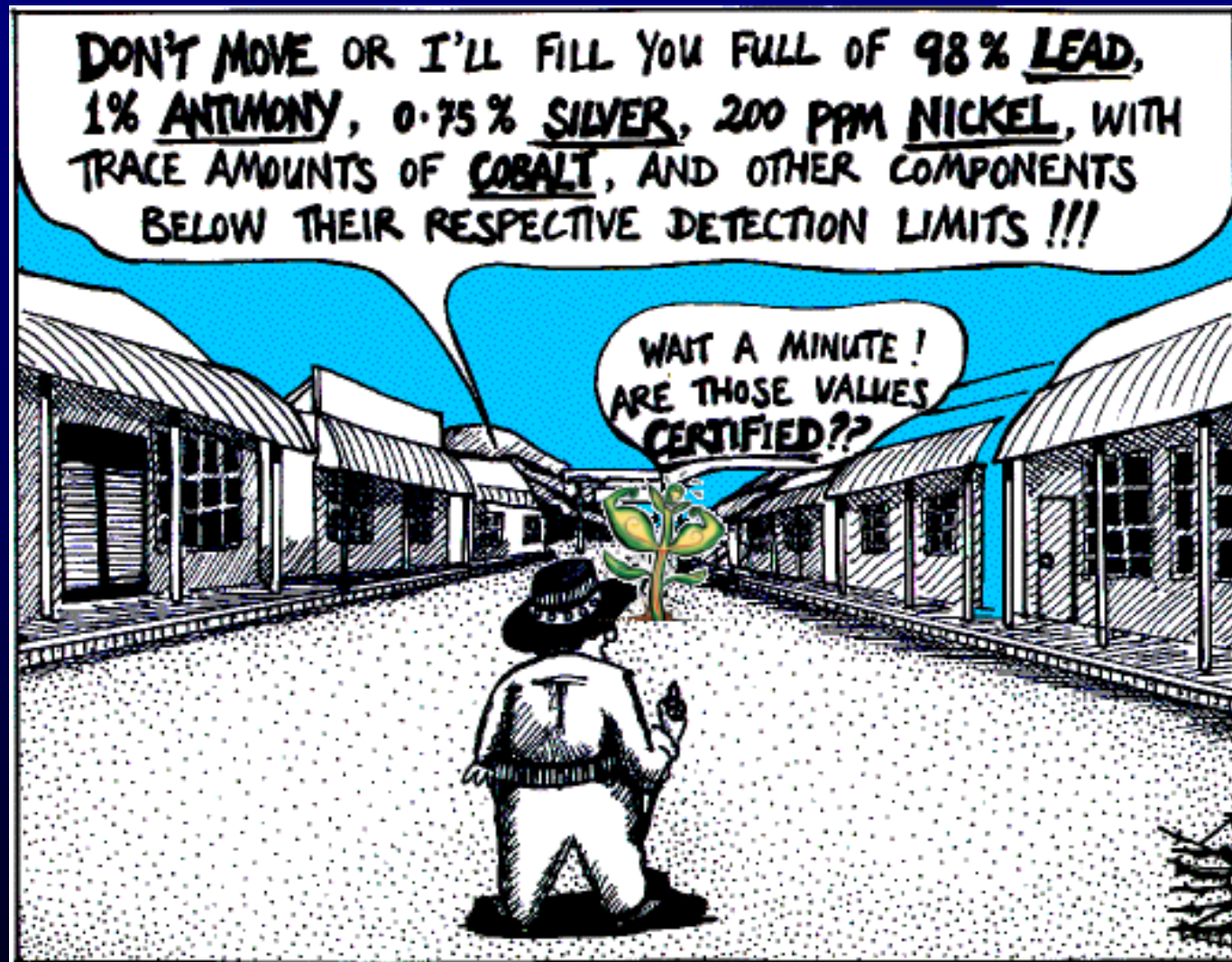


Trace metals as micronutrients

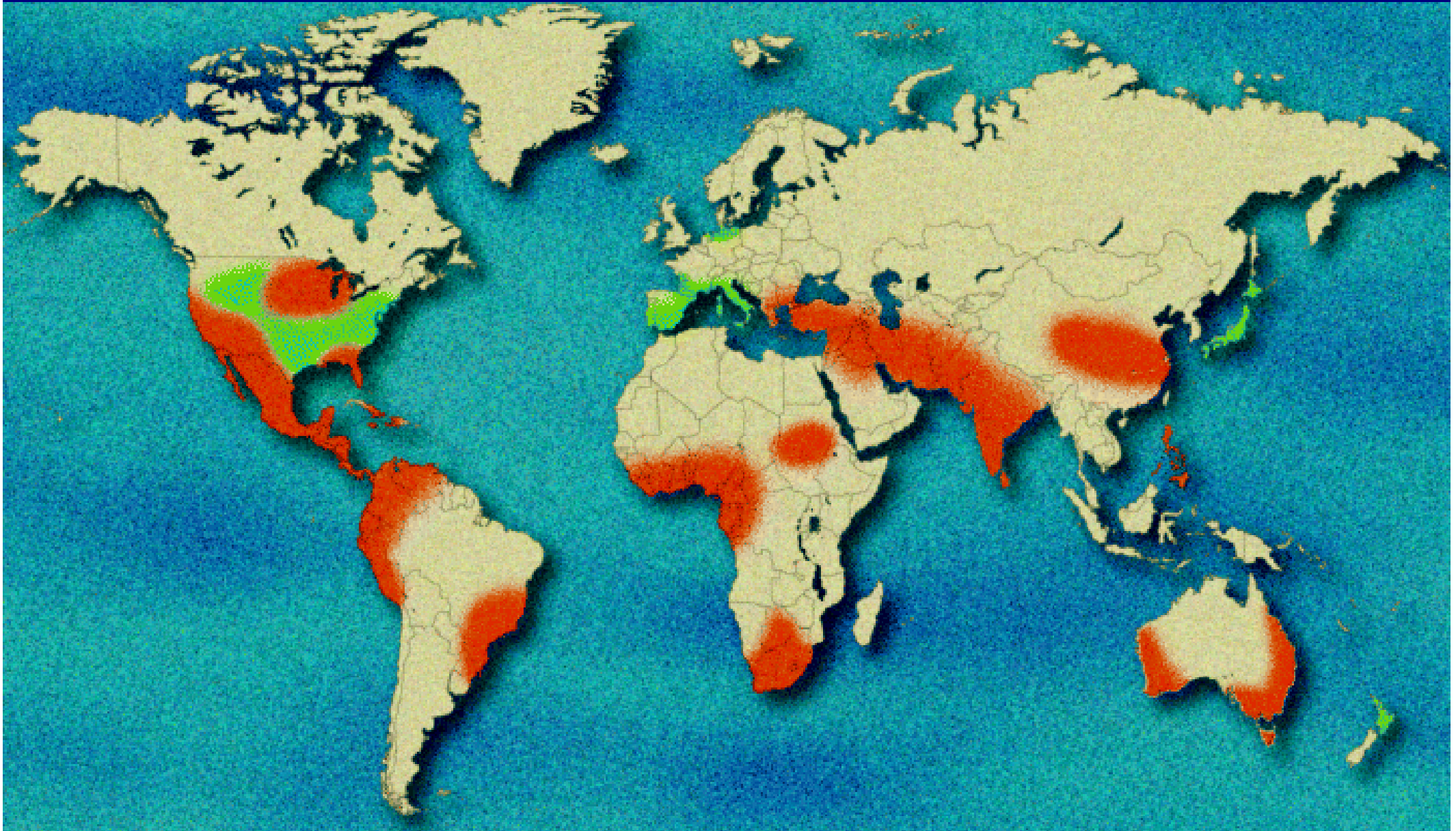


Metal uptake in the Wild West

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

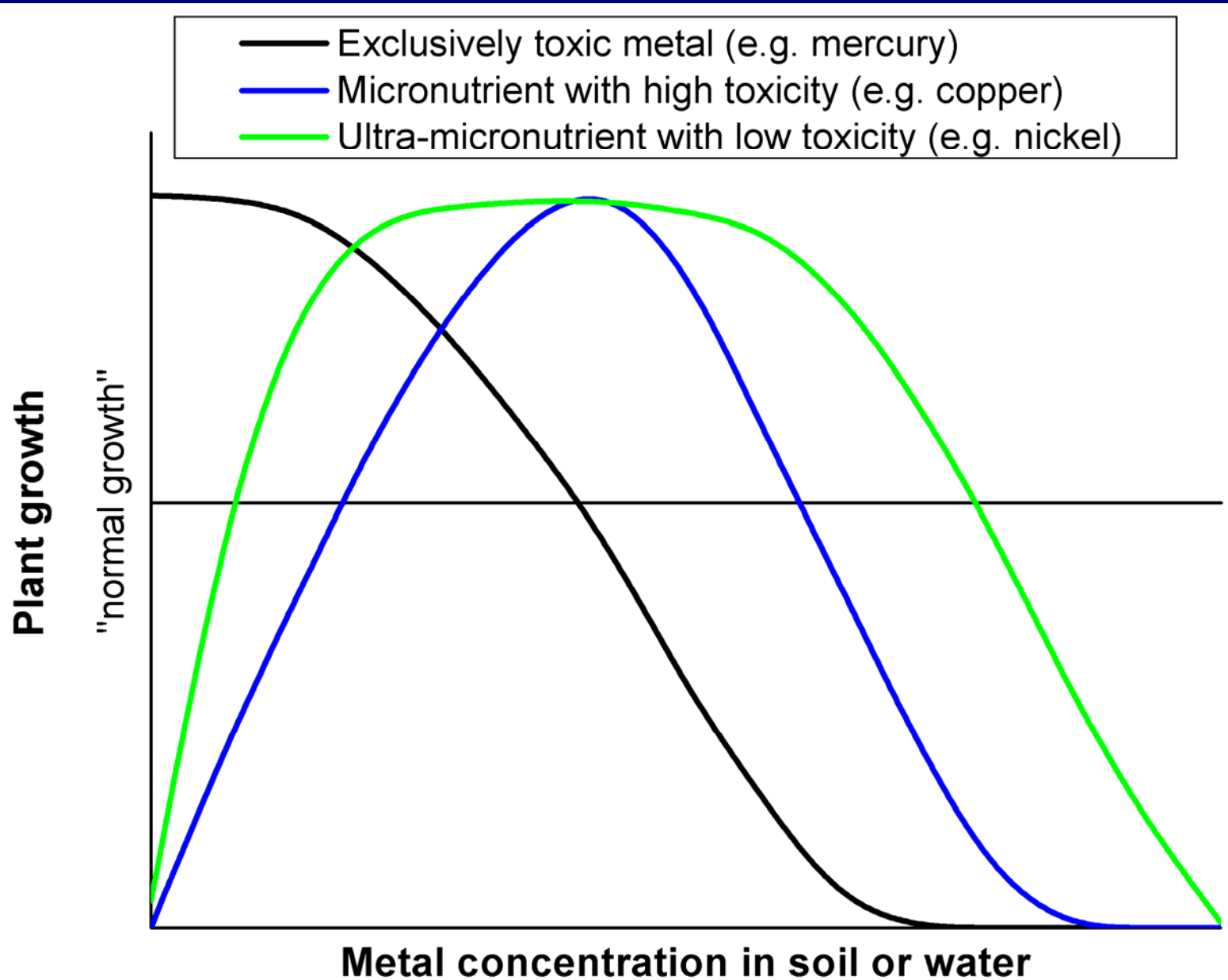
Heavy metal deficiency as a global problem of agriculture

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.

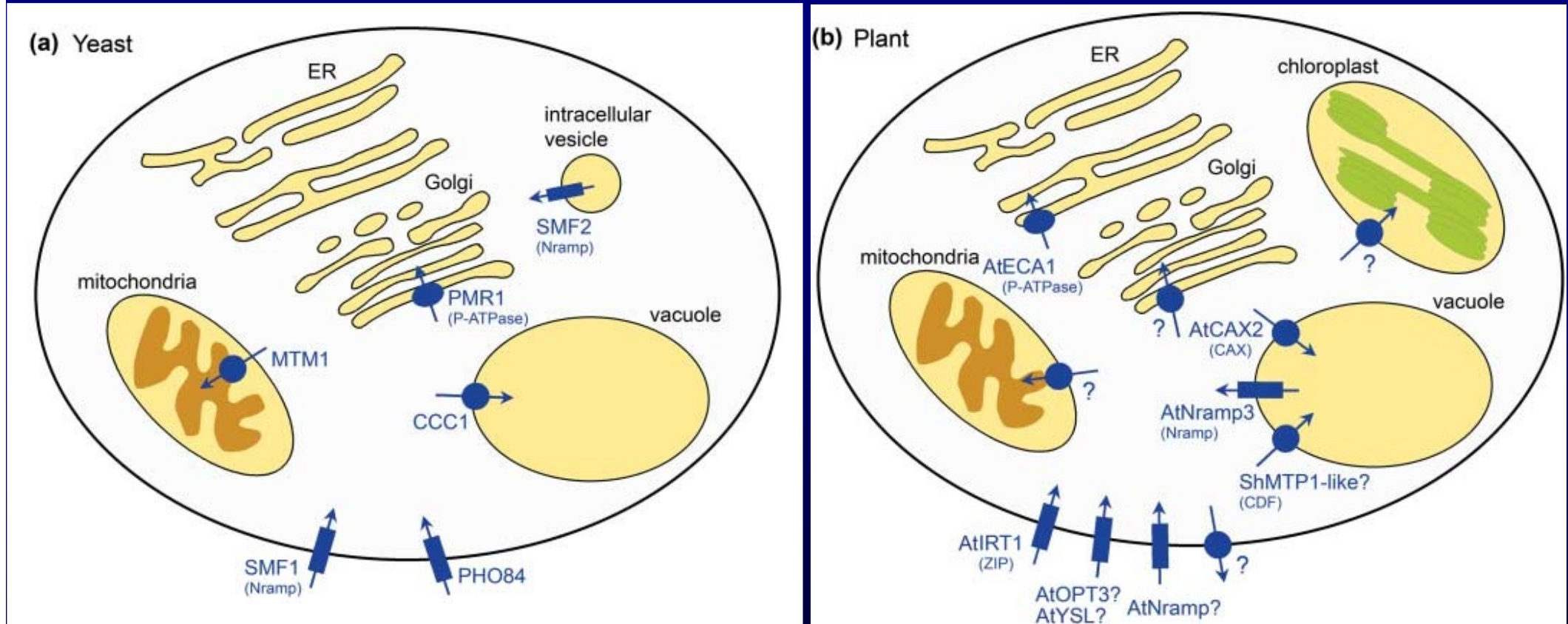
Dose-response principle for heavy metals



Mechanisms of metal uptake in Eucaryotes:

Main families of metal transport proteins

example: manganese transport in yeast and plants

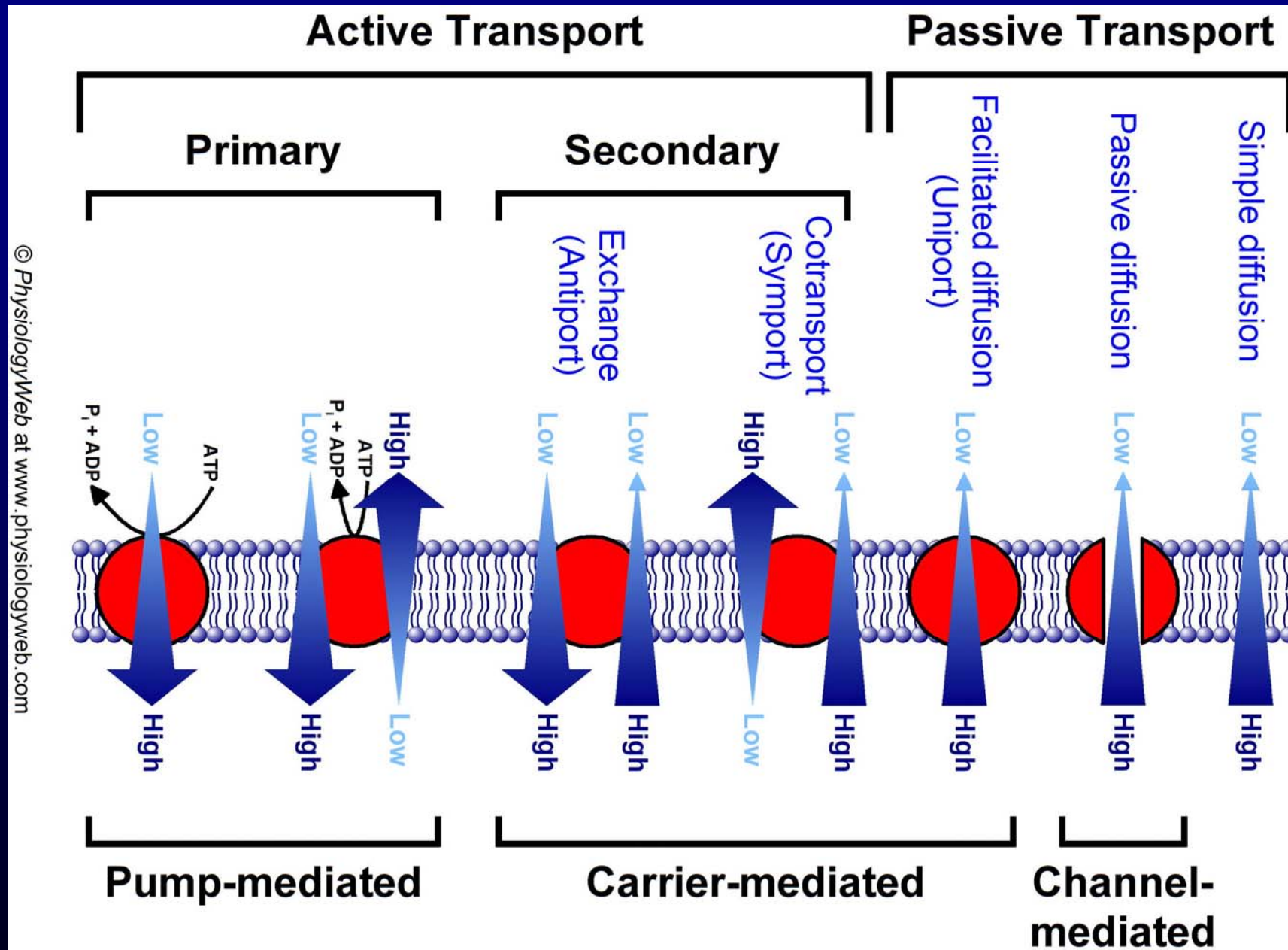


From: Pittman JK, 2005, NewPhytol167, 733-742

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 (**Nrap**-transporters)

Energetics and variants of metal transport



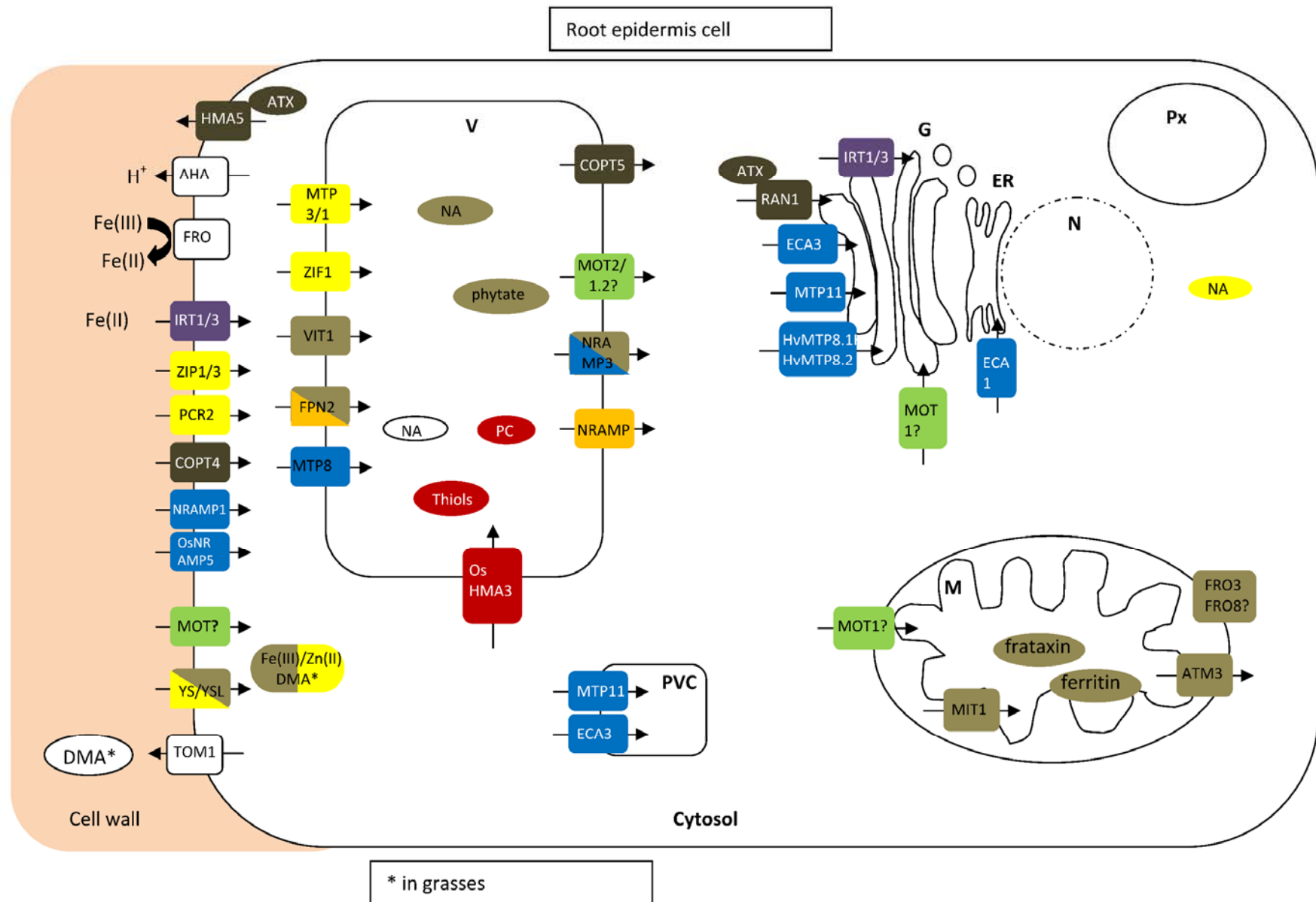
$$\Delta G = n_{\text{Ionen}} * R * T * \ln (c_{\text{inside}} / c_{\text{outside}}) + 3F (\varphi_{\text{outside}} - \varphi_{\text{inside}})$$

