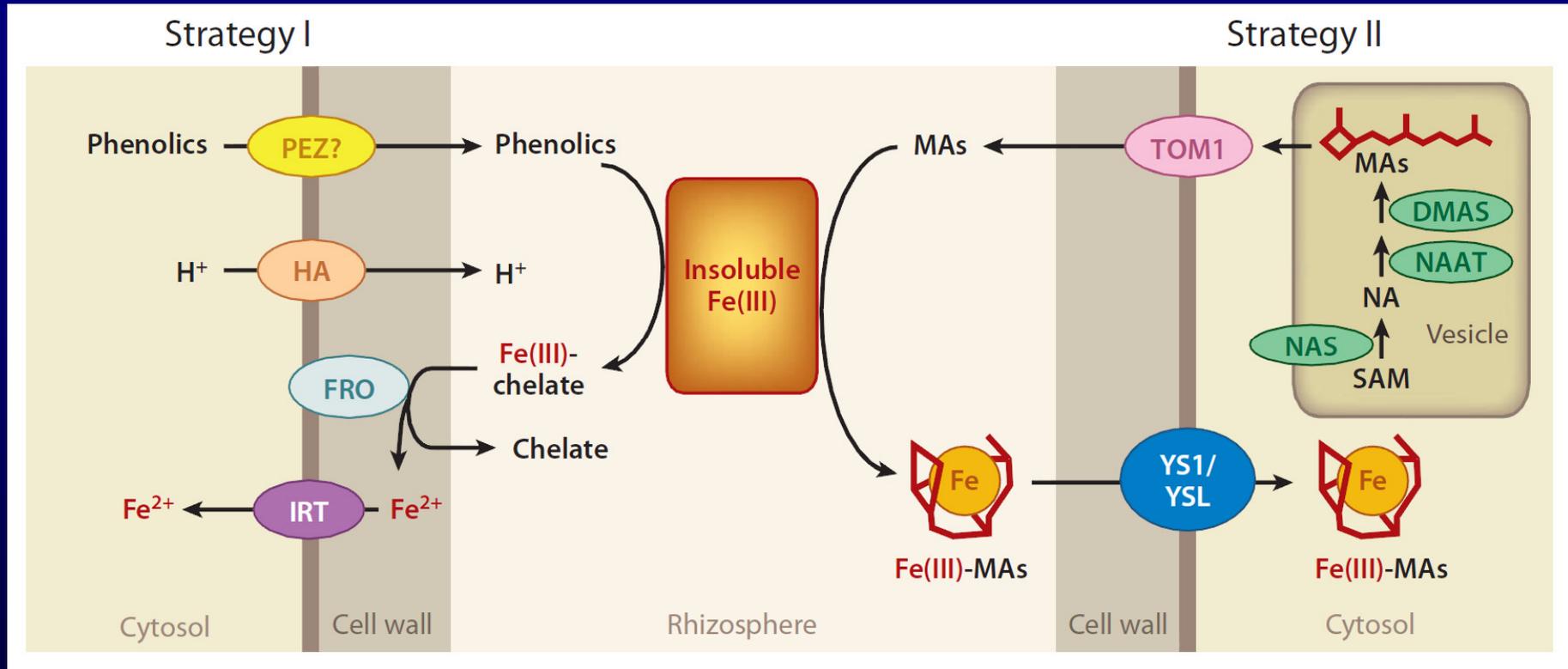
Iron concentrations at the surface (top picture) and in 1000m depth (bottom picture)



Source: [www-paoc.mit.edu](http://www-paoc.mit.edu)

# Mechanisms of iron uptake in plants

## Strategies of iron efficiency



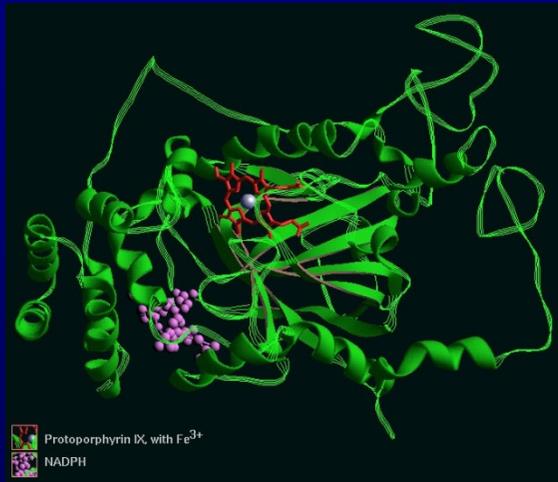
From: Kobayashi T, Nishizawa NK. 2012. Ann Rev Plant Biol 263, 131-152

### Strategies of making insoluble Fe(III) bioavailable

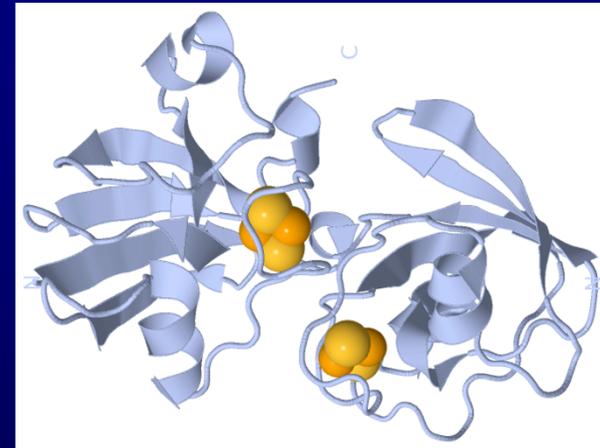
- Strategy I (most plants): use mostly of soil acidification and iron reductase at root surface
- Strategy II (grasses): use of secreted iron ligand mugeinic acid

# Examples of plant enzymes with iron in the active centre

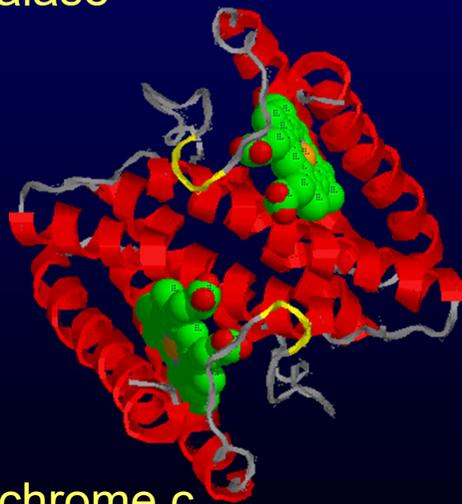
*Details on biochemistry & spectroscopy: lecture on iron+copper proteins!*