(R = gas constant, T = temperature, F = Faraday constant, φ = electrochemical potential)

Mechanisms of metal uptake in plants:

Different transport steps require different transporters

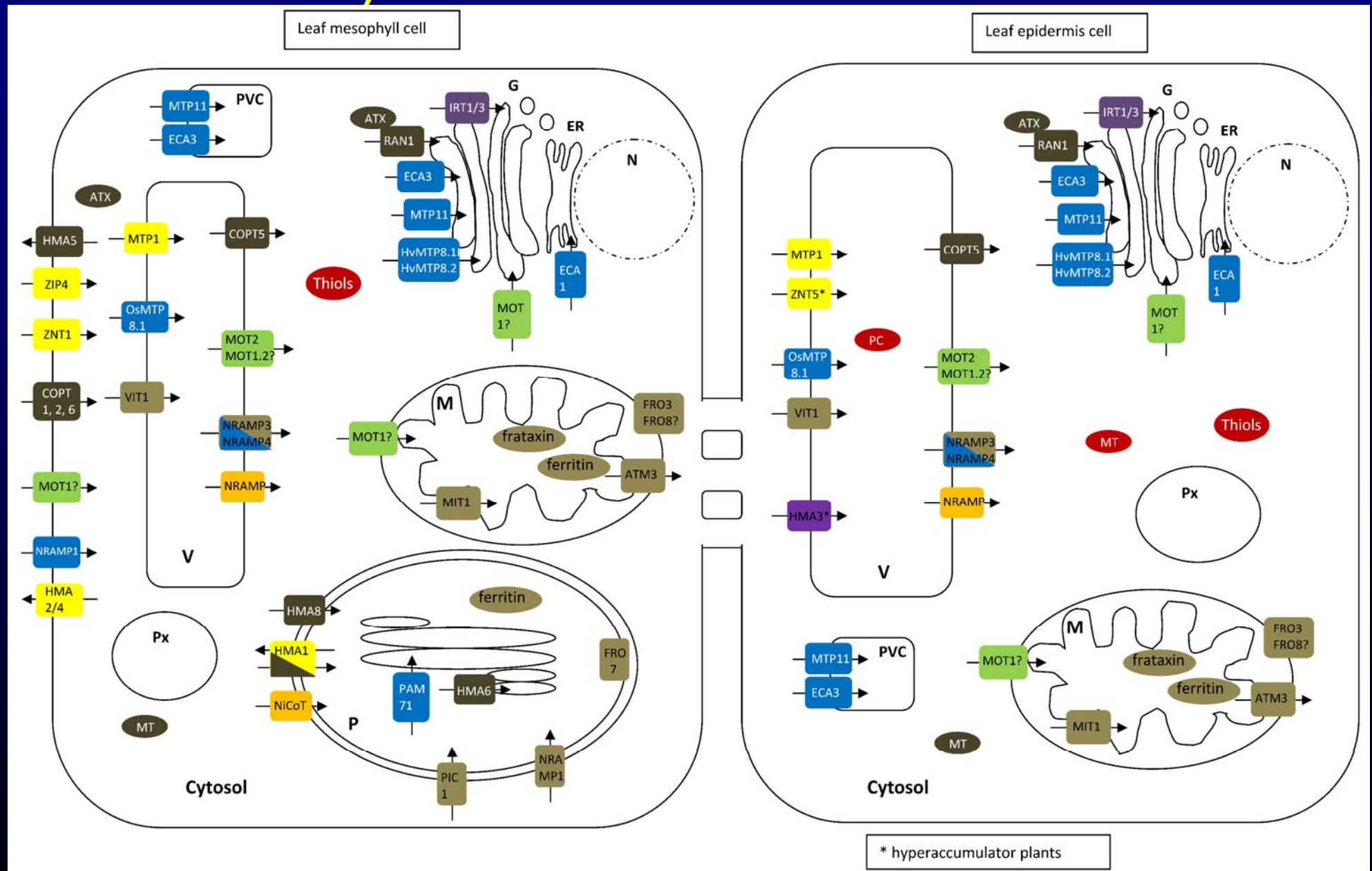
1) Root uptake and intracellular distribution



Mechanisms of metal uptake in plants:

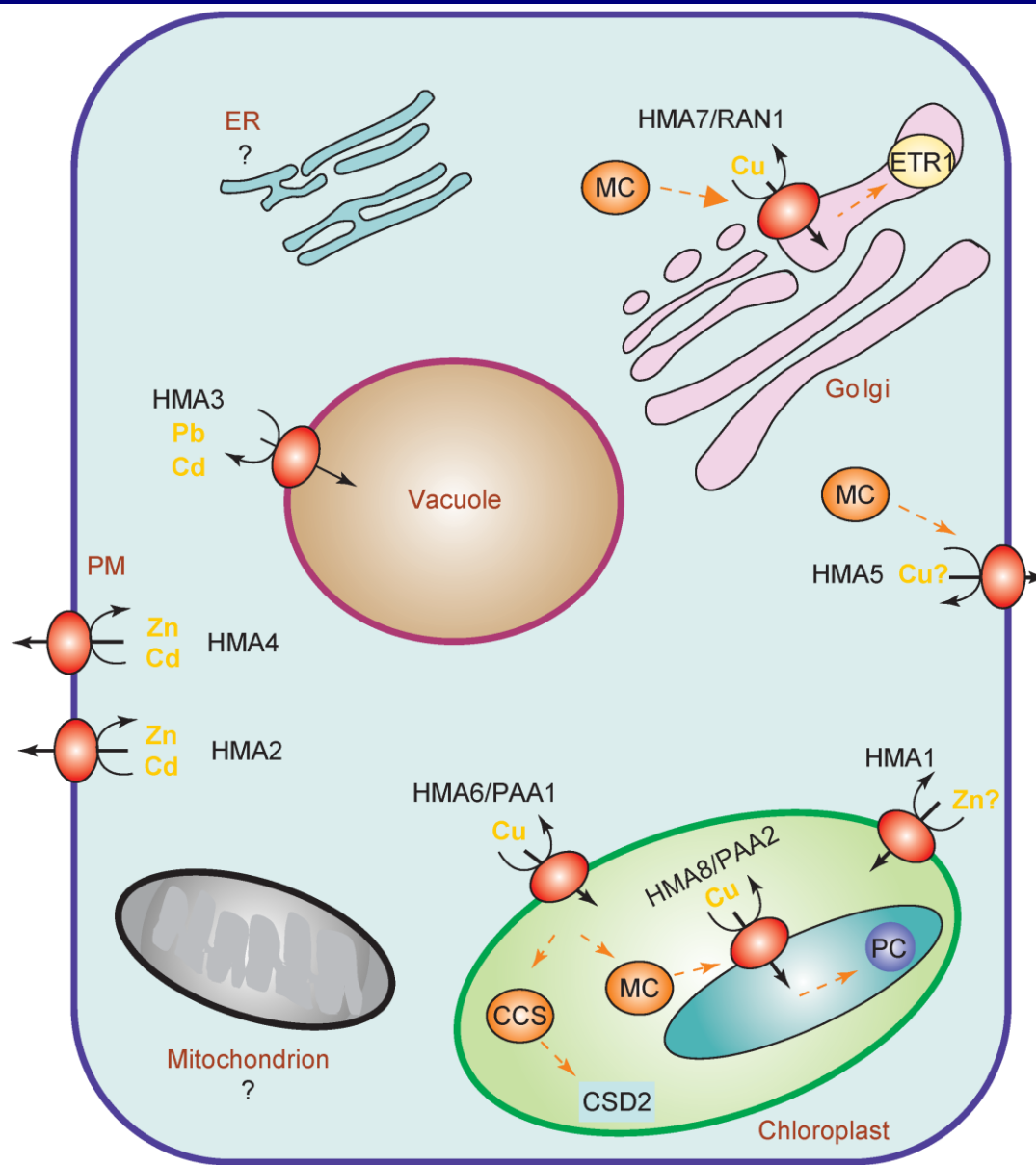
Different transport steps require different transporters

2) Distribution in shoot cells



Mechanisms of metal uptake+compartmentation in plants (I)

CPx-type (=P_{1B}-type) ATPases

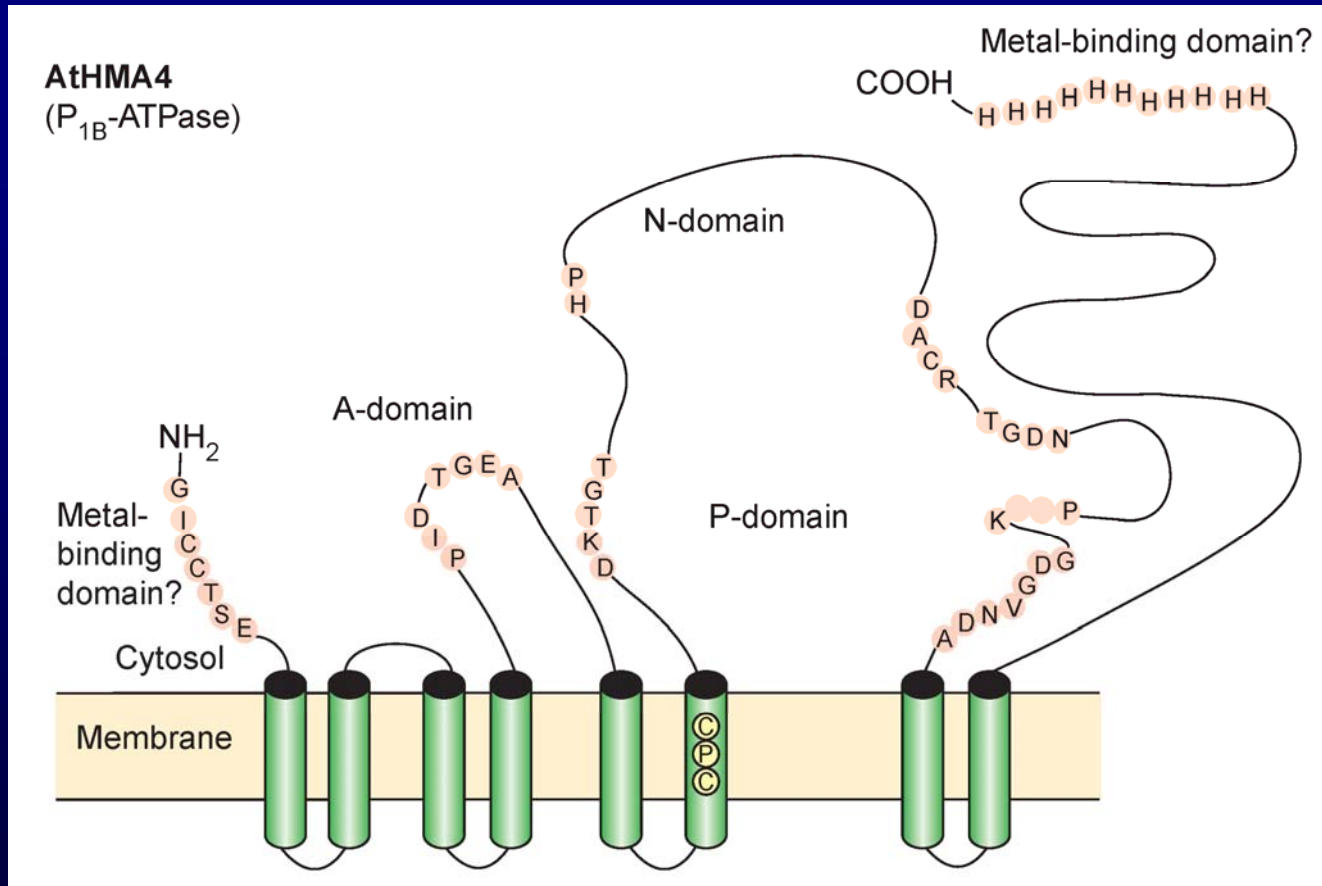


Functions (concluded mostly from differential expression studies)

- 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_{1B}-type) ATPases



From: Williams LE, Mills RF,
2005,
TrendsPlantSci 10,
491-502

Sequence characteristics

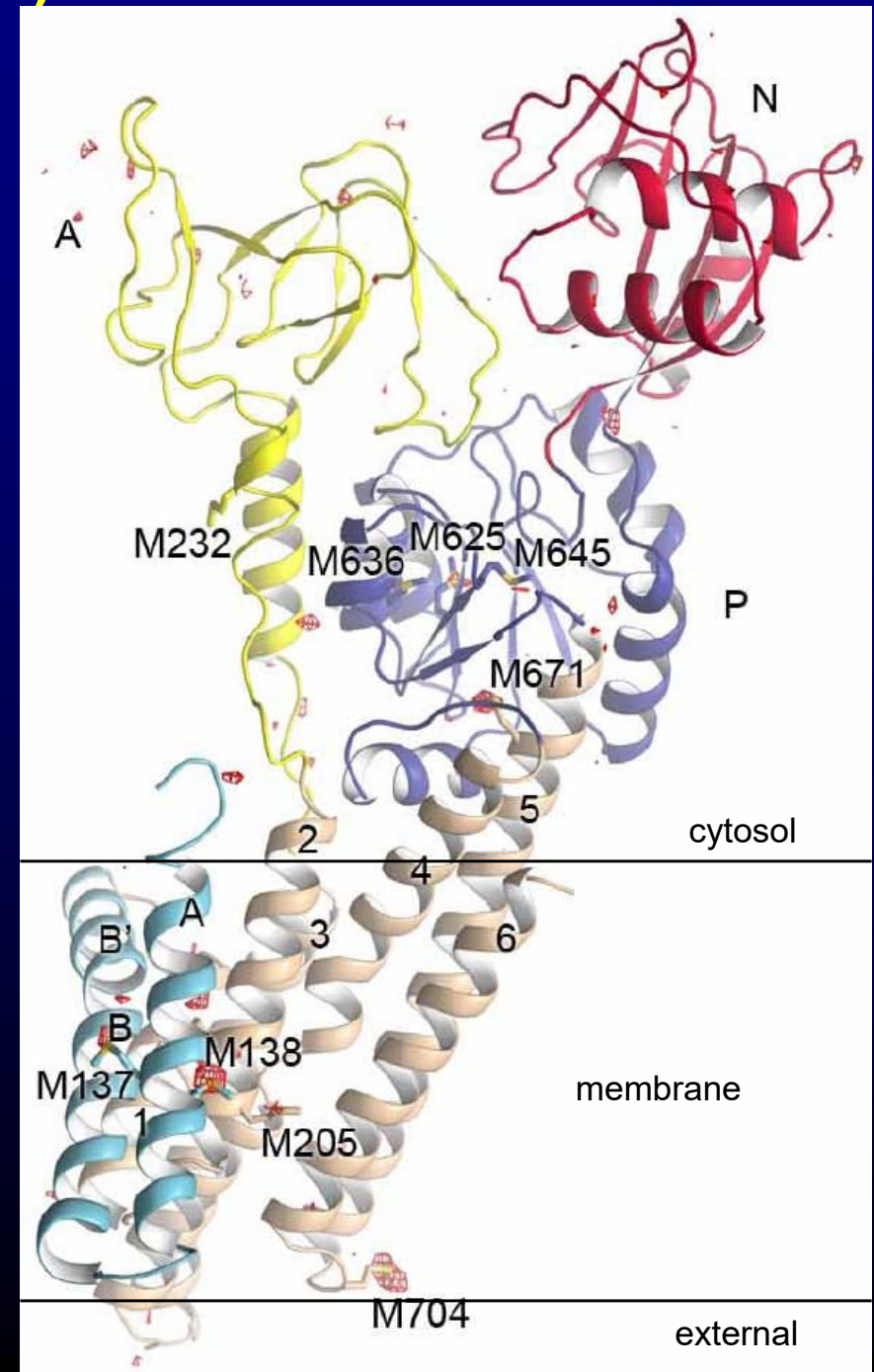
- CPx-motif in 6th transmembrane helix (→ Name!)
- Variable number of transmembrane helices
- MANY histidines and cysteines in sequence (→ e.g. 58 Cys in TcHMA4)
- Metal binding domain at N-terminus (in cytosol)
- Histidine repeat at C-terminus (in cytosol)

Mechanisms of metal uptake+compartmentation in plants (I)

CPx-type (=P_{1B}-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 Zn²⁺, not a larger complex
- high affinity Zn-binding site contains two cysteines



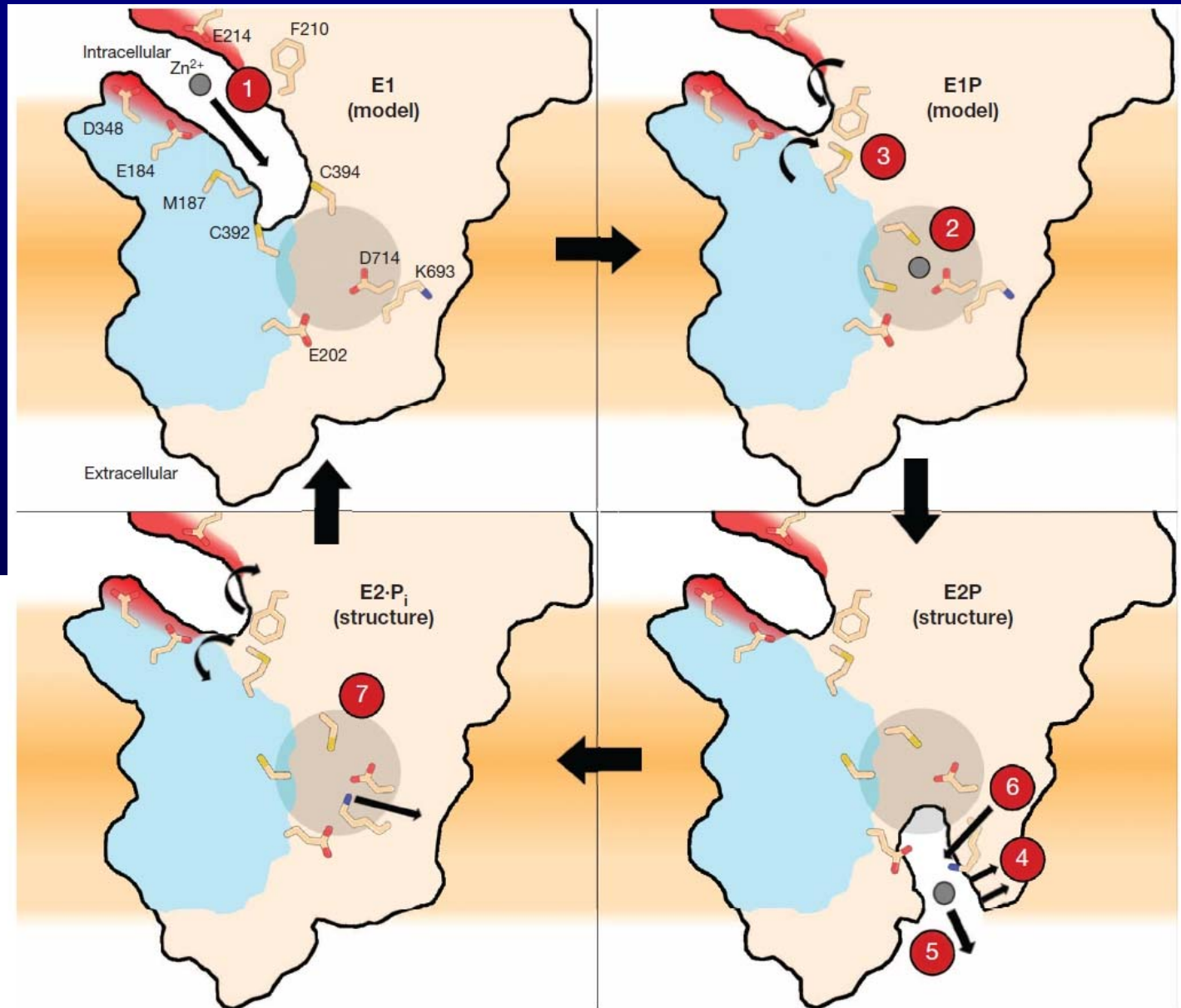
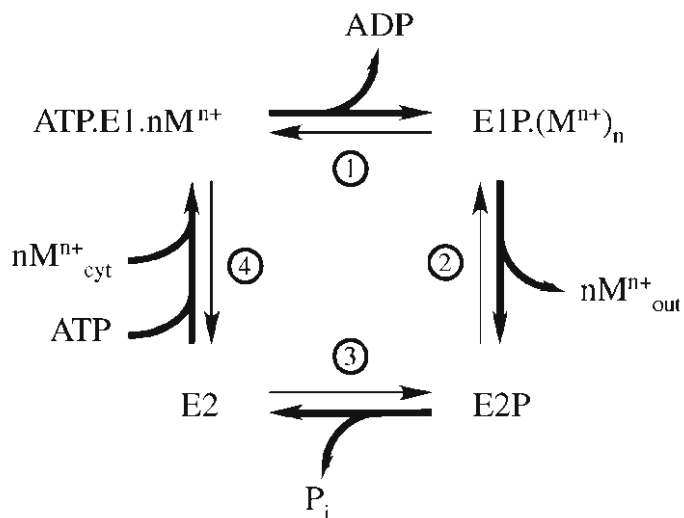
From: Wang K et al, 2014, Structure and mechanism of Zn-transporting P-type ATPases. Nature 514, 518-

Mechanisms of metal uptake+compartmentation in plants (I)

CPx-type (=P_{1B}-type) ATPases

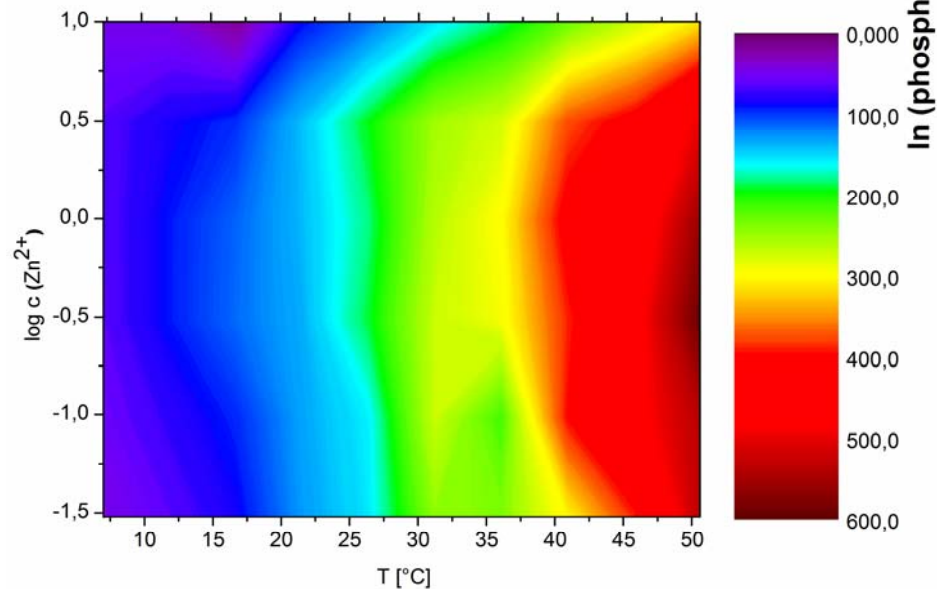
Mechanism

- 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 Zn²⁺
- pore closes after ATP hydrolysis

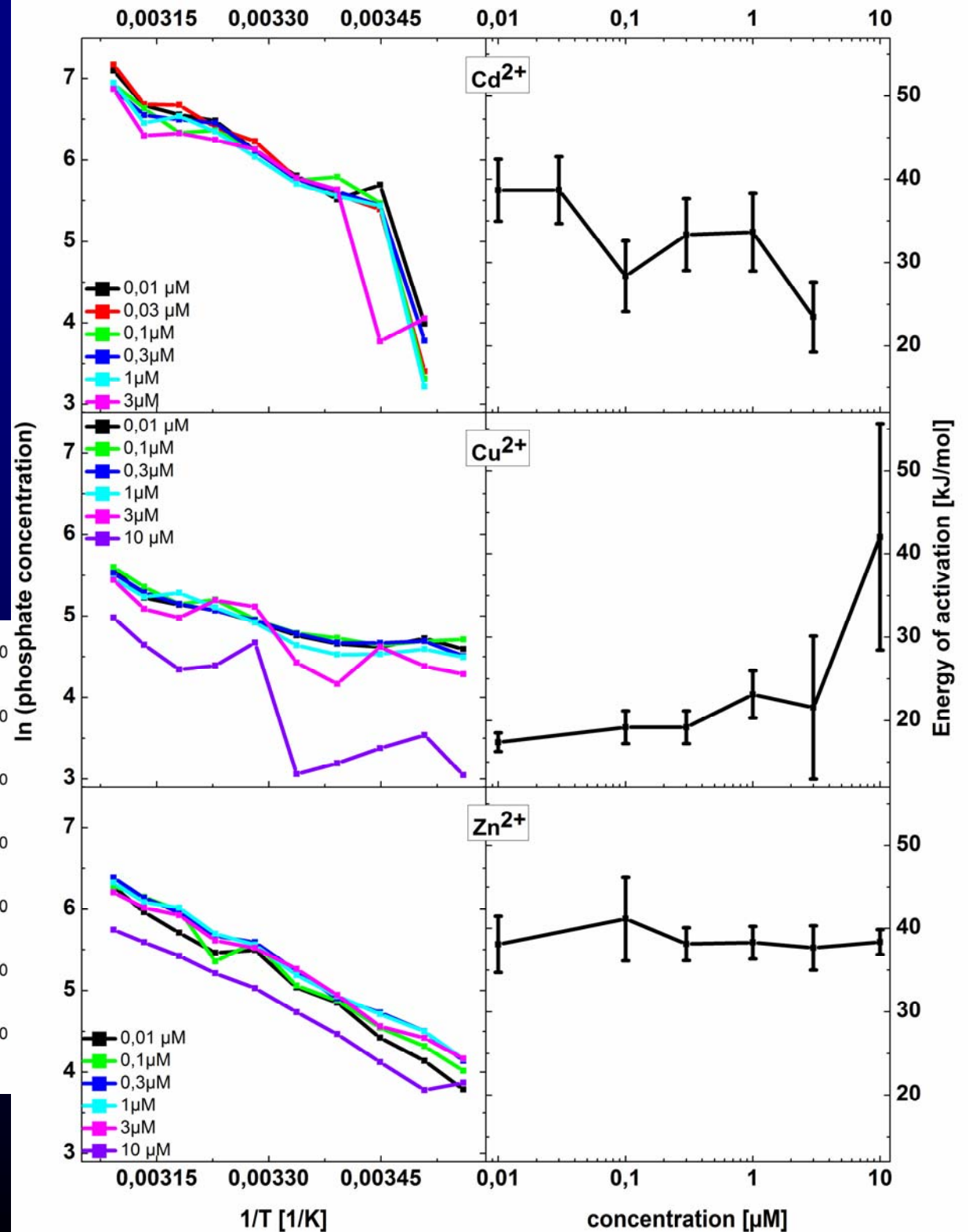


Activation and substrate inhibition of TcHMA4

- Activation energies for TcHMA4 (CPx = P_{1B} ATPase) are similar to other metal ATPases.
- Activation energy changes with the concentration and type of the metal to be pumped.

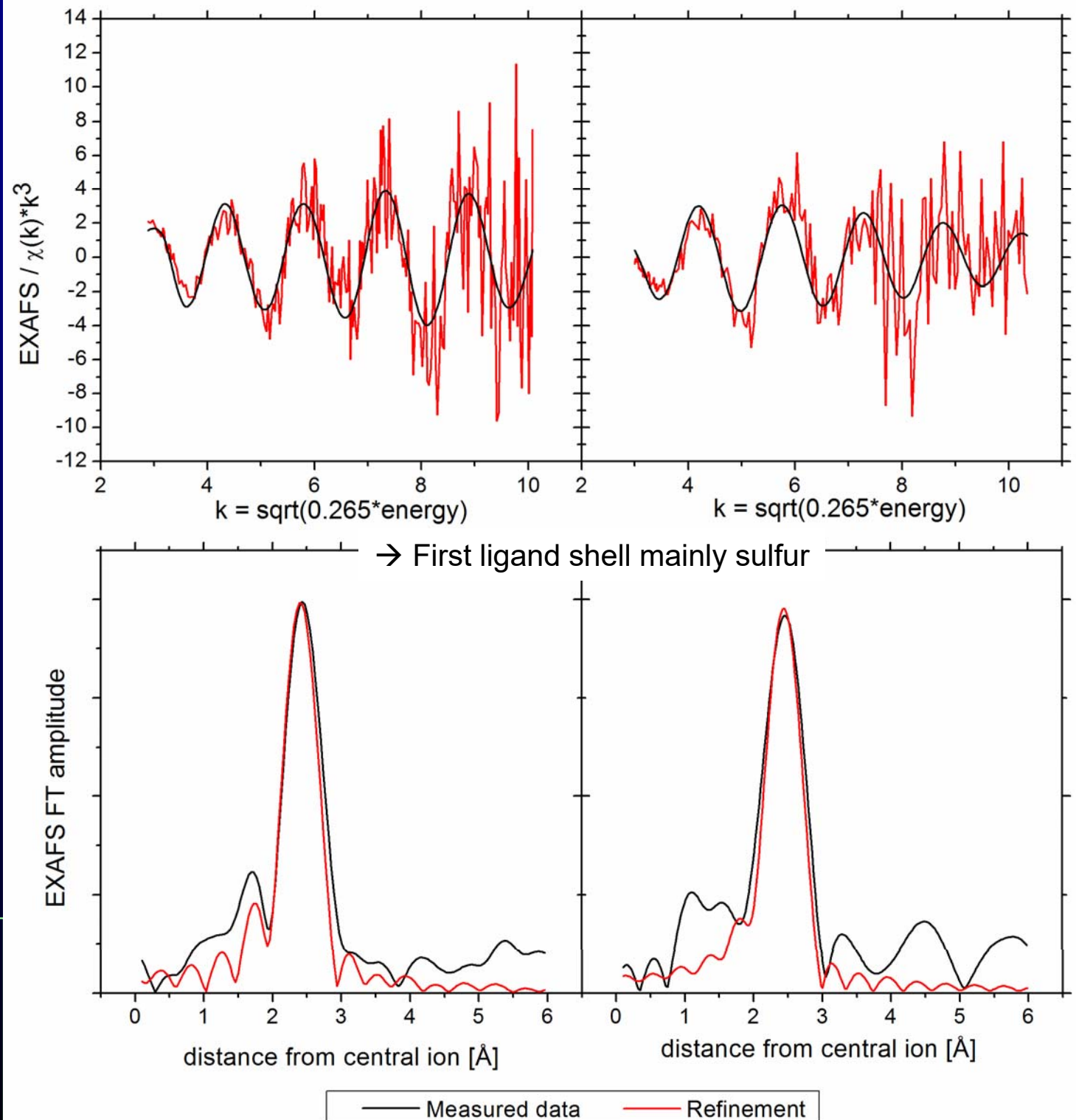


Leitenmaier B, Witt A, Witzke A, Stemke A, Meyer-Klaucke W, Kroneck PMH, Küpper H (2011)
 Biochimica et Biophysica Acta (Biomembranes) 1808, 2591-2599



EXAFS-analysis of TcHMA4

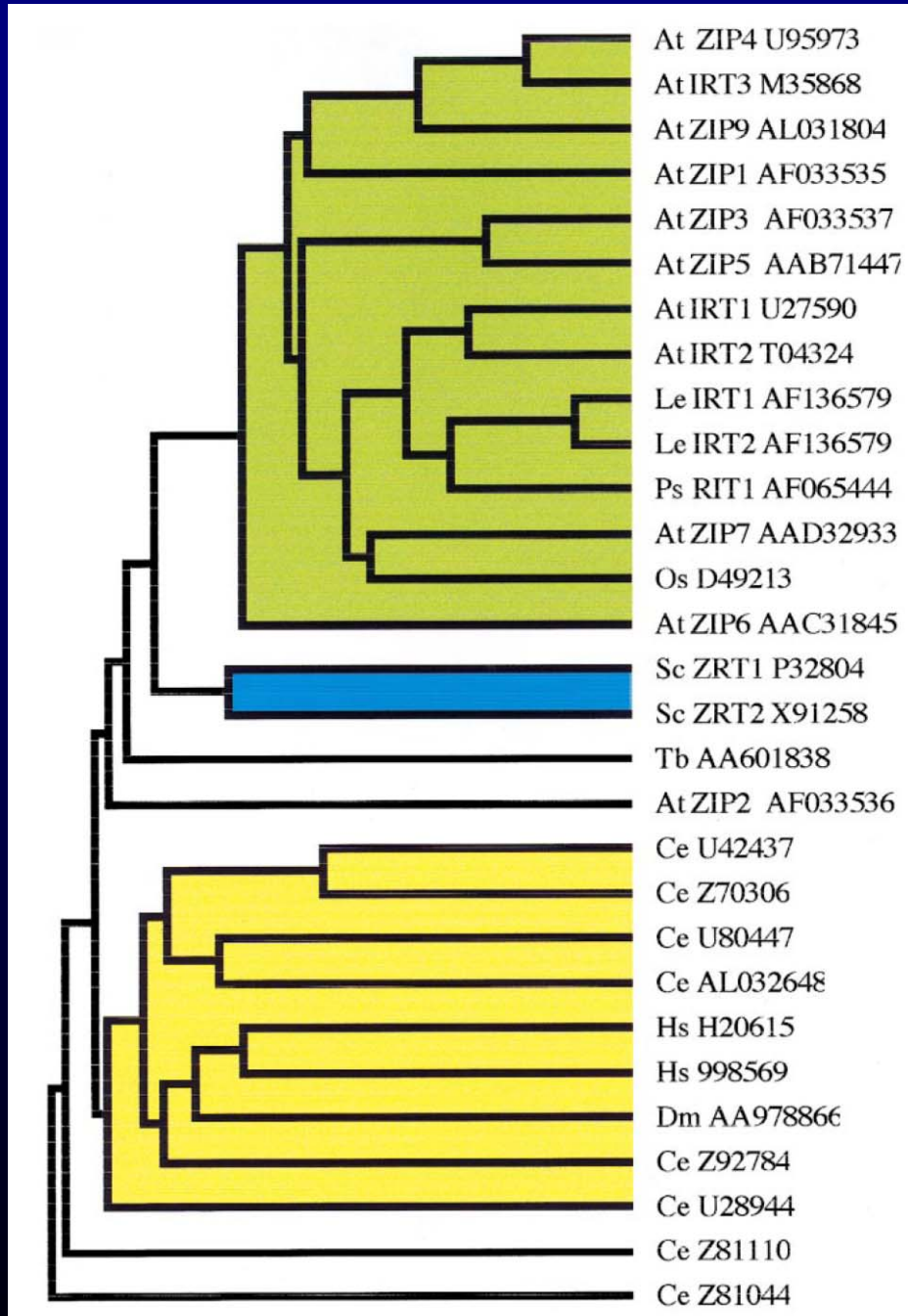
- at low Cd concentrations, the first ligand shell in this ATPase consists mainly of S (thiol groups from some of the 58 cysteines in the sequence)



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



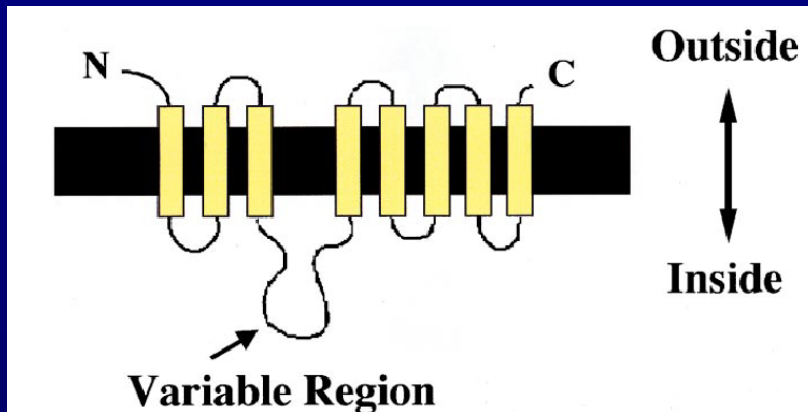
Likely Functions (deduced from expression studies)

- uptake of metals into cells over the cytoplasmatic 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

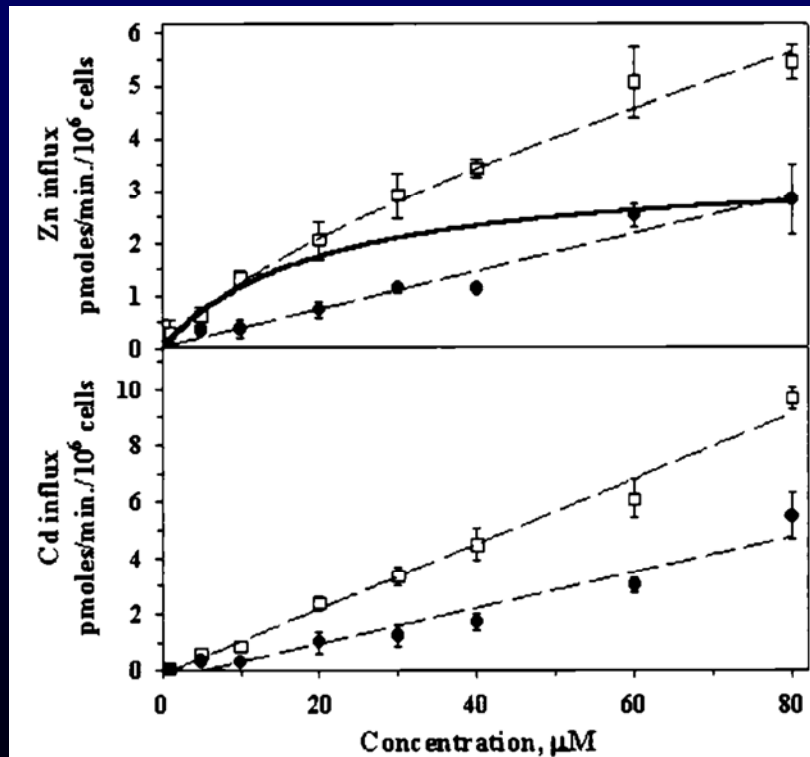


From:Guerinot ML, 2000, BBA 1465, 190-8

- Functions suggested by expression studies)**
- uptake of metals into cells over the cytoplasmatic 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



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

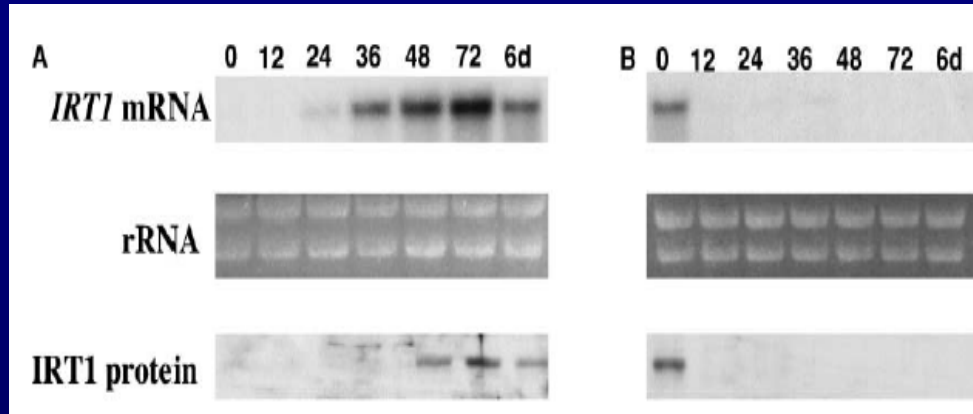
Characteristics revealed by yeast expression studies