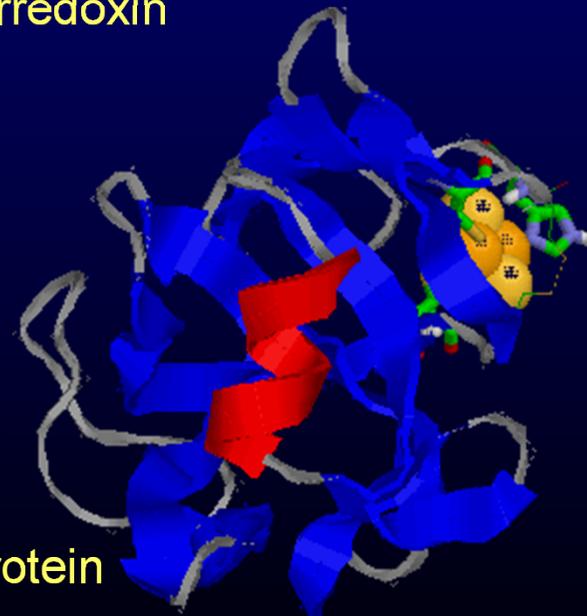
Catalase



Ferredoxin



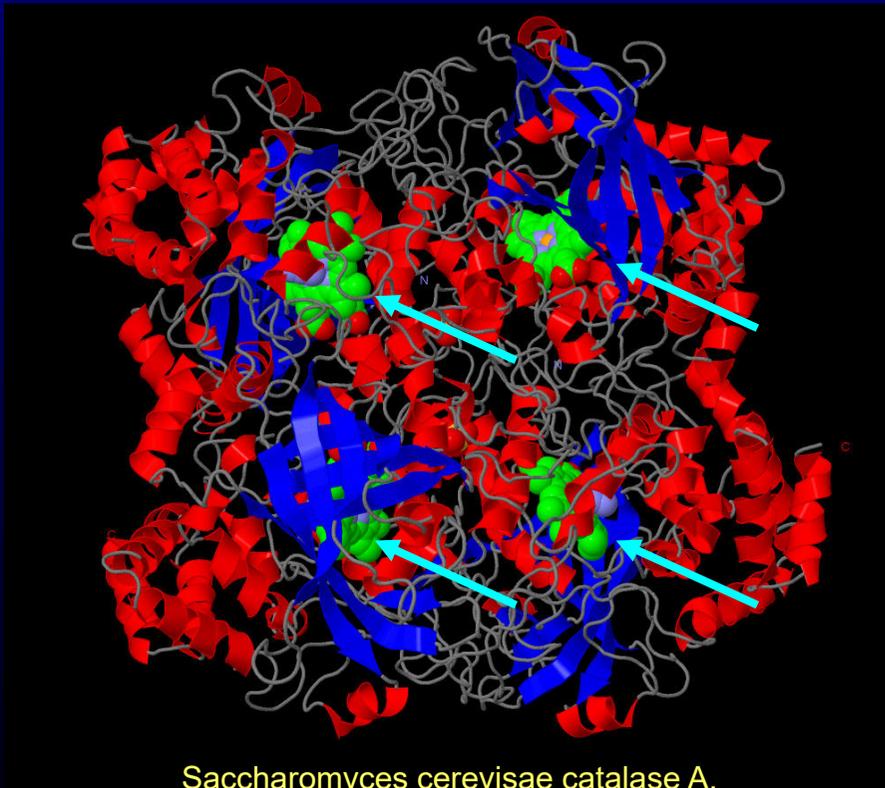
Cytochrome c



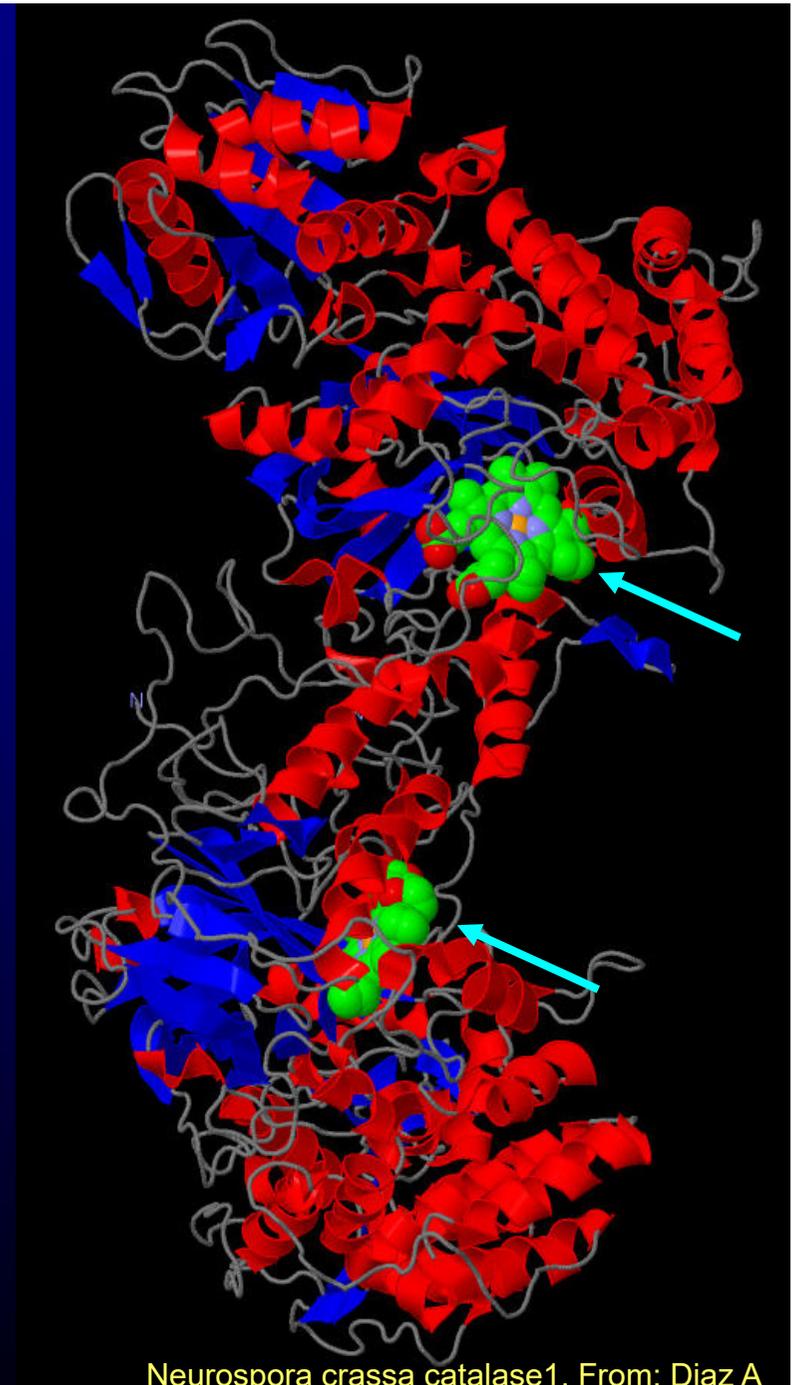
Rieske protein

# Catalases, some of the most important heme-iron enzymes: (a) function and structure

- Detoxify hydrogen peroxide ( $H_2O_2$ )
- Various forms found in all kinds of aerobic organisms from bacteria to plants and animals
- Iron always present as heme-iron



*Saccharomyces cerevisiae* catalase A,  
From: Maté JM et al, 1999, J Mol Biol 268, 135-49