- 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)

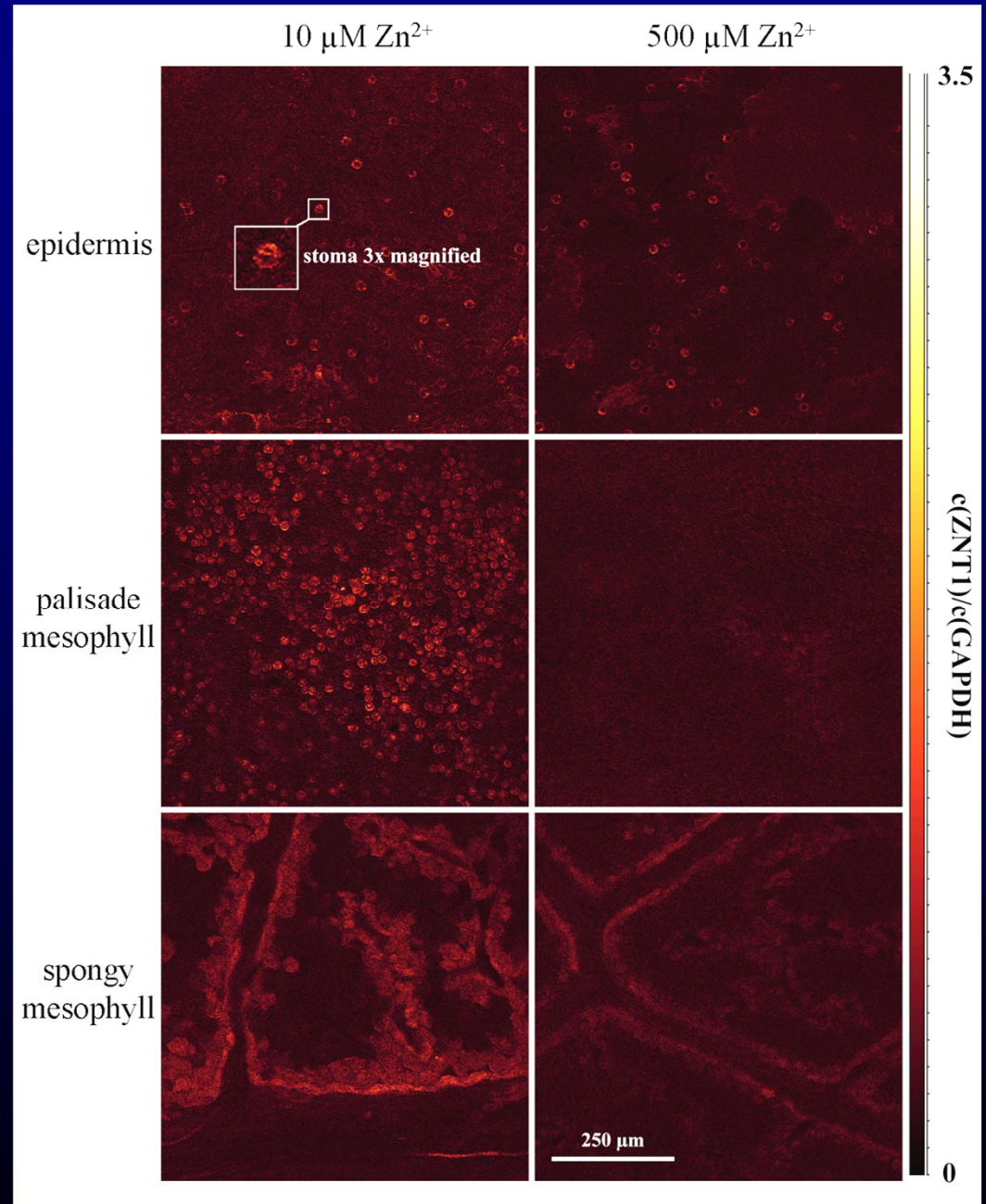


From: Connolly et al., 2002, PlantCell 14, 1347-57

Expression pattern

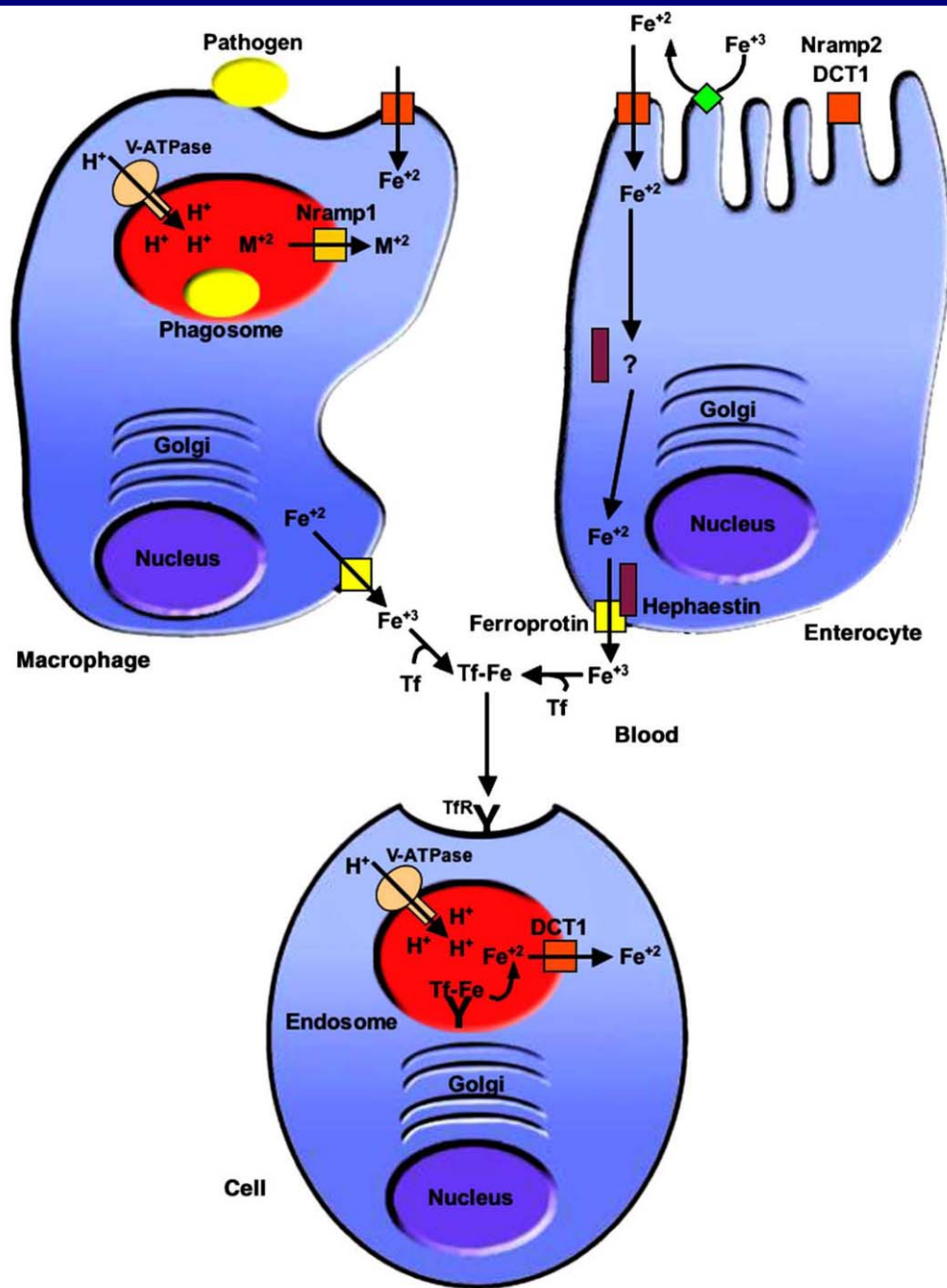
- expressed in most plants mainly under metal deficient conditions
- in metal hyperaccumulators rather strong expression at all metal levels
- expression mainly in metabolically active cells, not metal storage cells

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)

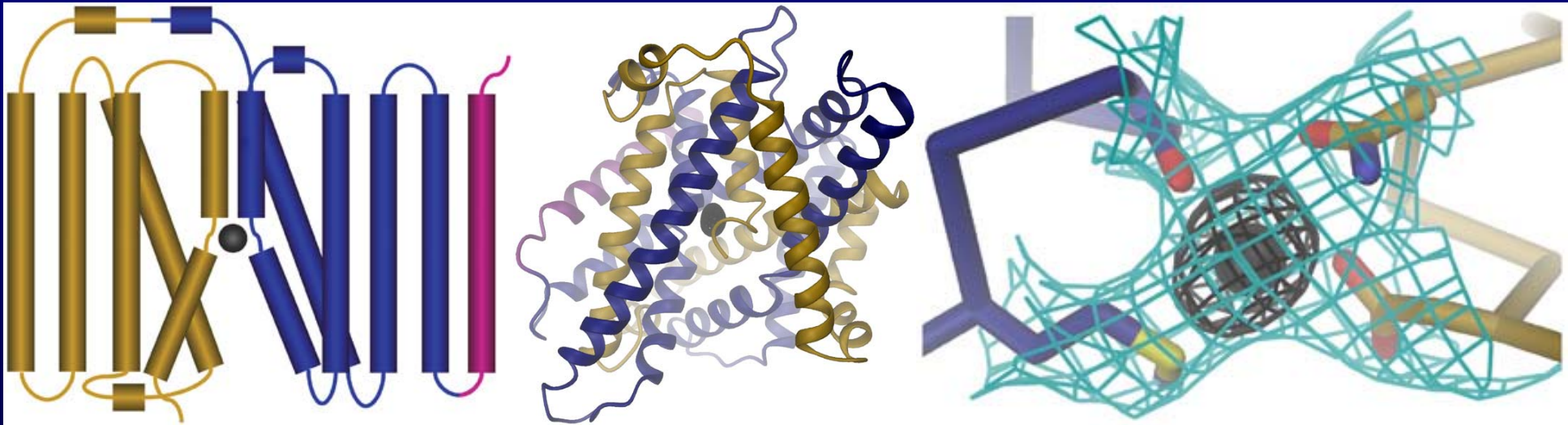


Likely Functions (deduced from expression studies)

- 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)



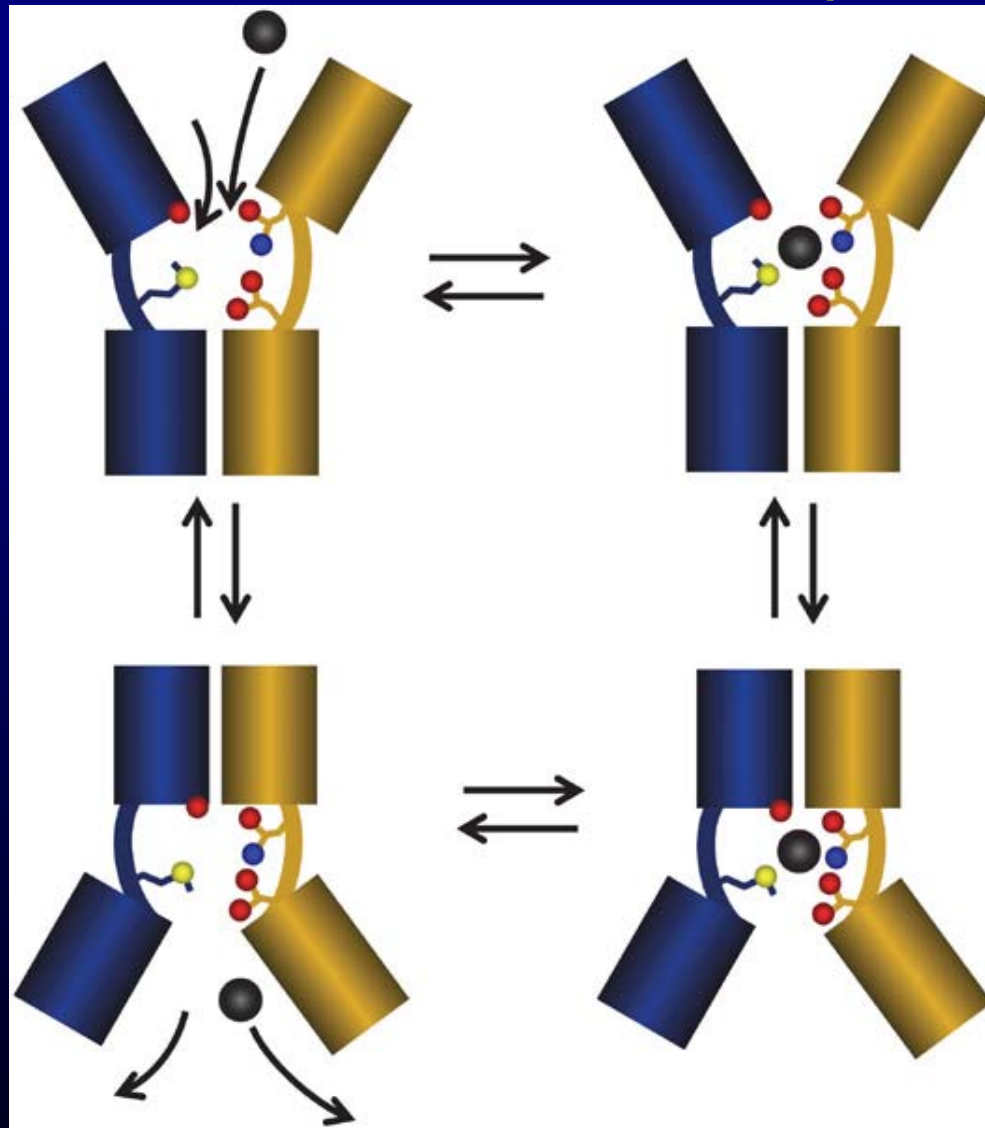
From: Ehrnstorfer IA, Geertsma ER, Pardon E, Steyaert J, Dutzler R. Nat Struct Mol Biol. 21, 990-6

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)

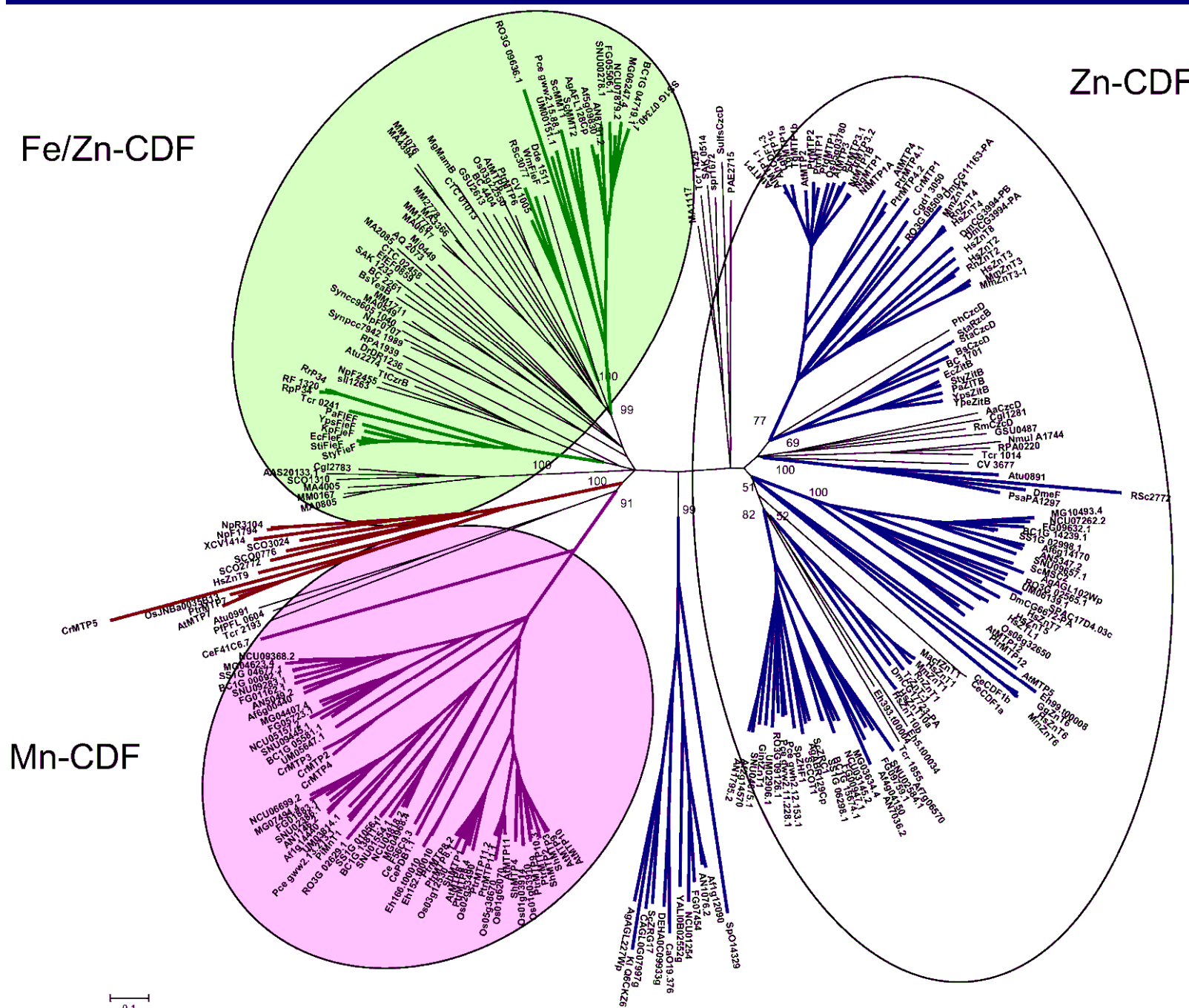
Natural resistance associated macrophage proteins (NRAMPs)



Mechanism deduced from crystal structure and enzyme kinetic studies

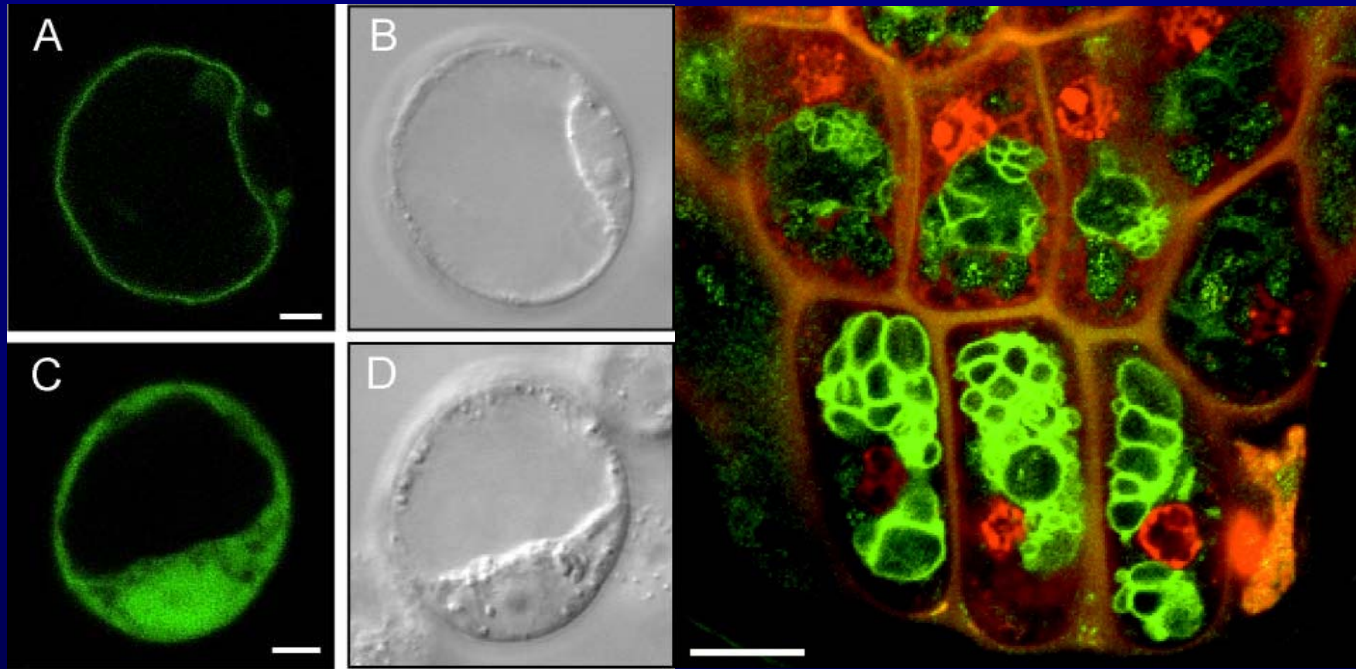
- proton symport with the electrochemical gradient drives metal translocation against the gradient
- binding of metal and proton induces a conformational change of the two halves of the helices 1 and 6 around a hinge in the metal binding site
- the conformational change closes the pore on the outer side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



- abundant in all eucaryotes, incl. humans, plants and fungi
- different subfamilies

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



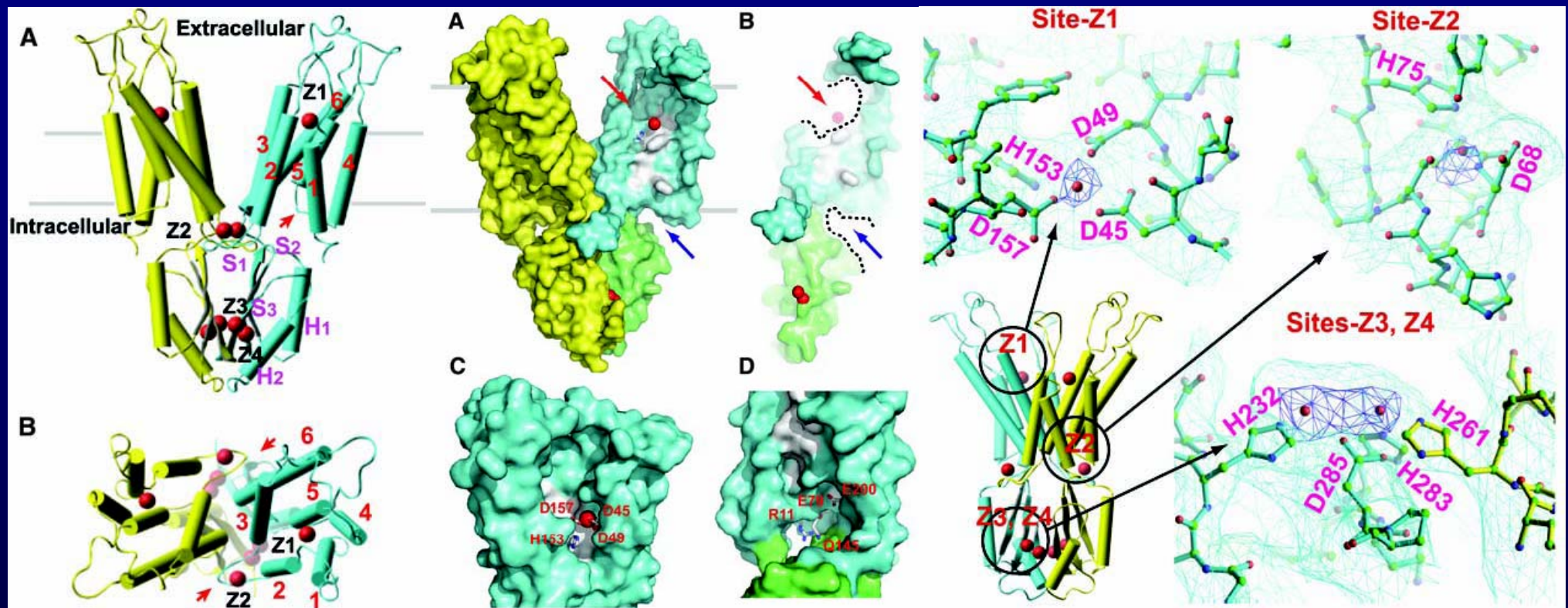
From: Kobae et al., 2004,
PlantCellPhysiol 45, 1749-58

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

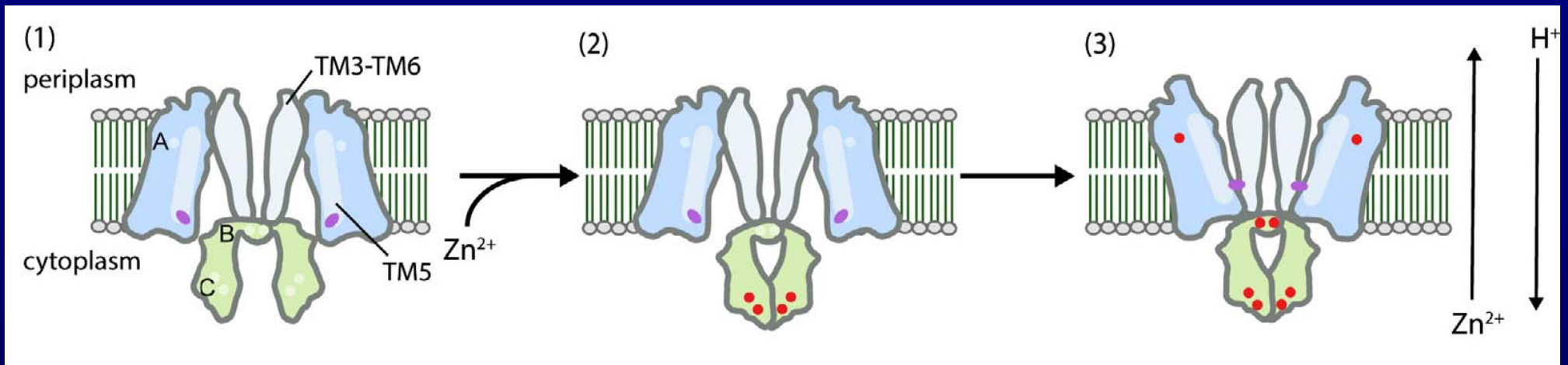


From: Lu M, Fu D. 2007. Structure of the zinc transporter YiiP. Science 317, 1746-8

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

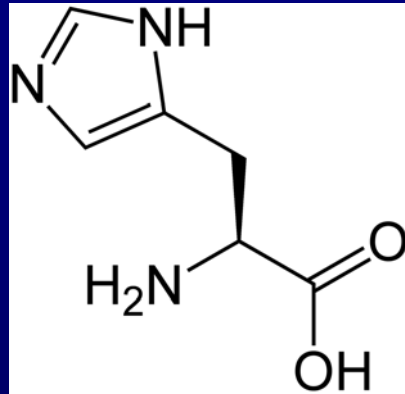


From: Kolaj-Robin O, Russell D, Hayes KA, Pembroke JT, Soulimane T. 2015. FEBS Lett. 589, 1283-95

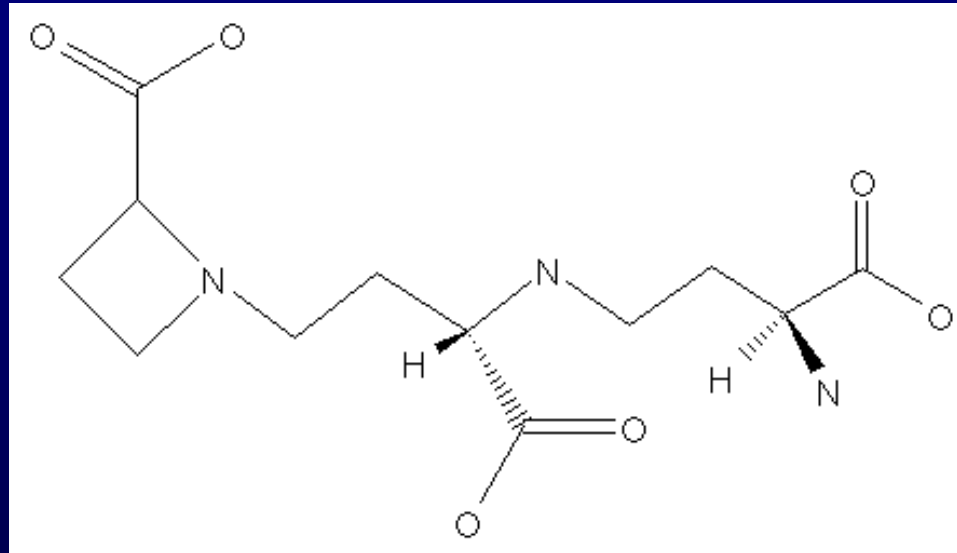
Mechanism concluded from structure and kinetic studies

- 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

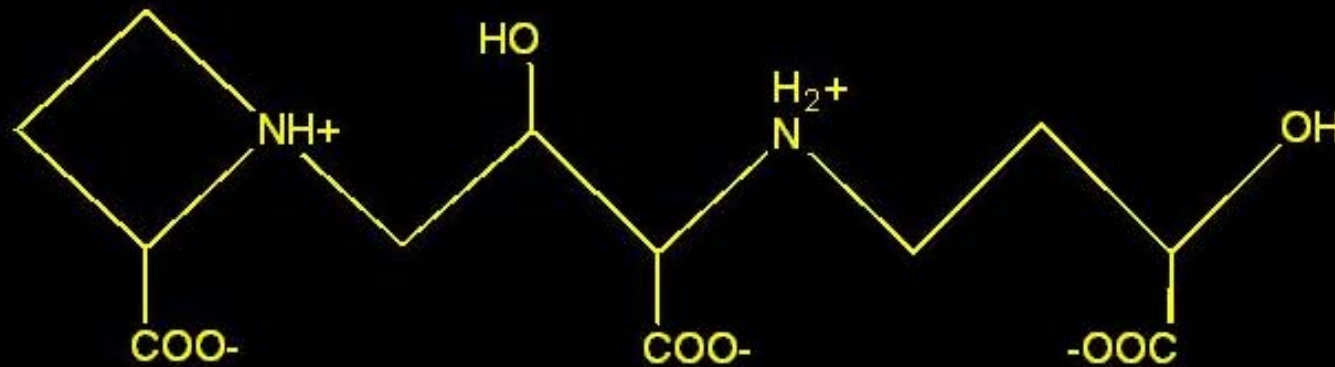
Mechanisms of metal uptake in plants (V): Long-distance transport ligands



Histidine



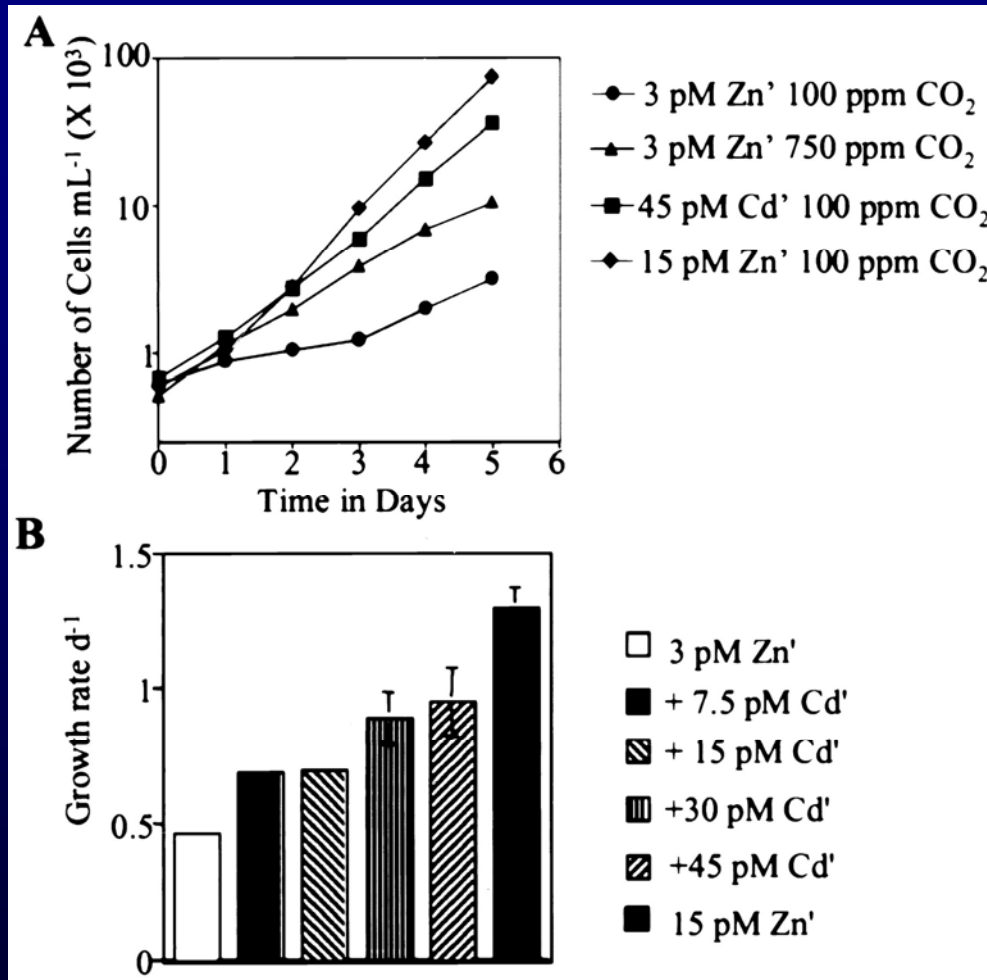
Nicotianamine



Mugineic acid

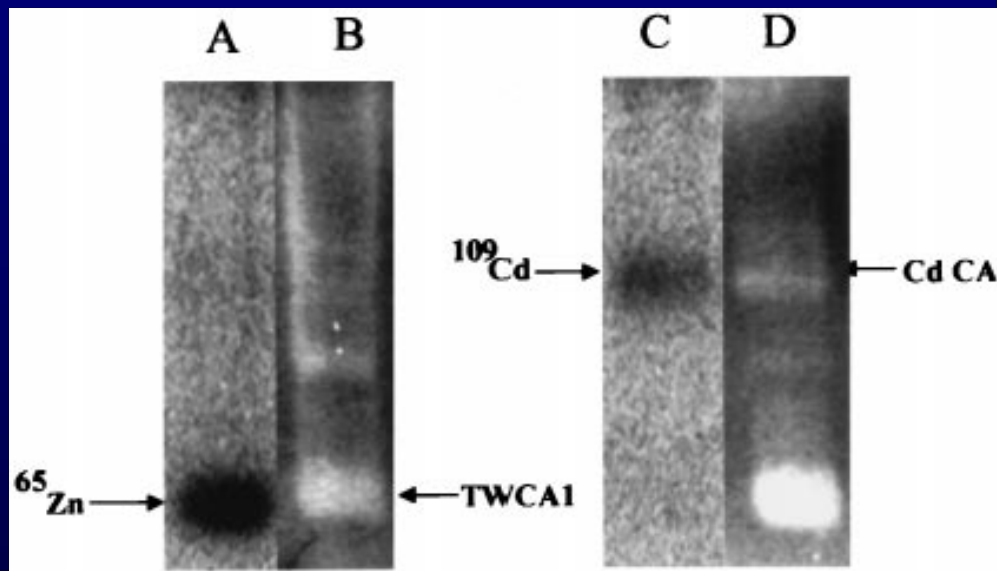
Now let's have a look at a few plant micronutrients

Cadmium as a micronutrient

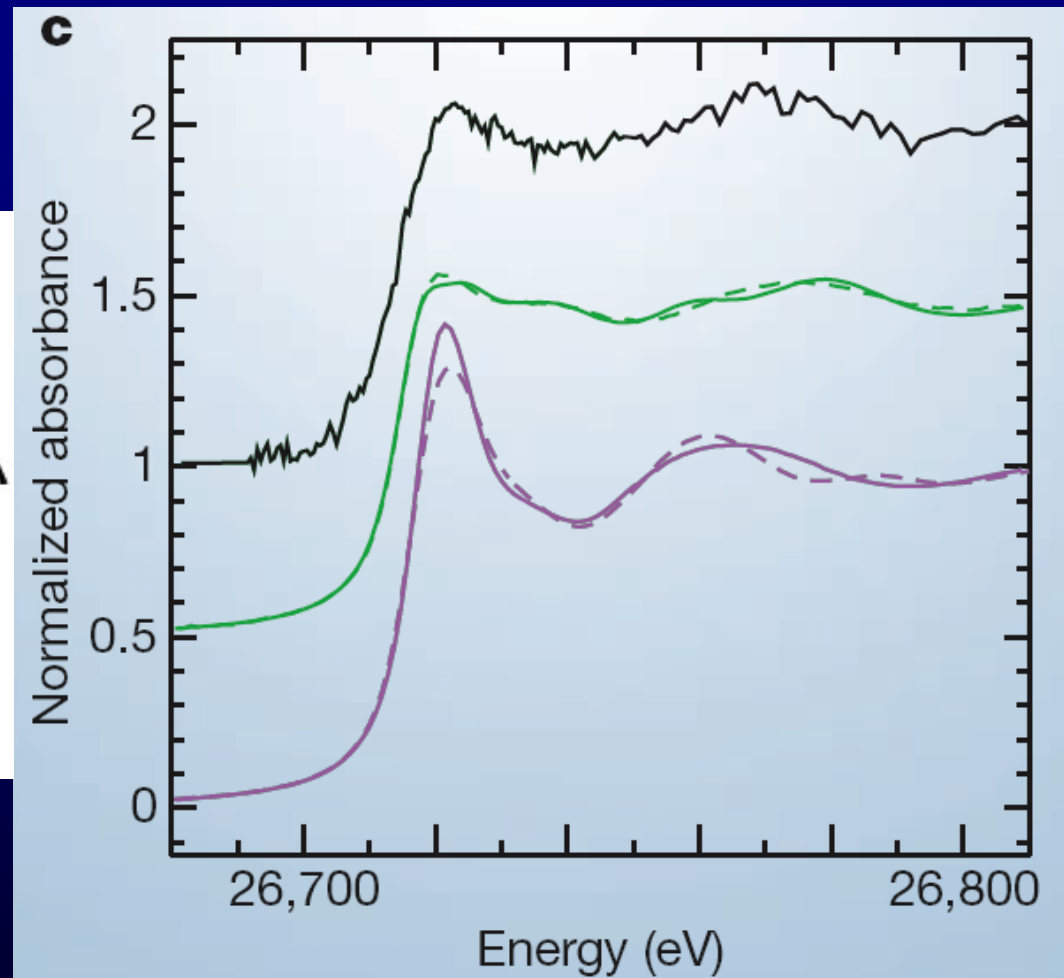


Cadmium as Plant-micronutrient in *Thalassiosira weissflogii*. A, B: growth of the algae. (Lane and Morel, 2000, PNAS97)

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



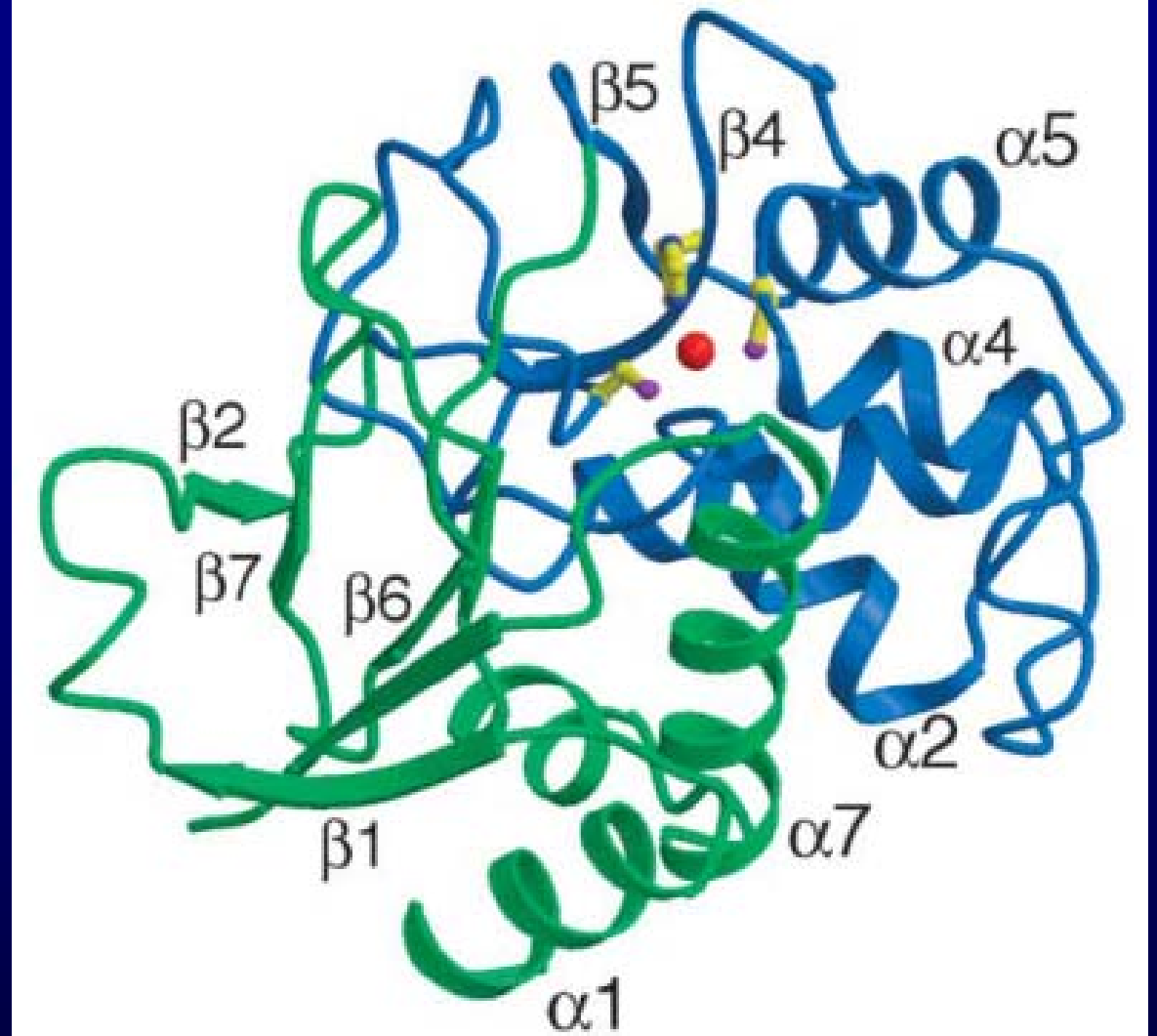
Size of the cadmium-carboanhydrase in comparison to the normal Zn-carboanhydrase (Lane and Morel, 2000, PNAS Vol. 97)