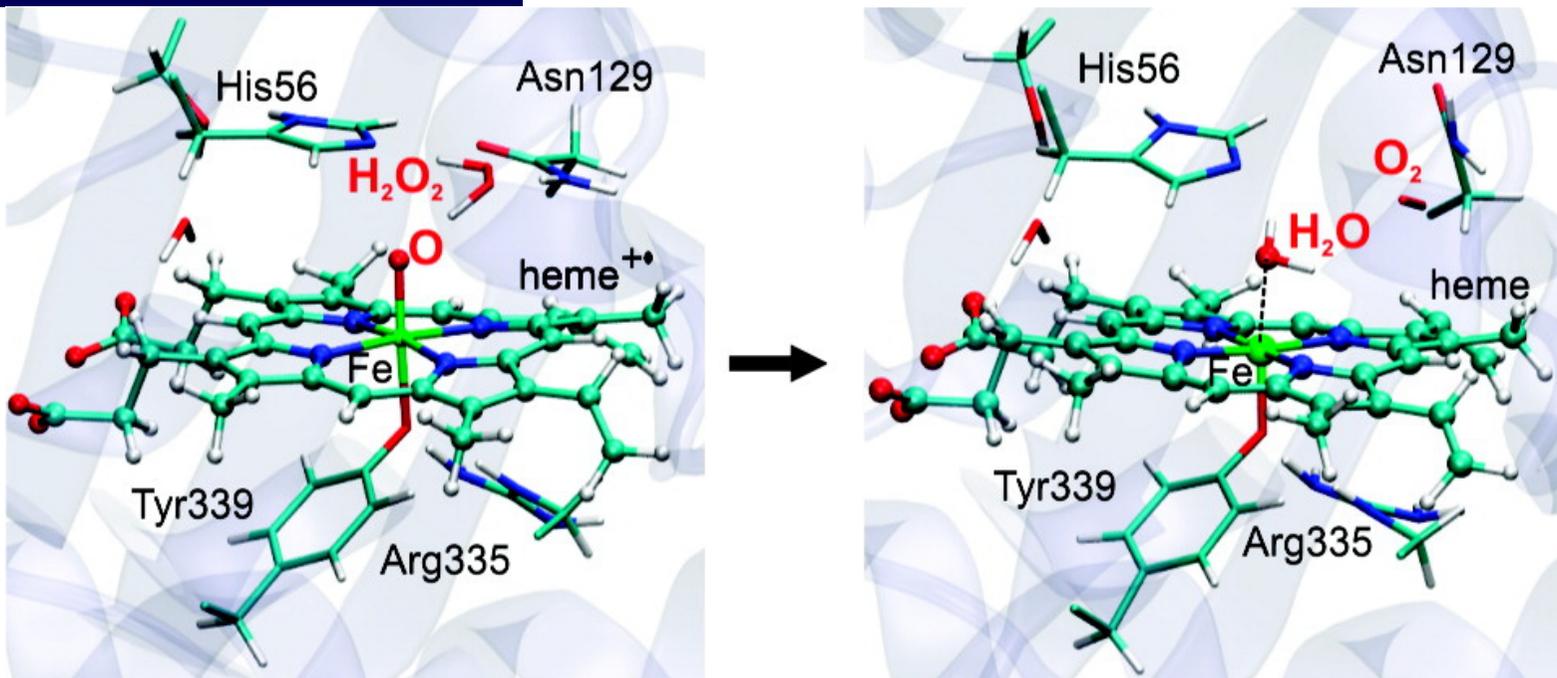
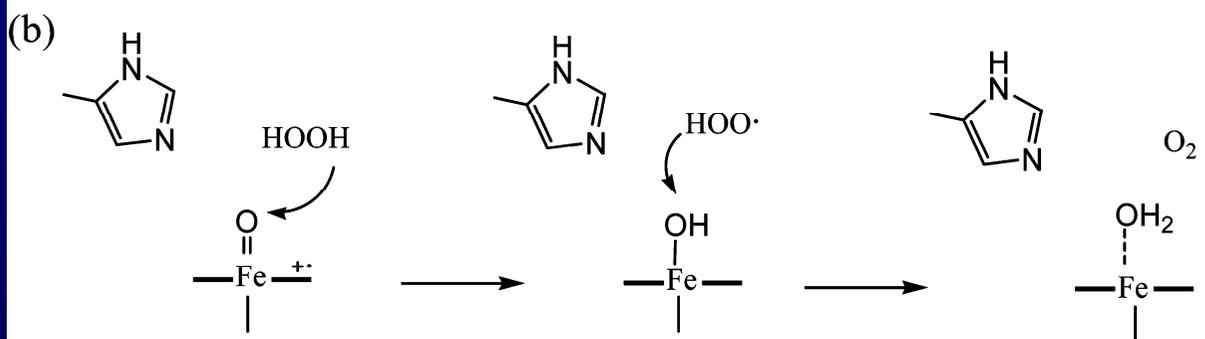
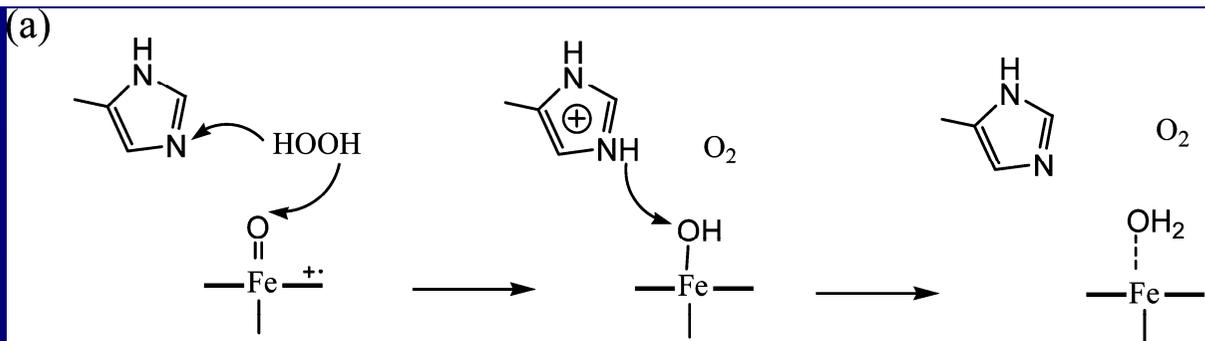


*Neurospora crassa* catalase 1, From: Diaz A  
et al, 2004, J Mol Biol 342, 971-85

# Catalases, heme-iron enzymes

## (b) catalysis

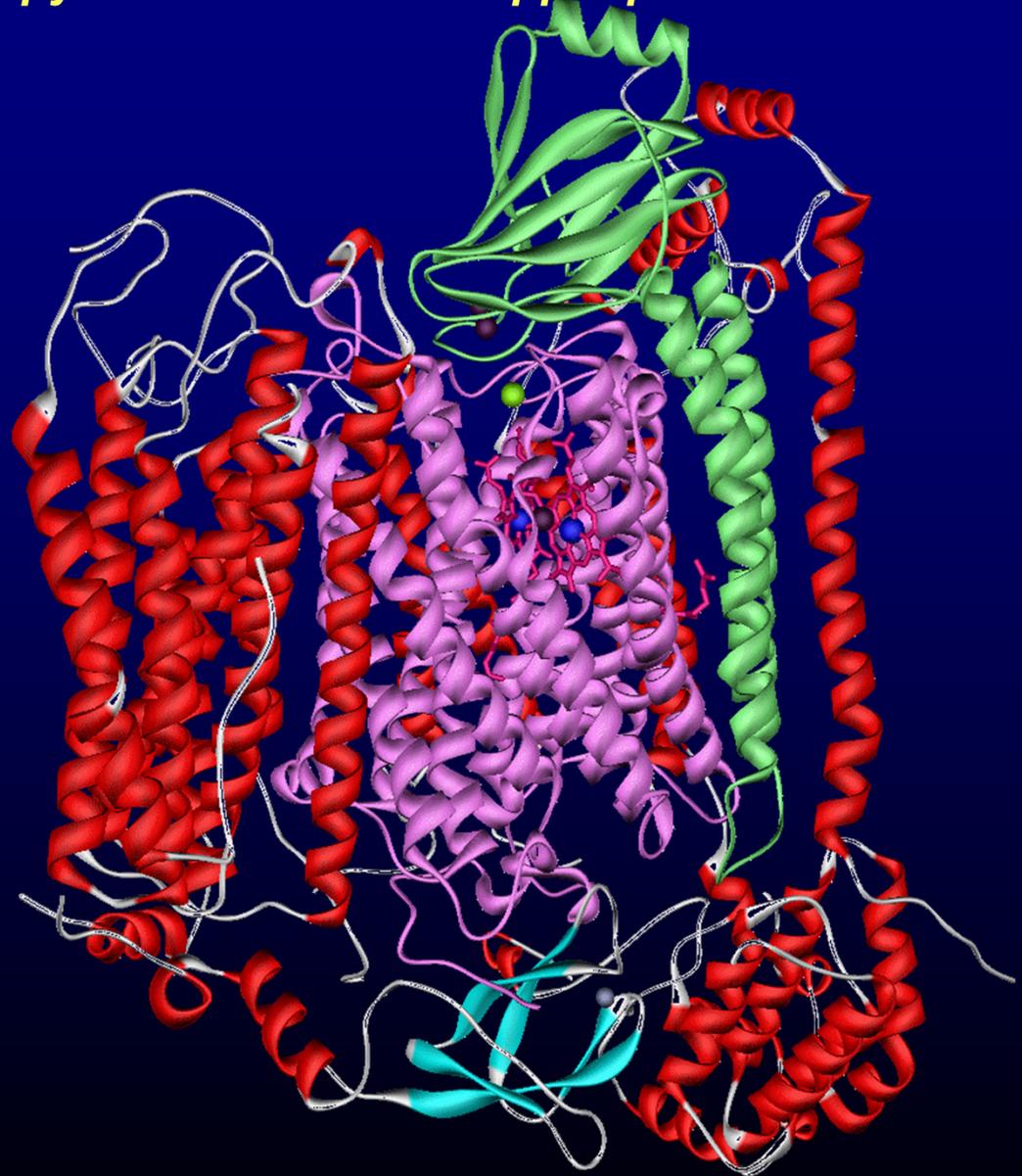
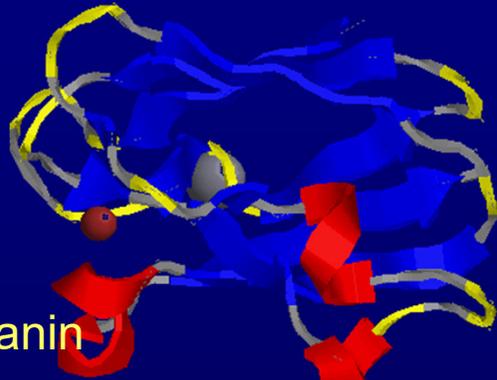
From: Alfonso-Prieto M et al., 2009, J. Am. Chem. Soc. 131, 11751-61.