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 α -helices and 9 β -sheets, Cd at the lower end of a funnel-like binding pocket
- Cd²⁺ is bound via three conserved amino acid residues: 2x cysteine and 1x histidine, plus 1x Water ($\rightarrow$ tetrahedral coordination). Further fixed water molecules nearby

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

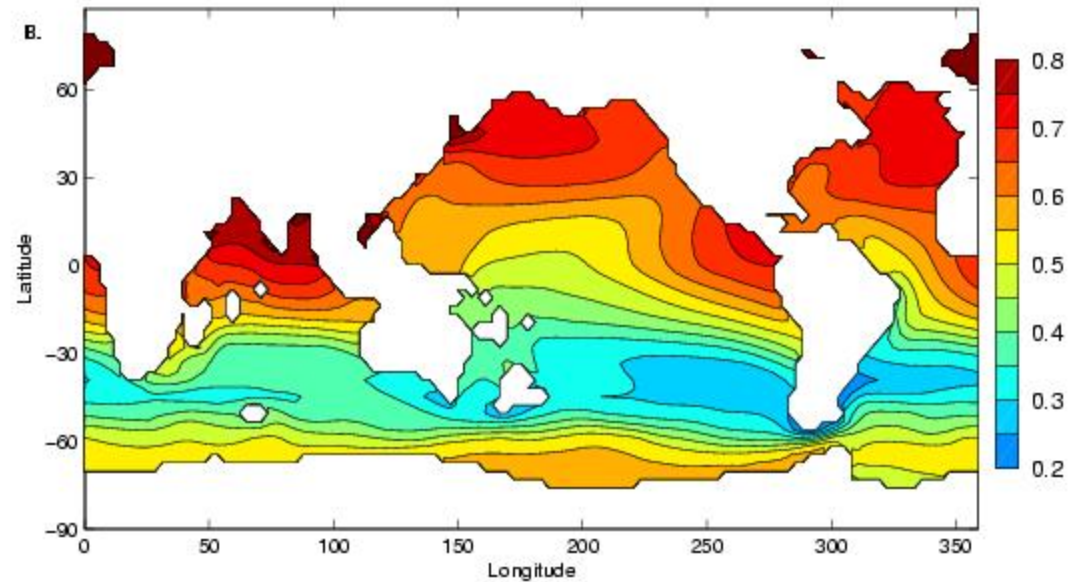
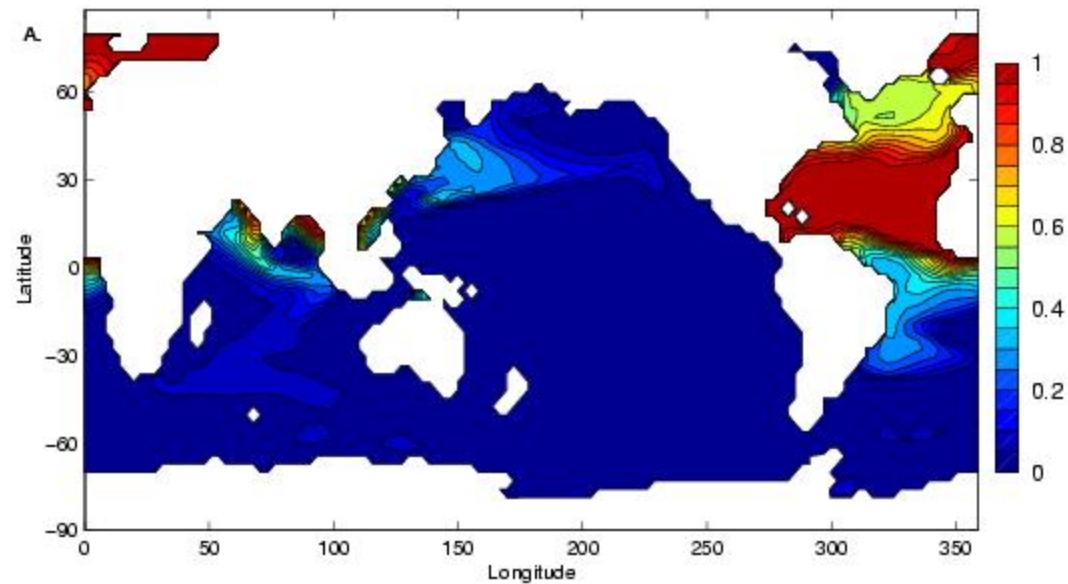


With 10 μM cadmium in the nutrient solution
--> healthy plants

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

Iron

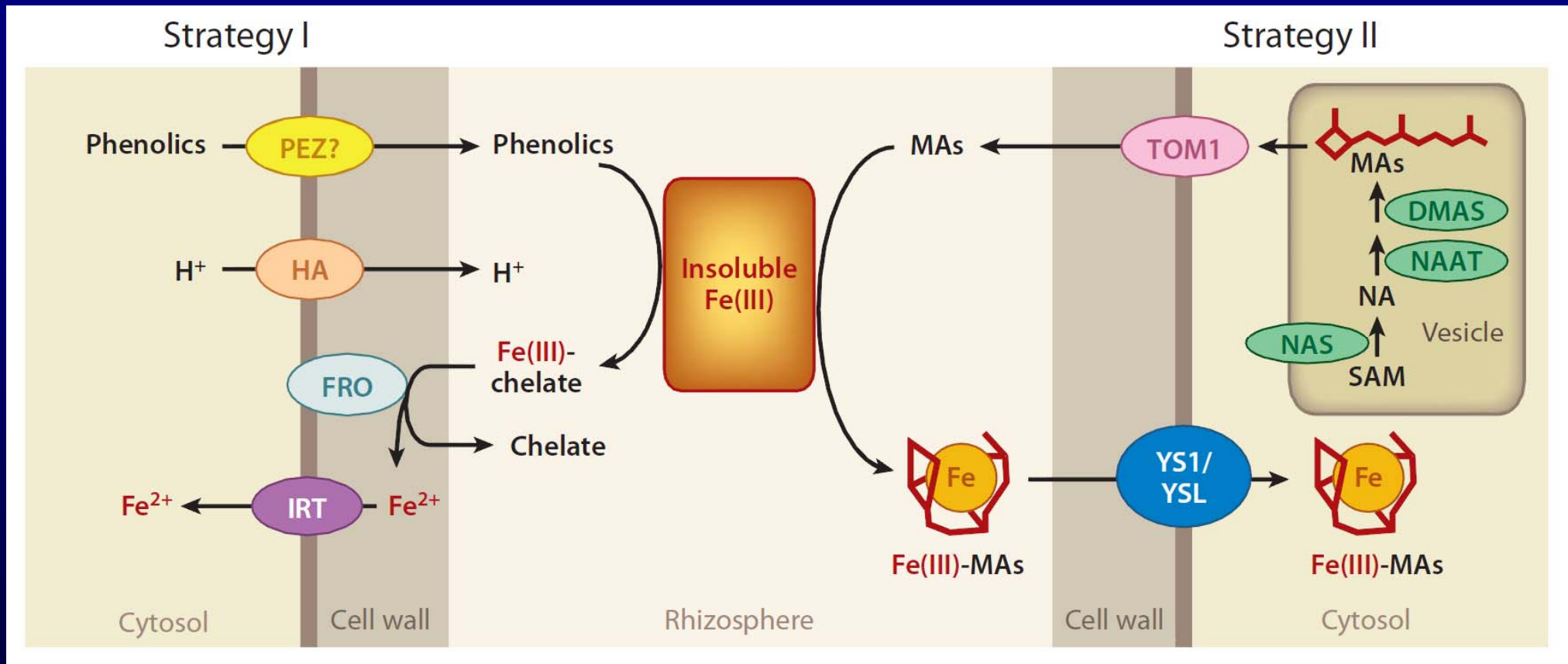
Iron concentrations at the surface (top picture) and in 1000m depth (bottom picture)



Source: www-paoc.mit.edu

Mechanisms of iron uptake in plants

Strategies of iron efficiency

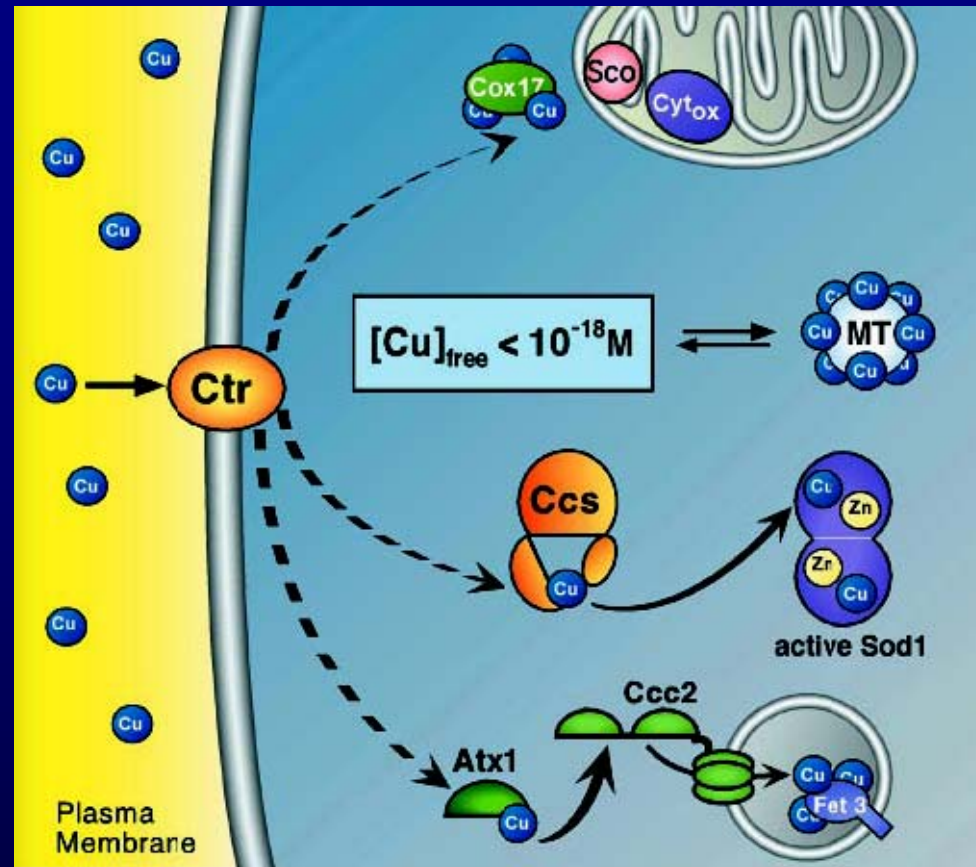


From: Kobayashi T, Nishizawa NK. 2012. Ann Rev Plant Biol 263, 131-152

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 mugineic acid

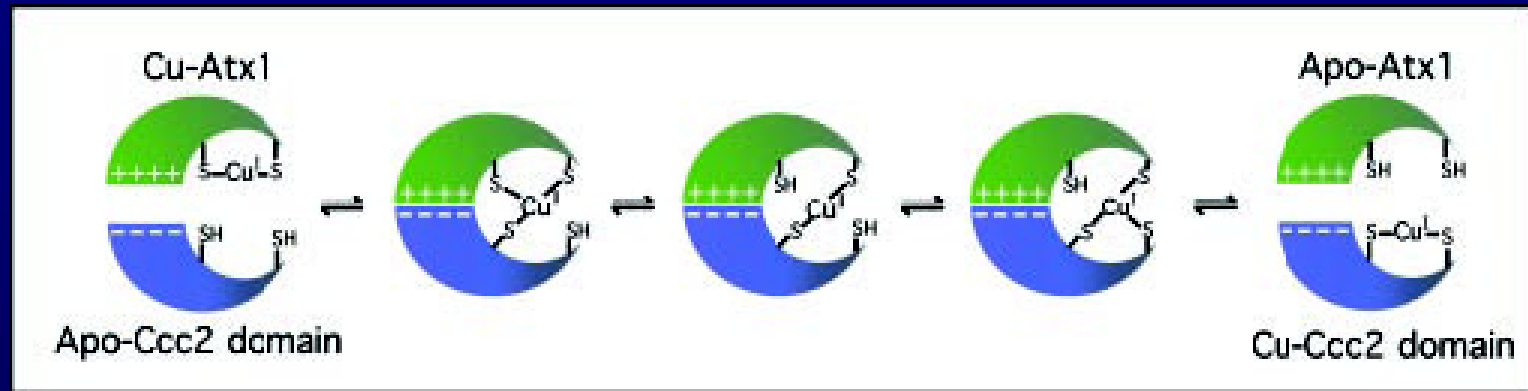
Copper delivery inside cellular compartments



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



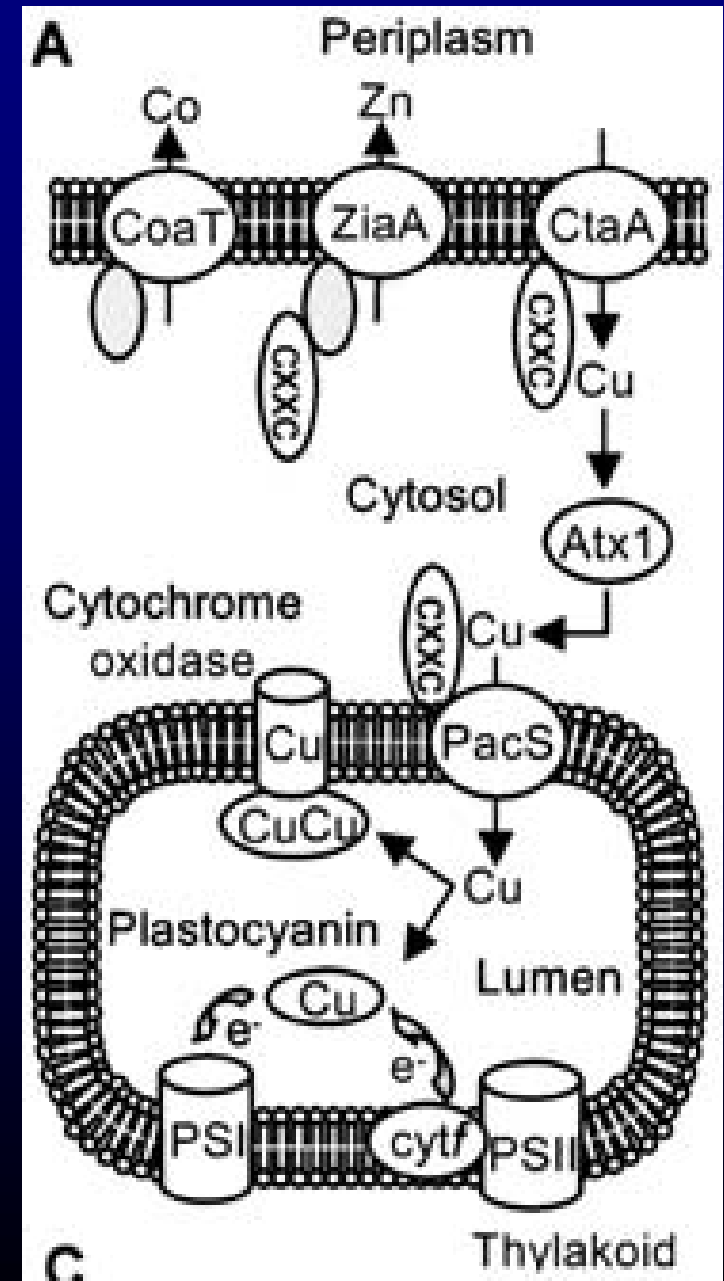
From: O'Halloran TV, Culotta VC, 2000, JBC275, 25057-60

- 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

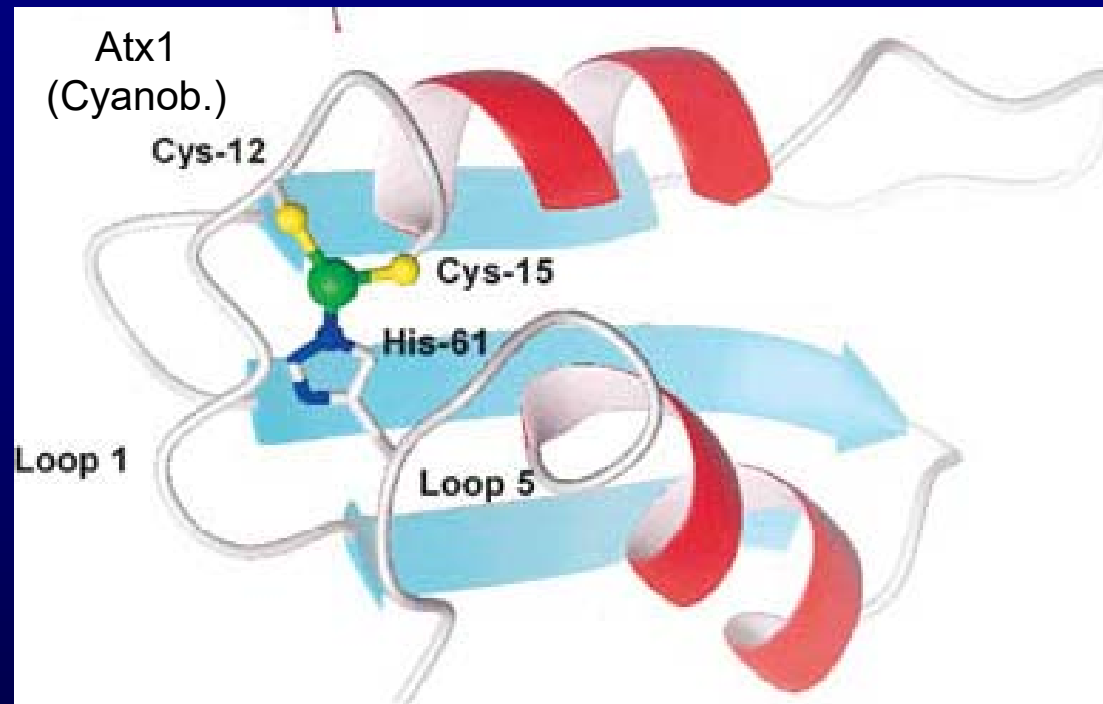
- CopZ in non-photosynthetic bacteria donates Cu to CopY transcription factor? Or Cu delivery to Cu-efflux transport ATPase CopB or copper influx ATPase CopA?
- Atx1 found to specifically shuttle copper to an intracellular CPx-type copper ATPase the thylakoid in cyanobacteria+plants
- CtaA+PacS ATPases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



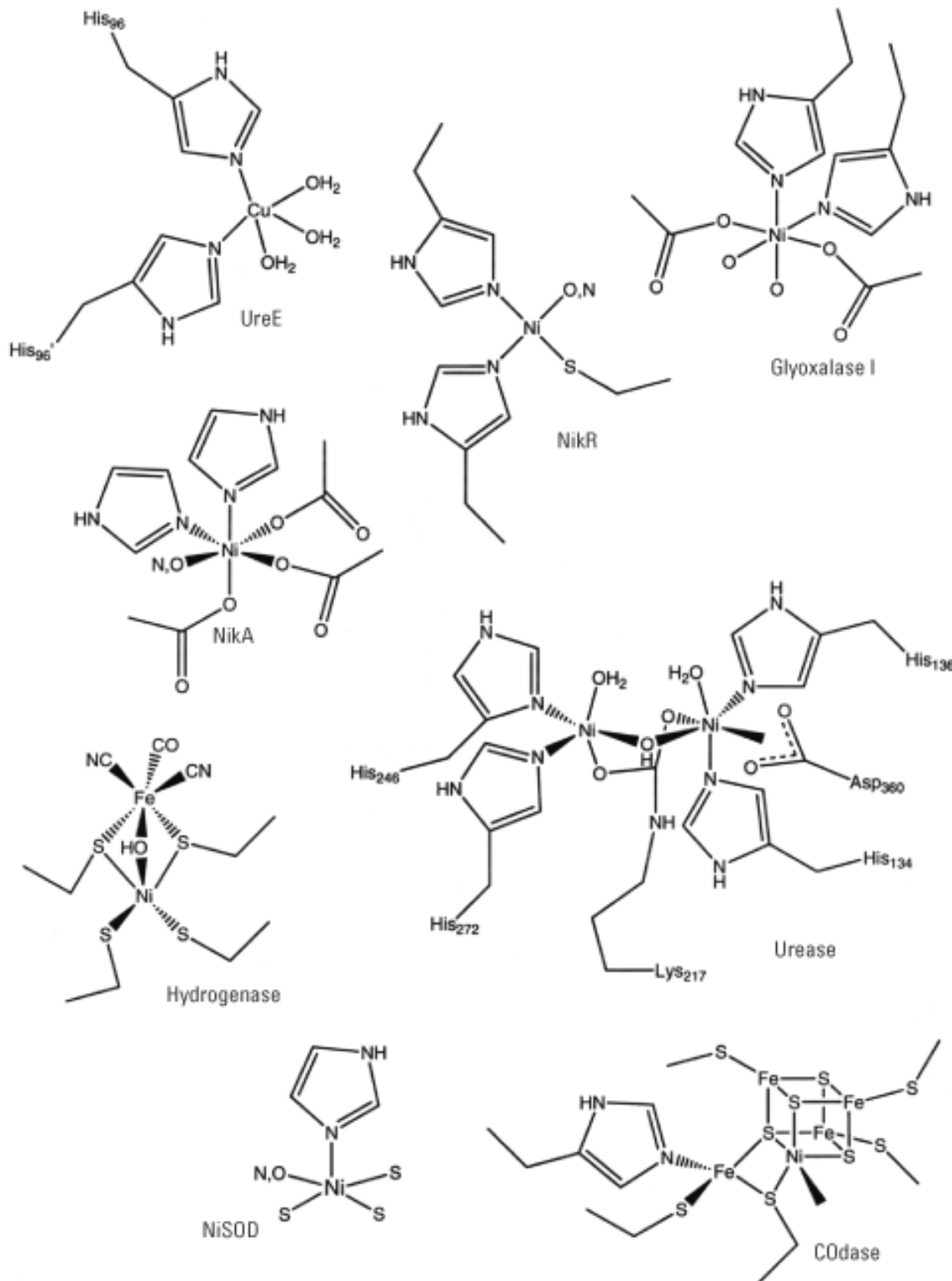
From: Borrelly GPM, et al., 2004, BiochemJ378, 293-7

- 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

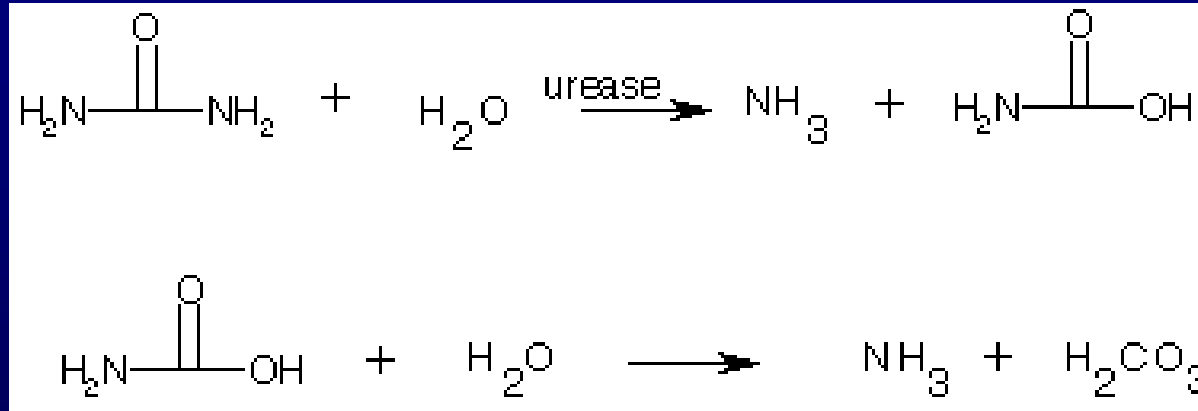
Characteristics

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



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

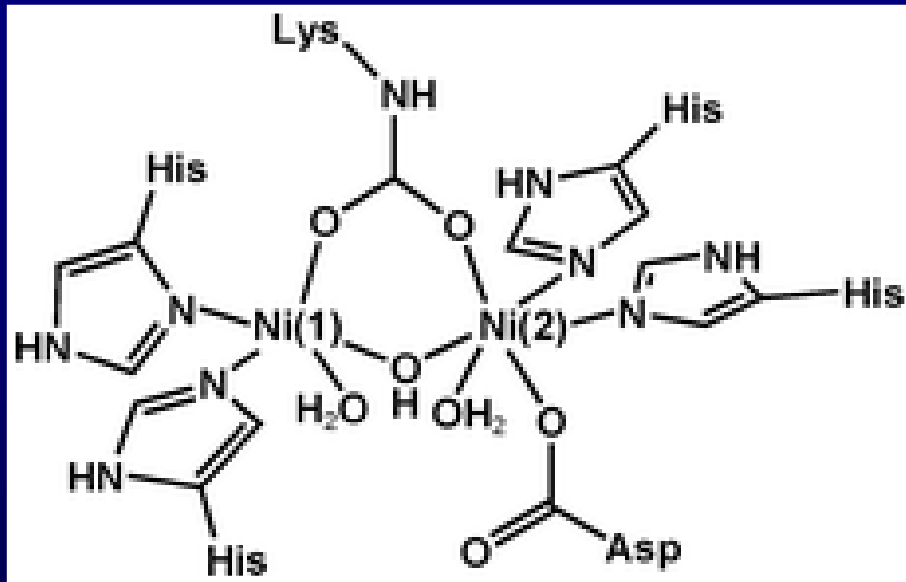
a) Function and occurrence



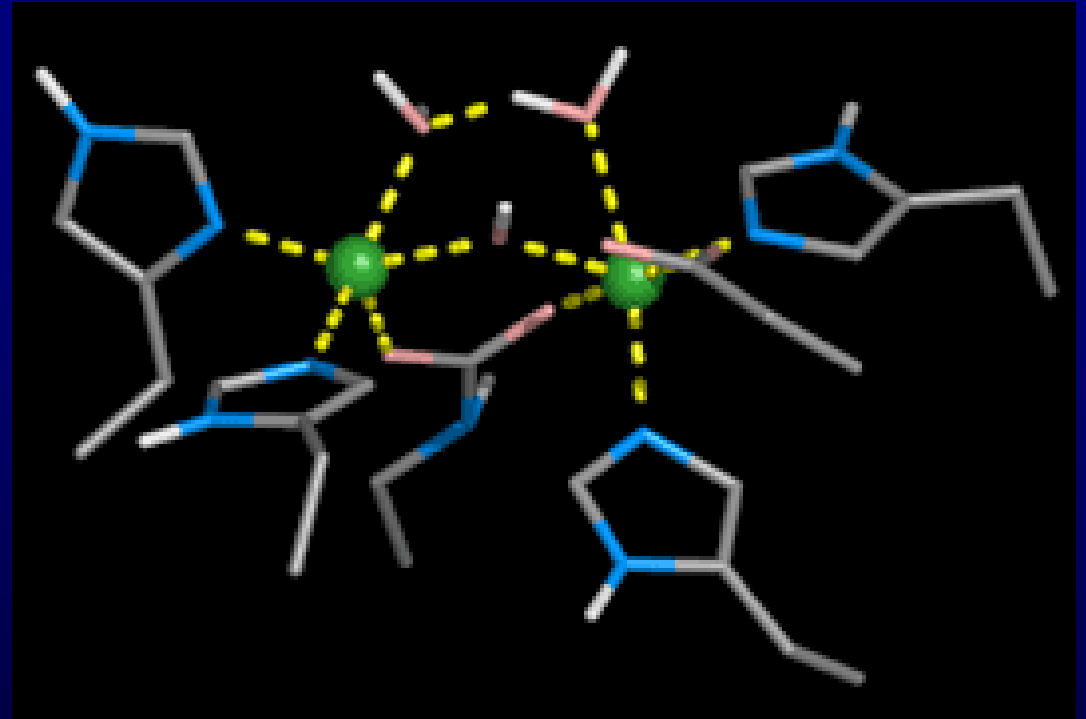
- Catalyses the decomposition of urea into carbon dioxide and ammonia
- In most organisms (plants, fungi, bacteria)
- Very important for metabolism: urea toxicity is one of the main mechanisms of damage caused by nickel deficiency in plants
- Very specific for urea as a substrate
- Rather fast: turnover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

Urease

b) Active site



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



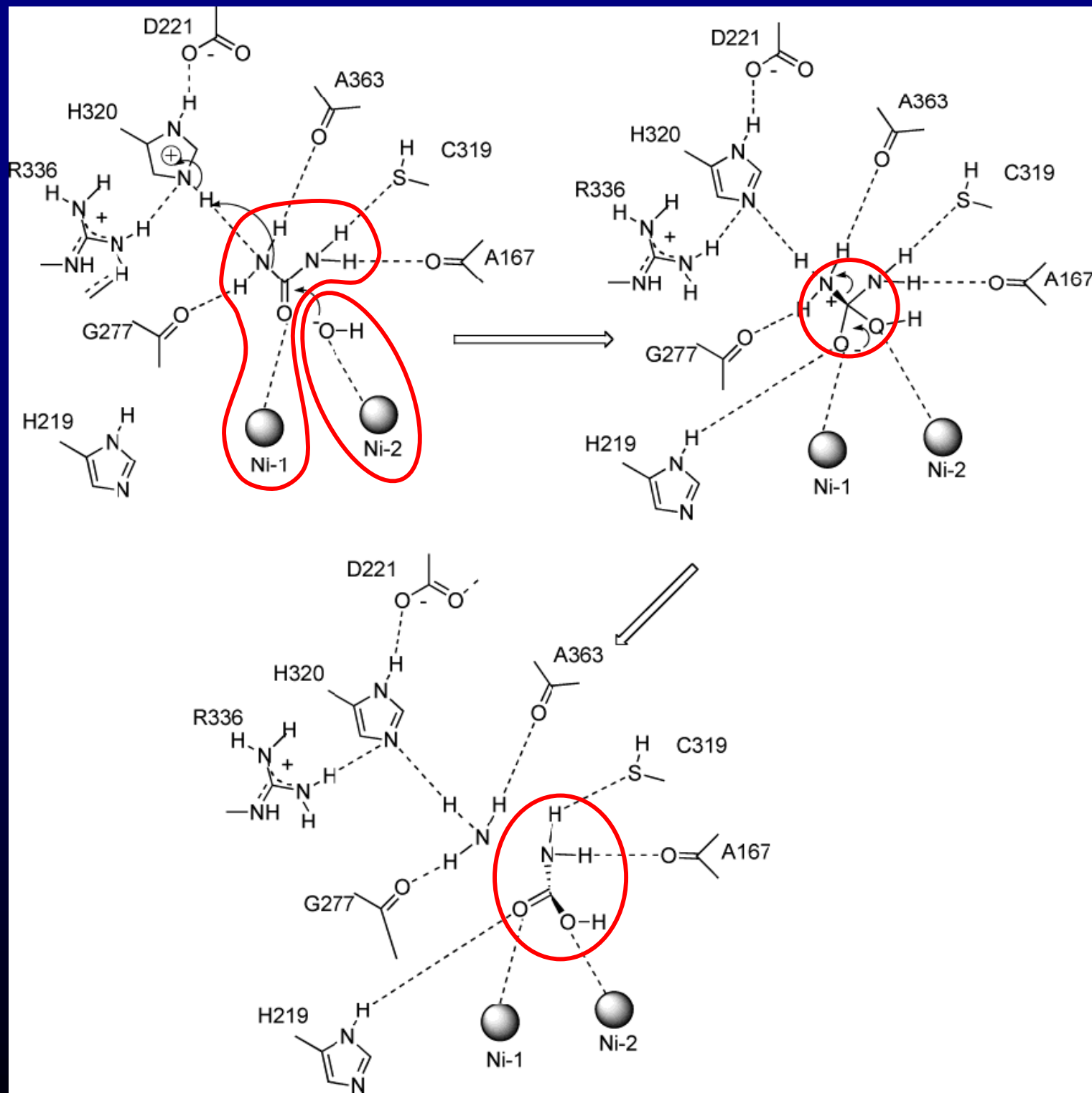
- two Ni²⁺ ions
- one Ni²⁺ bound by three fixed ligands (2 His-N and 1 Lys-O) and one water
- the other Ni²⁺ bound by four fixed ligands (2 His-N, 1 Asp-O and 1 Lys-O) and 1 water
- the two nickels are bridged by a water molecule

Urease

c) Mechanism

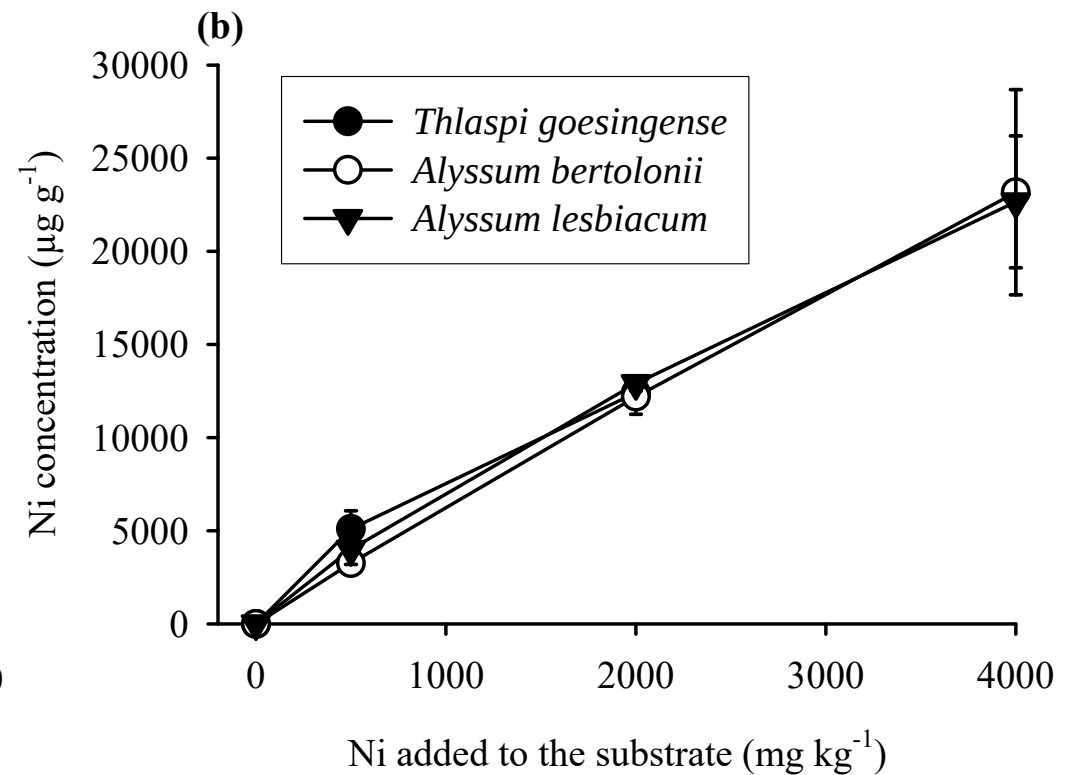
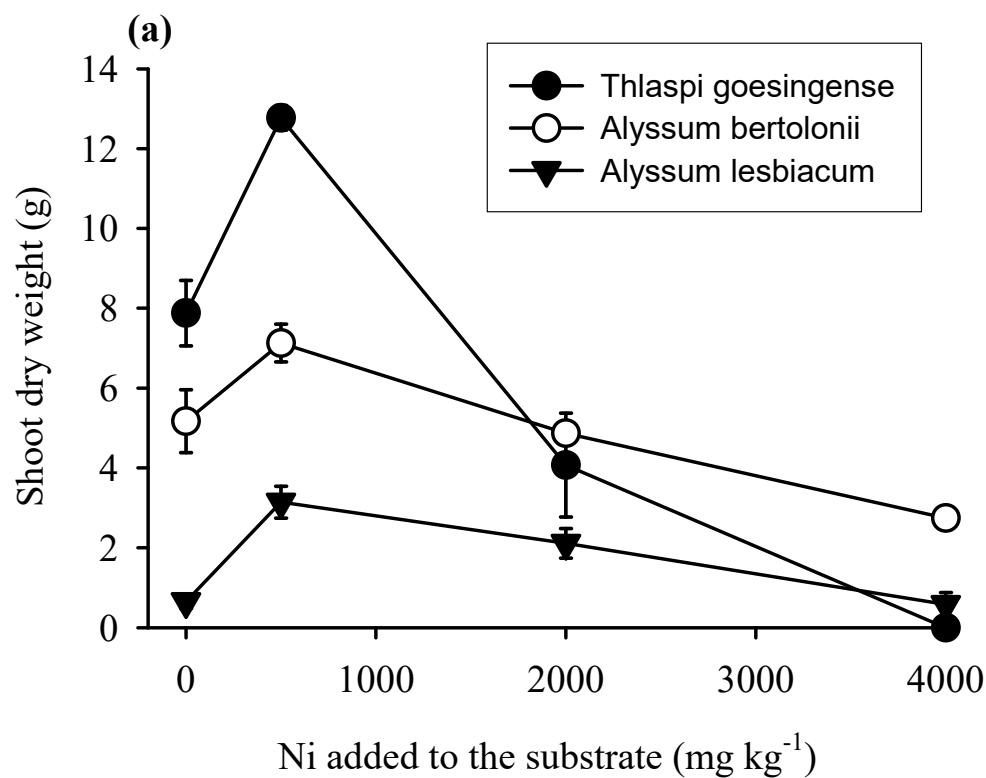
Steps

- 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

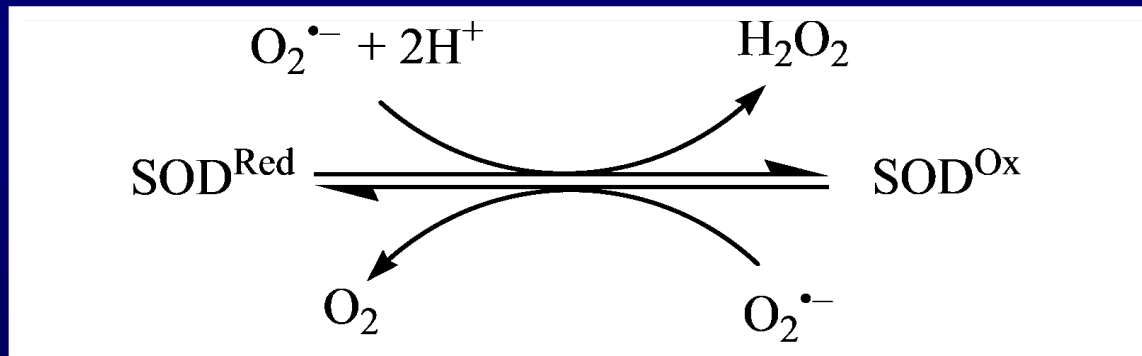


Alyssum lesbiacum

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

Nickel superoxide dismutase

(a) Function and occurrence



Characteristics

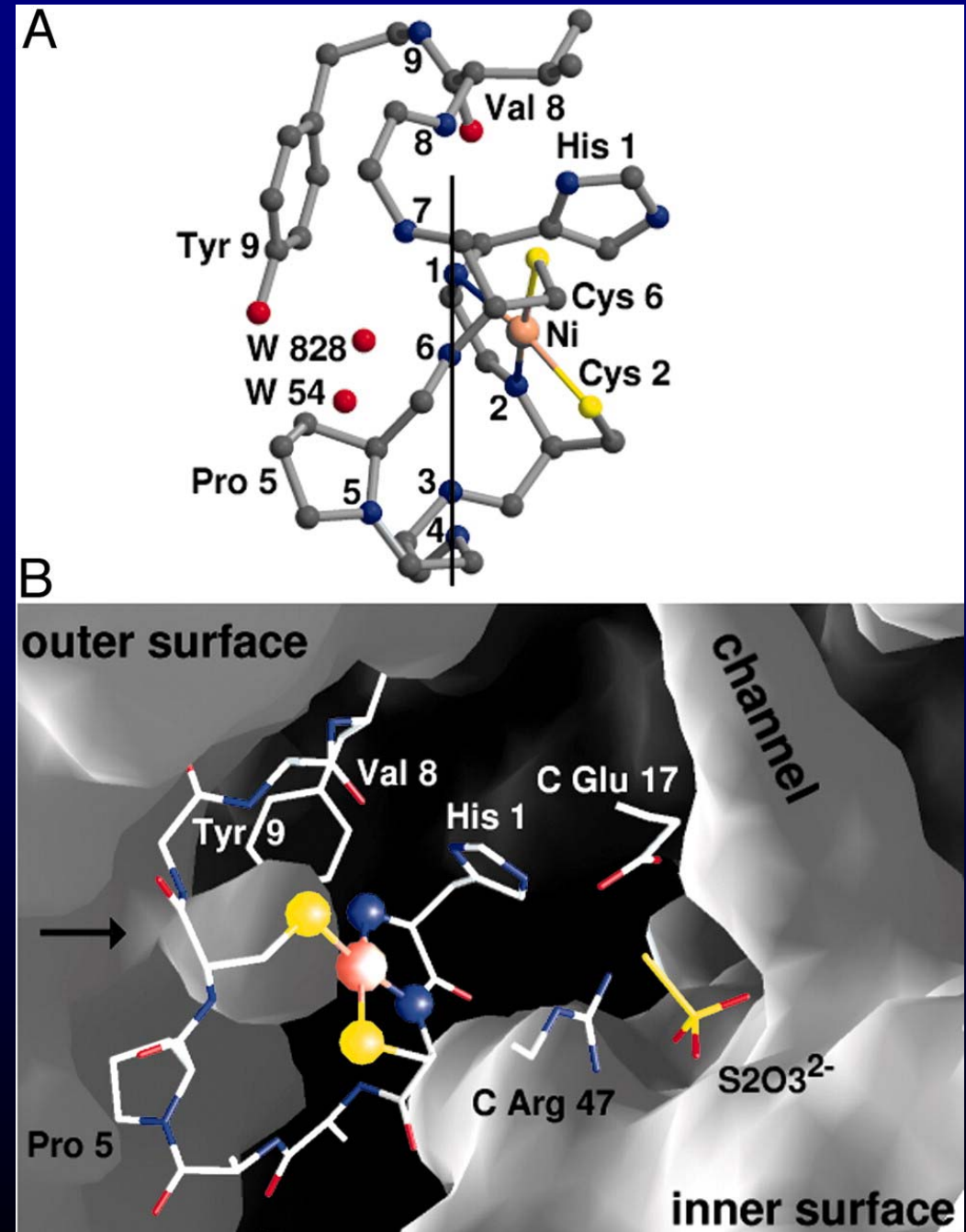
- Catalyses the detoxification of superoxide ($\text{O}_2^{\bullet-}$) by disproportionation into dioxygen (O_2) and hydrogen peroxide (H_2O_2)
- Ni-SOD is found in cyanobacteria and in *Streptomyces* (eubacteria), other SOD's are usually Cu/Zn, Fe or Mn enzymes