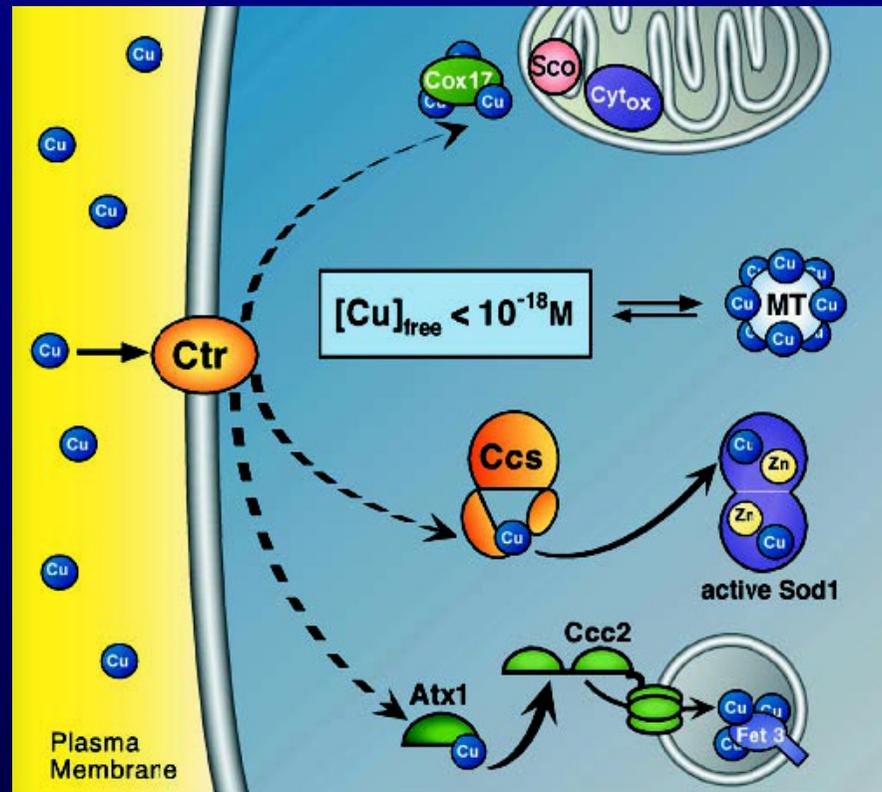
# Selected enzymes with copper in the active centre

*Details on biochemistry & spectroscopy: lecture on iron+copper proteins!*

- Plastocyanin
- Cytochrome c oxidase 3 Cu (binuclear site and mononuclear site)
- SOD = Superoxide dismutase



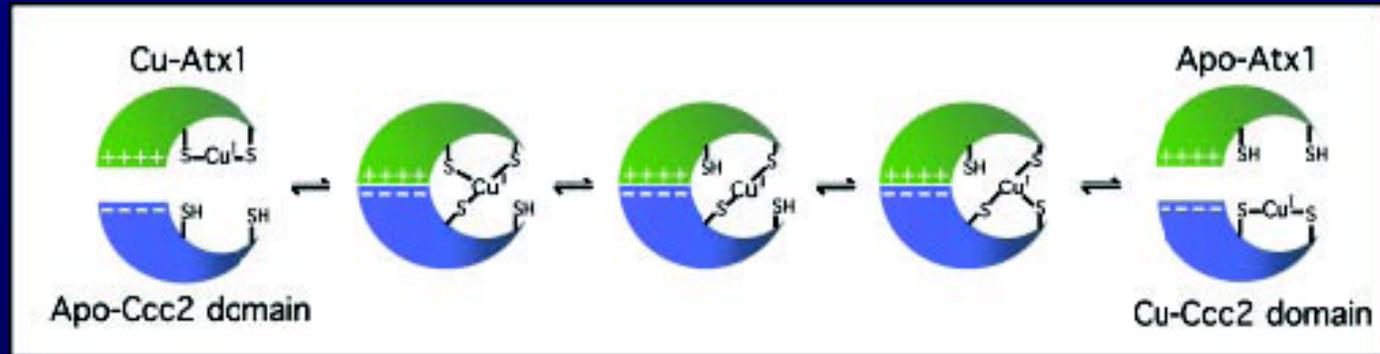
# Copper delivery inside cellular compartments



From: O'Halloran TV, Culotta VC, 2000, JBC275, 25057-60

- confusing large number of names for homologous proteins in different organisms
- REALITY: just 3 really different (non-homologous) Cu-chaperones are well known, some more proteins are postulated to be Cu-chaperones

# Copper delivery to the Golgi and thylakoid: ATX1 = HAH1 = ATOX1 = CopZ $\approx$ CCH (a) occurrence



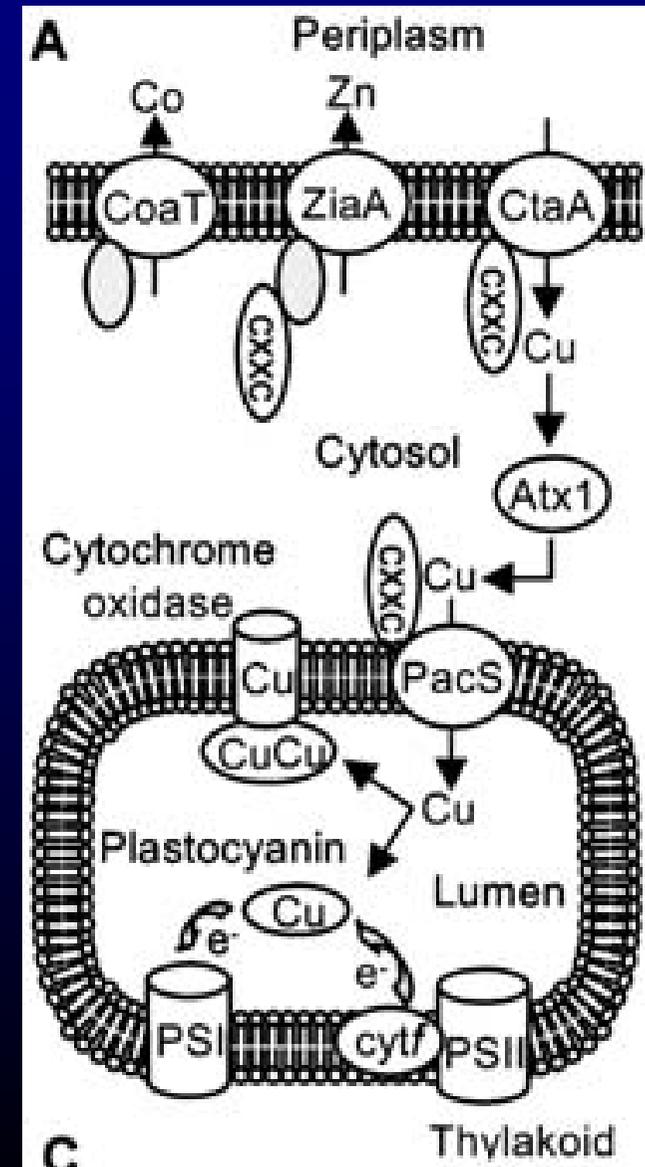
From: O'Halloran TV, Culotta VC, 2000, JBC275, 25057-60