2. Nickel superoxide dismutase

(c) active site

Characteristics

- Ni 5-6-coordinated, axial N-ligand(s) artefactually (x-ray damage!) lost when Ni^{3+} is reduced to Ni^{2+} during x-ray data collection
- Ni coordination by the amino group of His-1, the amide group of Cys-2, and the thiolate group of Cys-2 and of Cys-6
- sulphur (thiolate) ligation makes otherwise biologically redox-indert nickel redox-active ($2+/3+$)
- Accessibility of active site limited by Pro-5 and Tyr-9 → specificity for small molecules!

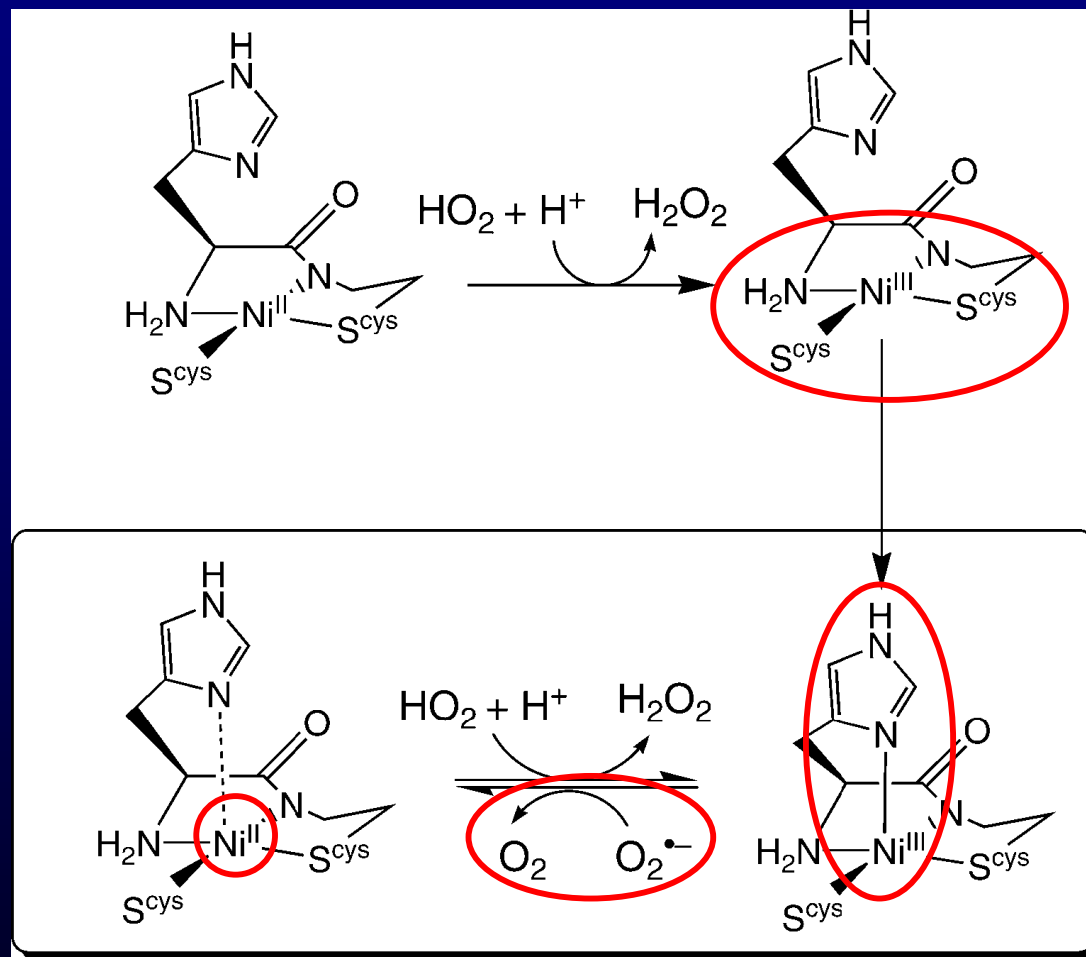


2. Nickel superoxide dismutase

(d) Mechanism

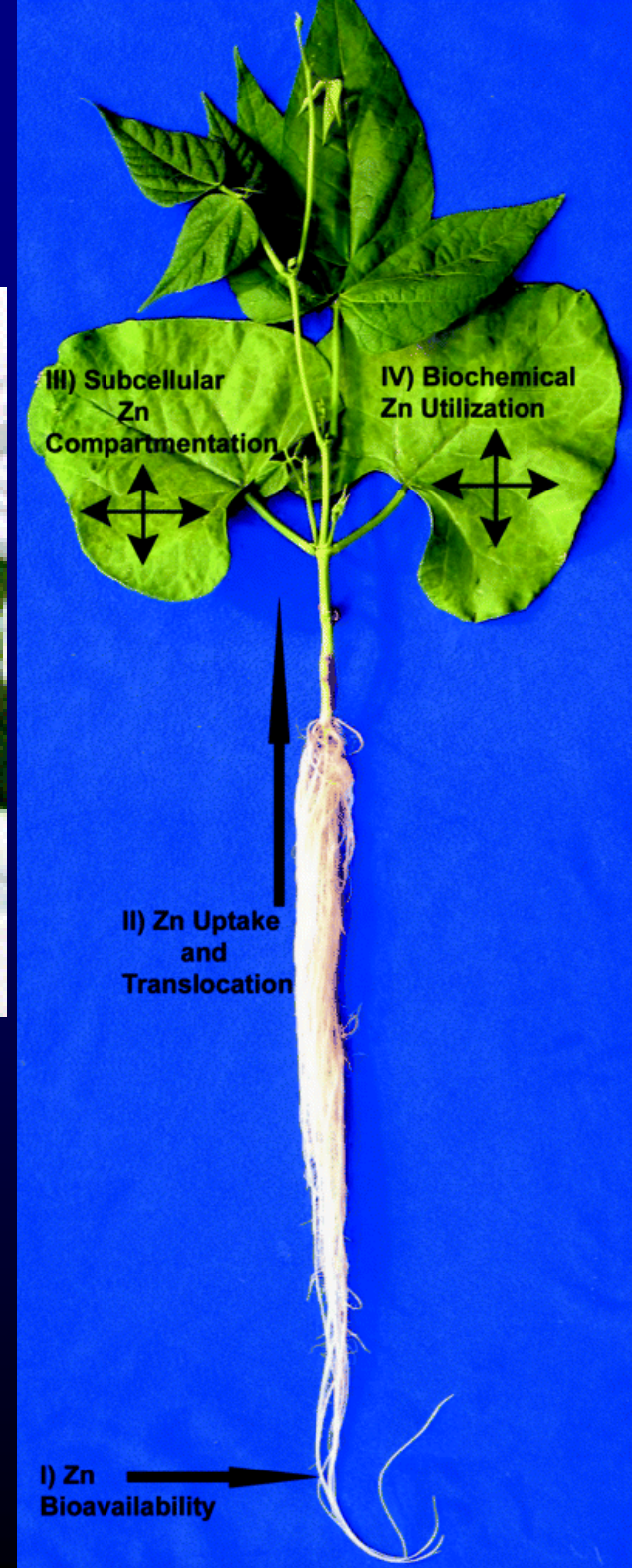
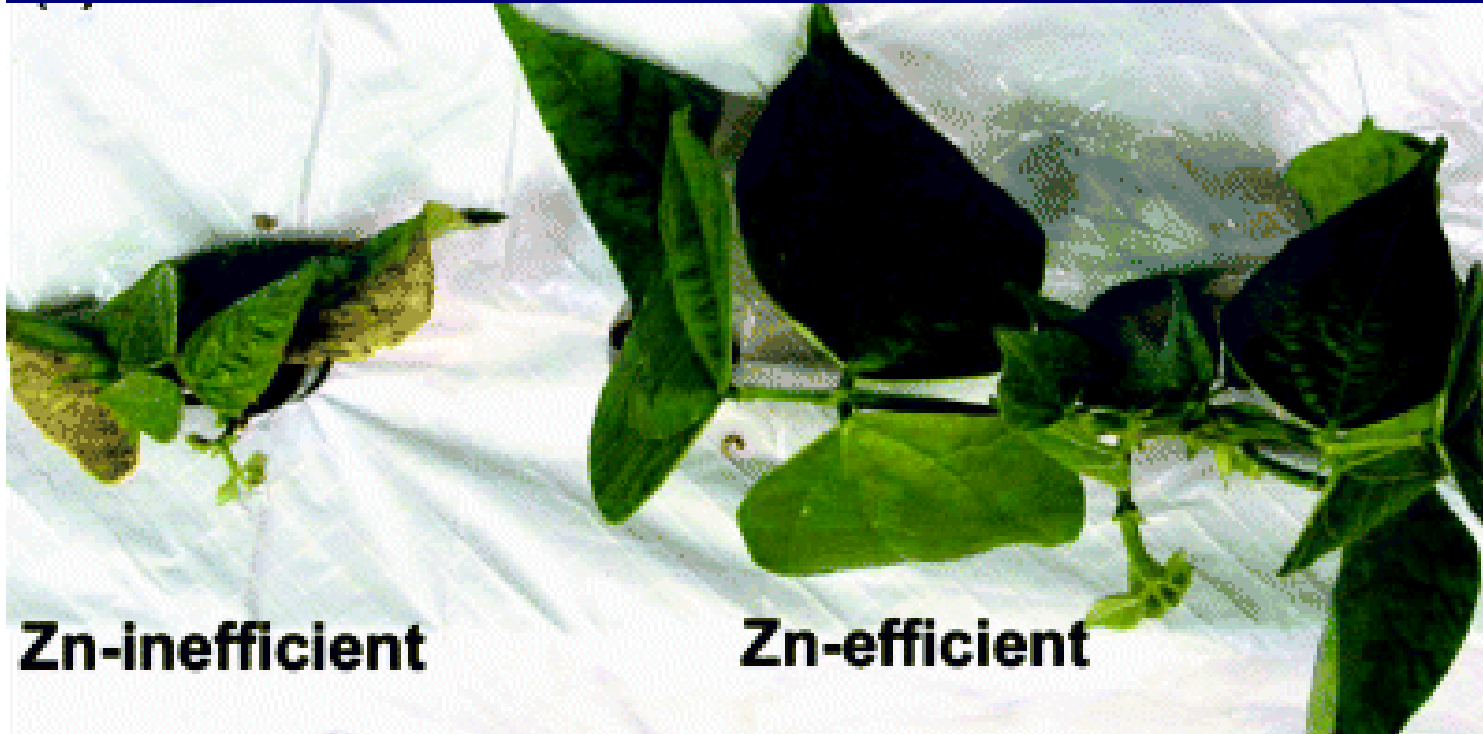
Steps and Characteristics

- Oxidation of the four-coordinate Ni^{2+} center to Ni^{3+} (unusual!)
- Rapid imidazole coordination. Once the imidazole is coordinated to the oxidized Ni^{3+} center it will remain ligated throughout catalysis
- Re-reduction of Ni^{3+} to Ni^{2+}
- the axial H(1) imidazole enhances the activity of NiSOD e.g. by reducing structural reorganisation during catalysis (→ enhances speed!)



From: Neupane KP et al., 2007, JACS129, 14605-18

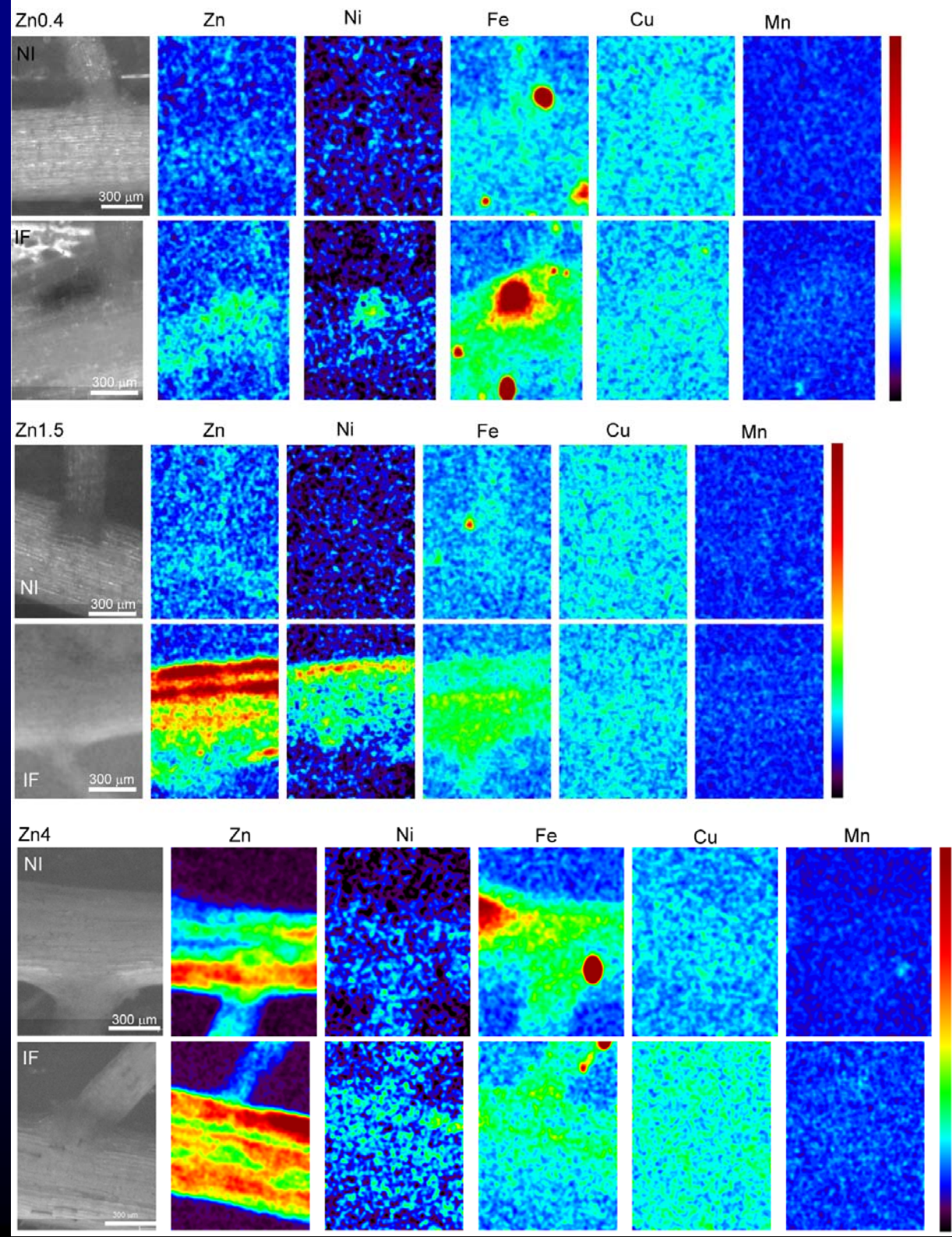
Zinc efficiency



From: Hacısalıhoğlu 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.

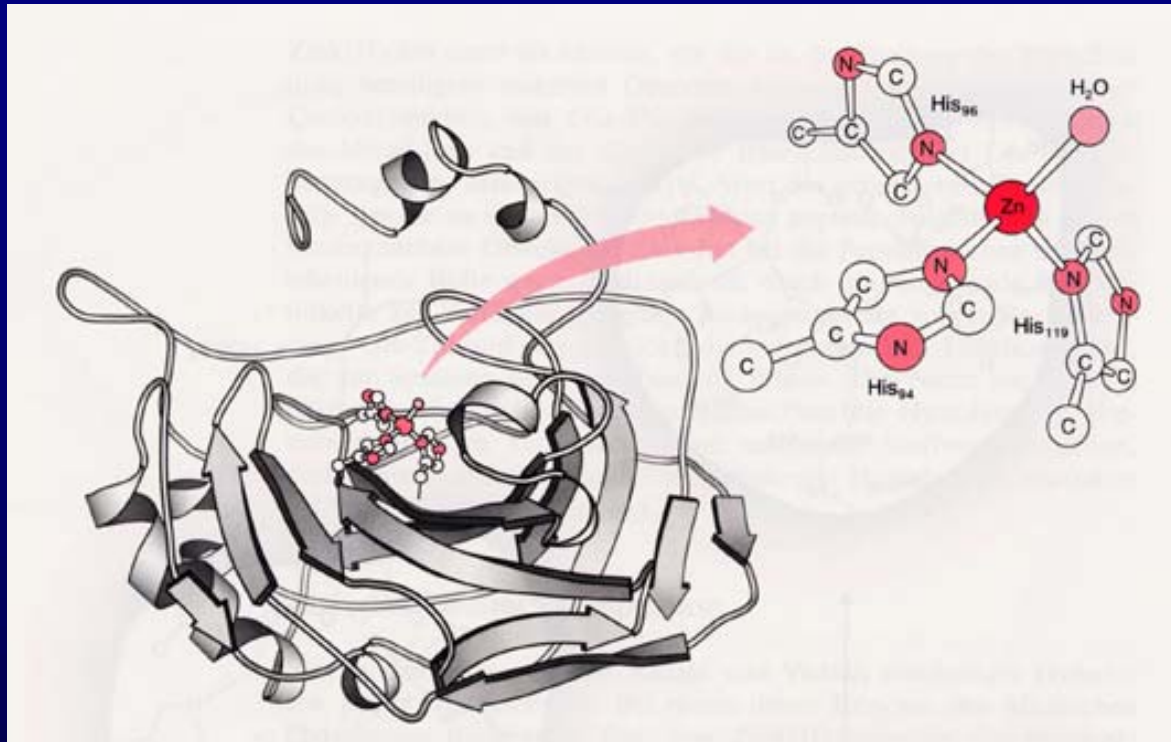
Emerging field: Role of trace metals in plant defence response to biotic stress

- Local Zn mobilization in response to pathogen Phomopsis in soybean roots revealed by μ XRF imaging of living roots.
- Still unknown (ongoing work): regulatory mechanism, genes involved,...



Morina F, Mijovilovich A, Koloniuk I, Pecnik A, Novak O, Gruz J, Küpper H (2021) Journal of Experimental Botany DOI: doi.org/10.1093/jxb/erab052

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