- ATX1 found in yeast originally as a gene involved in protection against oxidative damage;
- human homologue: HAH1 = ATOX1
- bacterial homologue: CopZ
- cyanobacterial and homologues: Atx1
- similar to plant CCH

# Copper delivery to the Golgi and thylakoid: ATX1 = HAH1 = ATOX1 = CopZ

## (b) function in bacteria, cyanobacteria+plants

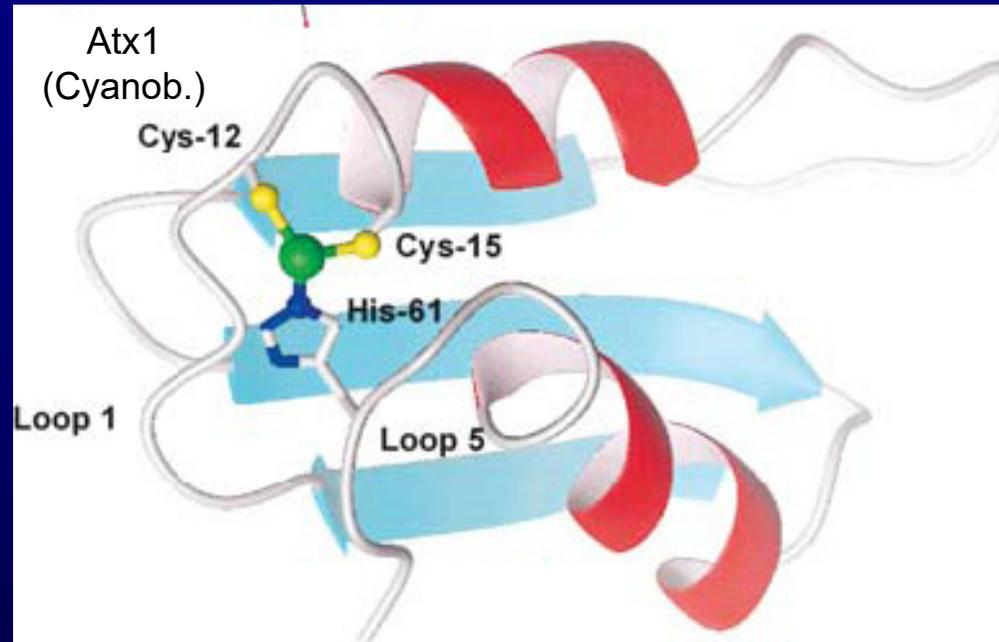
- CopZ in non-photosynthetic bacteria donates Cu to CopY transcription factor? Or Cu delivery to Cu-efflux transport ATPase CopB or copper influx ATPase CopA?
- Atx1 found to specifically shuttle copper to an intracellular CPx-type copper ATPase the thylakoid in cyanobacteria+plants
- CtaA+PacS ATPases deliver Cu for plastocyanine across thylakoid membrane



## Copper delivery to the Golgi and thylakoid:

**ATX1 = HAH1 = ATOX1 = CopZ = Atx1**

### (c) Cu-binding in bacterial+cyanobacterial+plant version



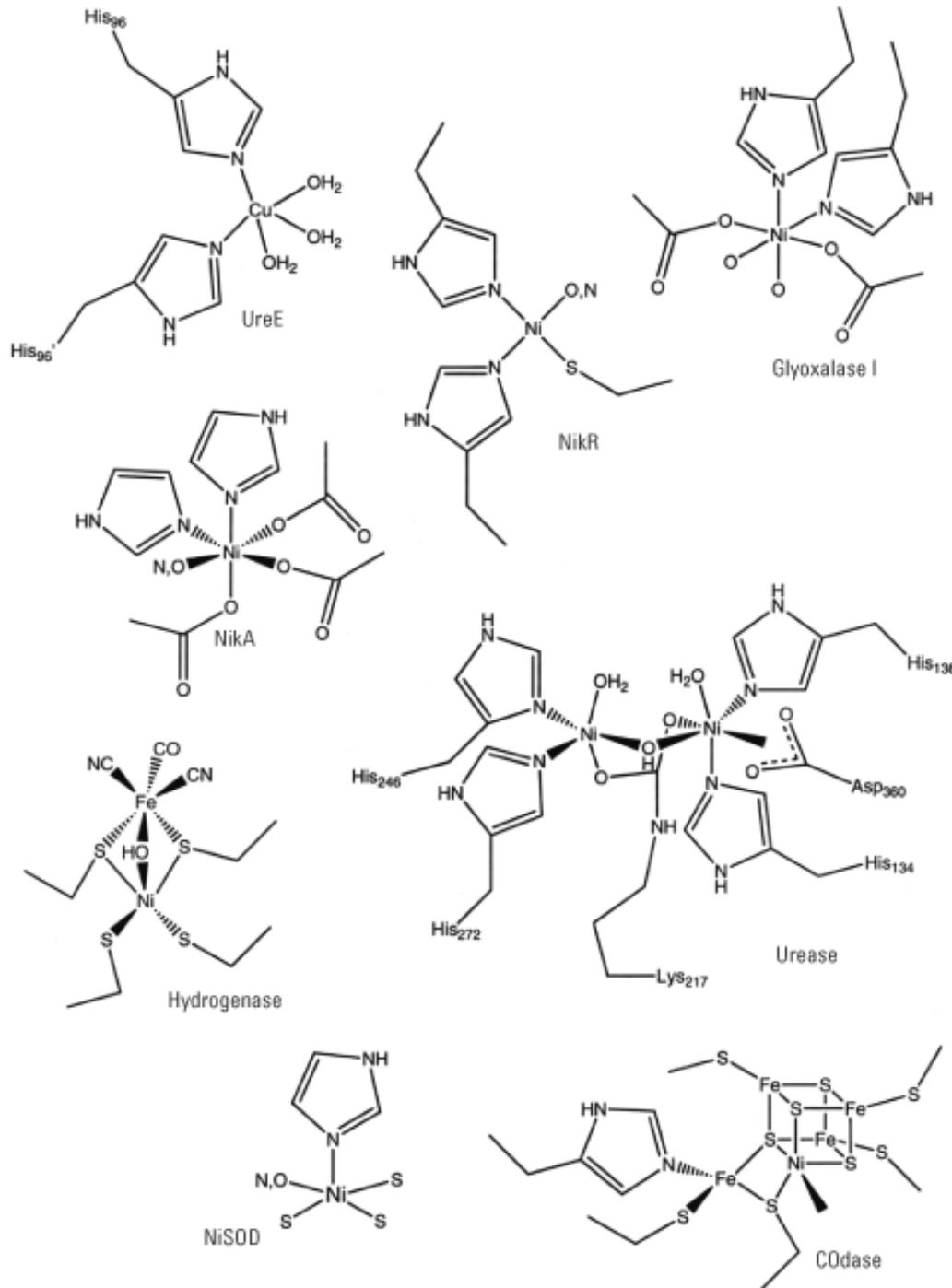
From: Borrelly GPM, et al., 2004, BiochemJ378, 293-7

- Atx1 binds a single Cu(I) ion like ATX1
- Atx1: like in the yeast+animal proteins, Cu-binding via two Cys in the sequence MT/HCXXC, **BUT** additional histidine61 from loop 5
- the additional histidine shifts Atx1 binding affinity towards CtaA by reducing affinity for PacS → trafficking of Cu(I) from one CtaA to the PacS
- other features like in yeast+animal proteins

# Examples of nickel complexes in proteins

## Characteristics

- Nickel is usually bound by nitrogen (mainly histidine), sulphur (cysteine) and oxygen ligands
- usually 5 (4-6) ligands

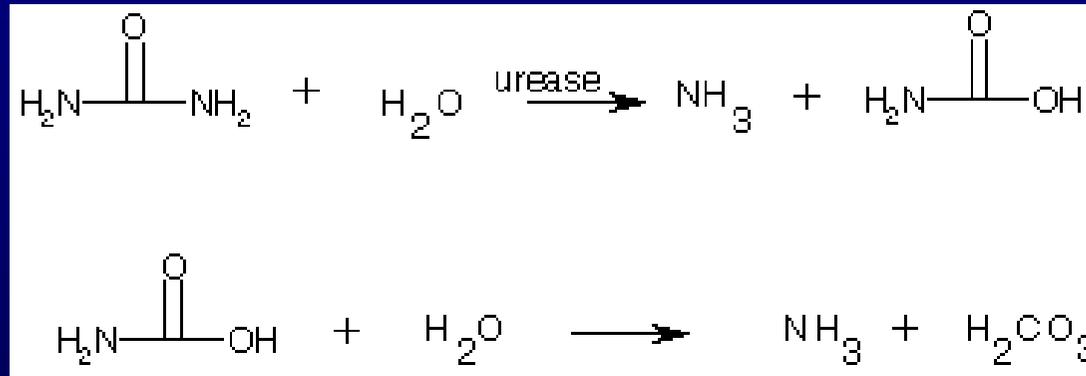


From:

Carrington PE et al, 2002, EnvHealthPers110, 705-

# Best known (and most important?) Ni-enzyme: Urease

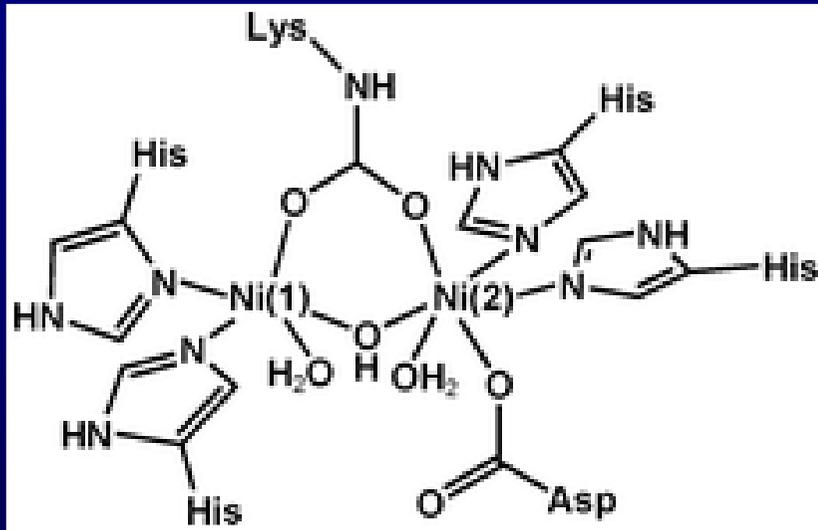
## a) Function and occurrence



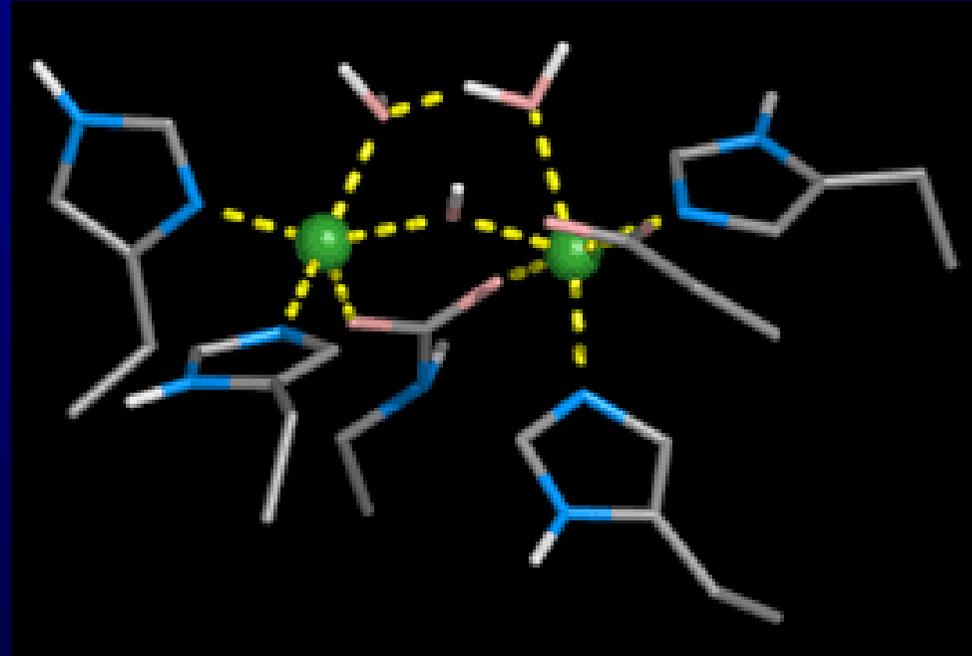
- Catalyses the decomposition of urea into carbon dioxide and ammonia
- In most organisms (plants, fungi, bacteria)
- Very important for metabolism: urea toxicity is one of the main mechanisms of damage caused by nickel deficiency in plants
- Very specific for urea as a substrate
- Rather fast: turnover rate  $k_{\text{cat}}$  around  $3,500 \text{ s}^{-1}$

# Urease

## b) Active site



From: Lee WZ et al., 2008,  
DaltonTrans, 2538-41



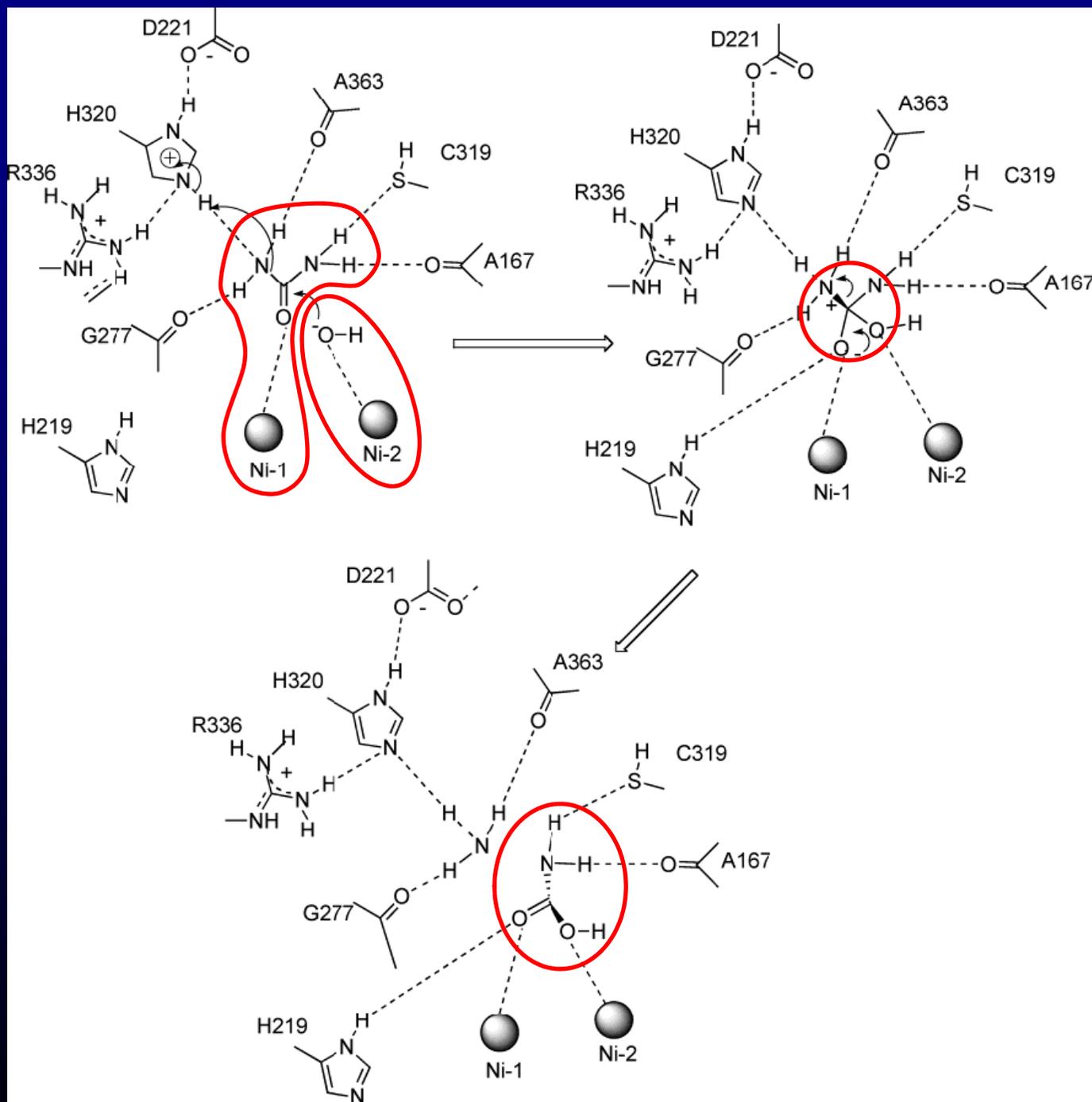
- two Ni<sup>2+</sup> ions
- one Ni<sup>2+</sup> bound by three fixed ligands (2 His-N and 1 Lys-O) and one water
- the other Ni<sup>2+</sup> bound by four fixed ligands (2 His-N, 1 Asp-O and 1 Lys-O) and 1 water
- the two nickels are bridged by a water molecule

# Urease

## c) Mechanism

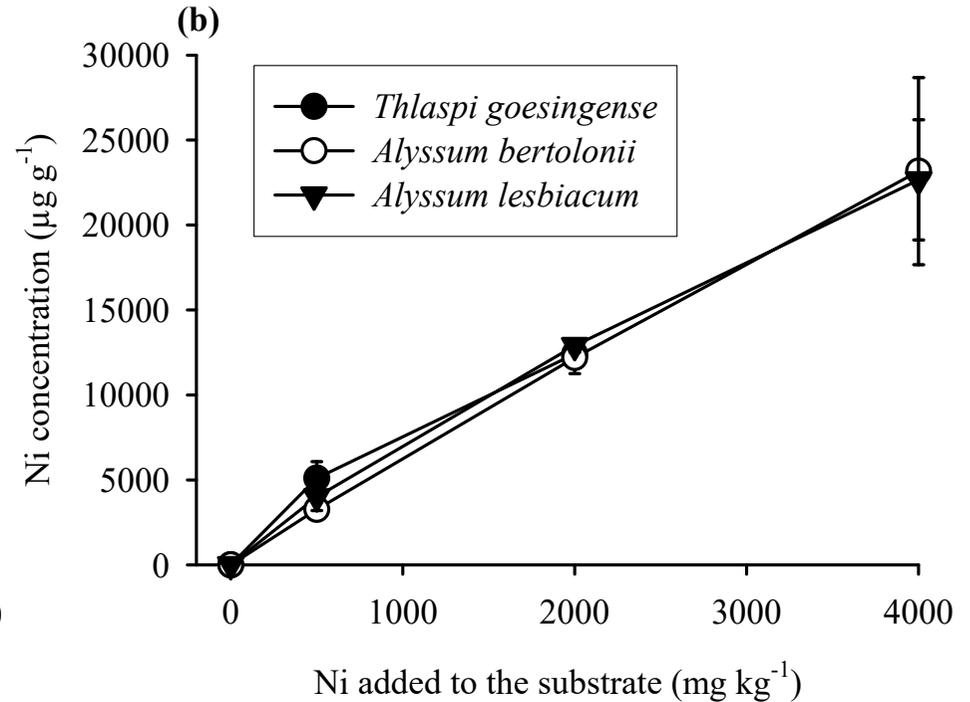
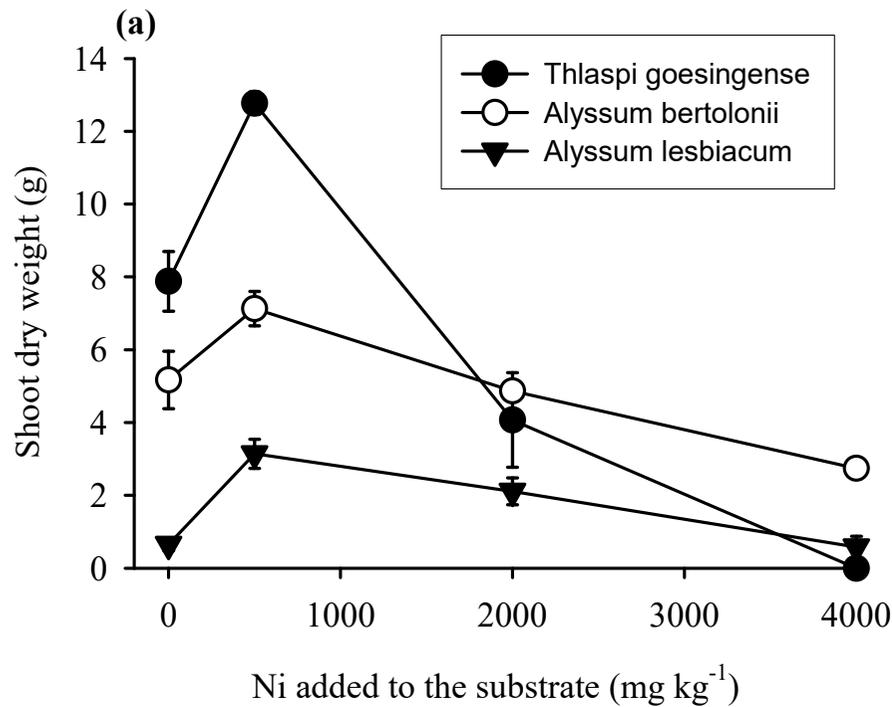
### Steps

- Nickel 1 binds urea at the oxygen
- Nickel 2 binds water
- tetrahedral intermediate
- after cleavage of the C-N bond, carbamic acid is bound to the nickels



From: Karplus PA, Pearson MA, Hausinger RP, 1997, Acc Chem Res 30, 330-37, modified by Estiu G, Merz KM, 2004, JACS126, 11832-42

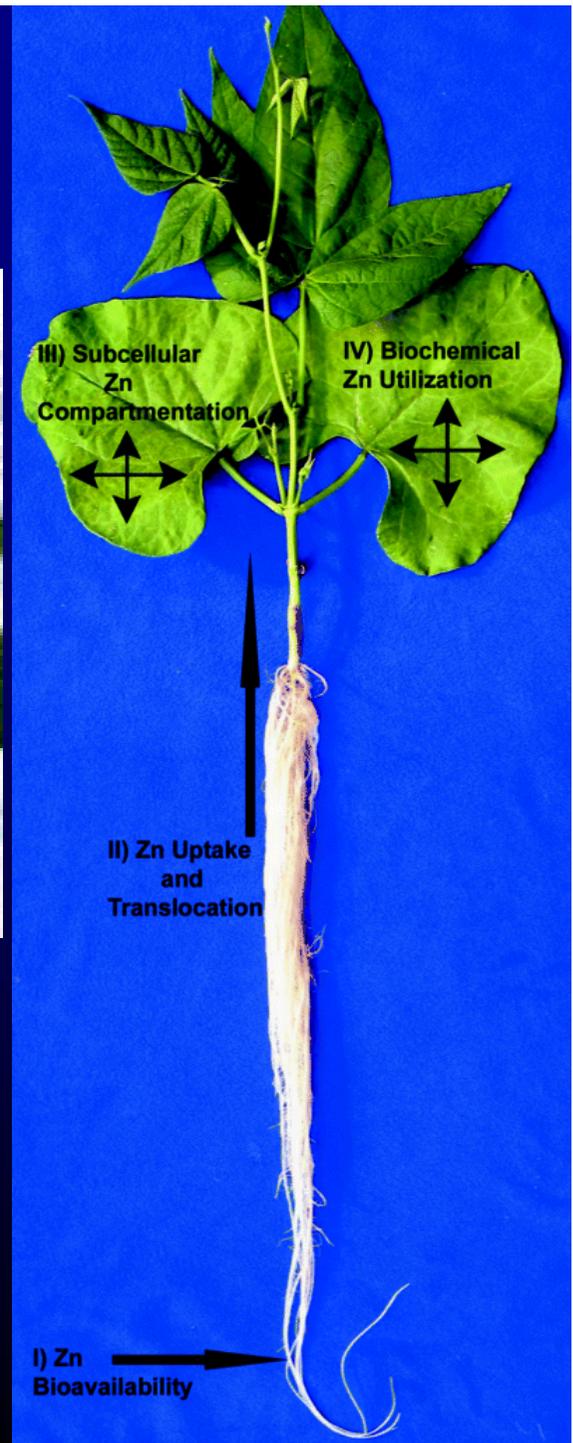
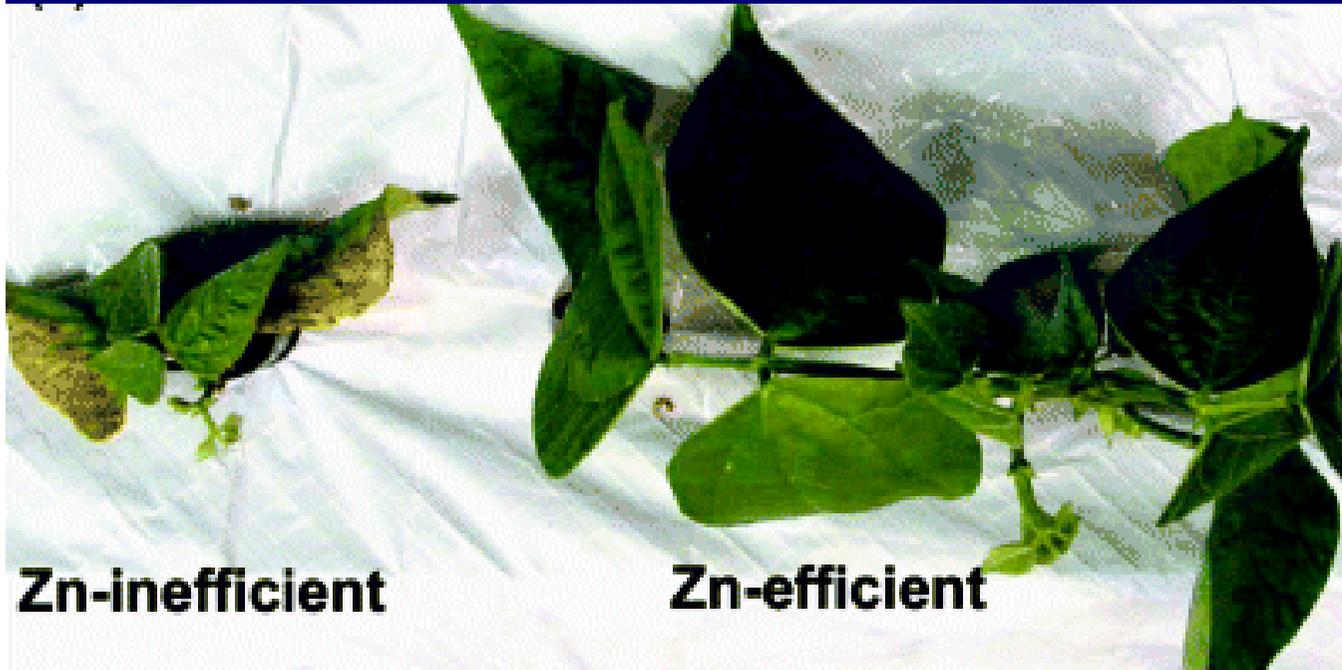
# Nickel deficiency in Ni-Hyperaccumulators



*Alyssum lesbiacum*

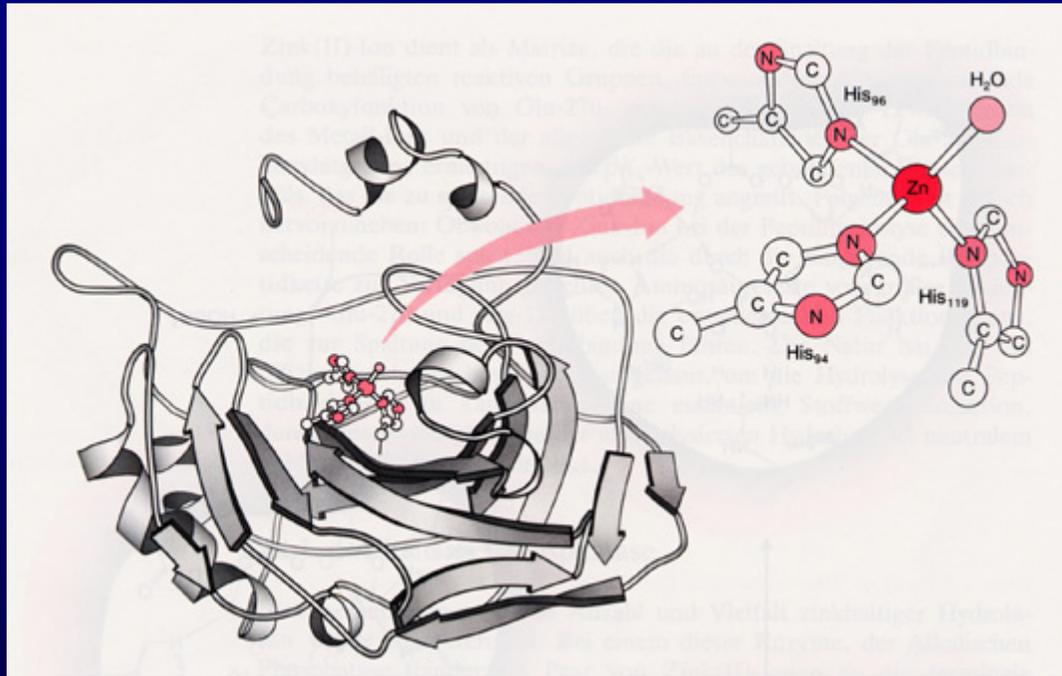
Küpper H, Lombi E, Zhao FJ, Wieshammer G, McGrath SP  
(2001) J Exp Bot 52 (365), 2291-2300

# Zinc efficiency



From: Hacisalihoglu G, Kochian, LV. How do some plants tolerate low levels of soil zinc?  
Mechanisms of zinc efficiency in crop plants.  
New Phytologist 159 (2), 341-350.

# Selected important plant enzymes with zinc in their active centre



Carboanhydrase → details in the lecture about photosynthesis related metal proteins

Zinc finger-motive

Tyrosin phosphatase

**All slides of my lectures can be downloaded  
from my workgroup homepage**

Biology Centre CAS → Institute of Plant Molecular Biology → Departments  
→ Department of Plant Biophysics and Biochemistry,  
*or directly*

[http://webserver.umbr.cas.cz/~kupper/AG\\_Kuepper\\_Homepage.html](http://webserver.umbr.cas.cz/~kupper/AG_Kuepper_Homepage.html)