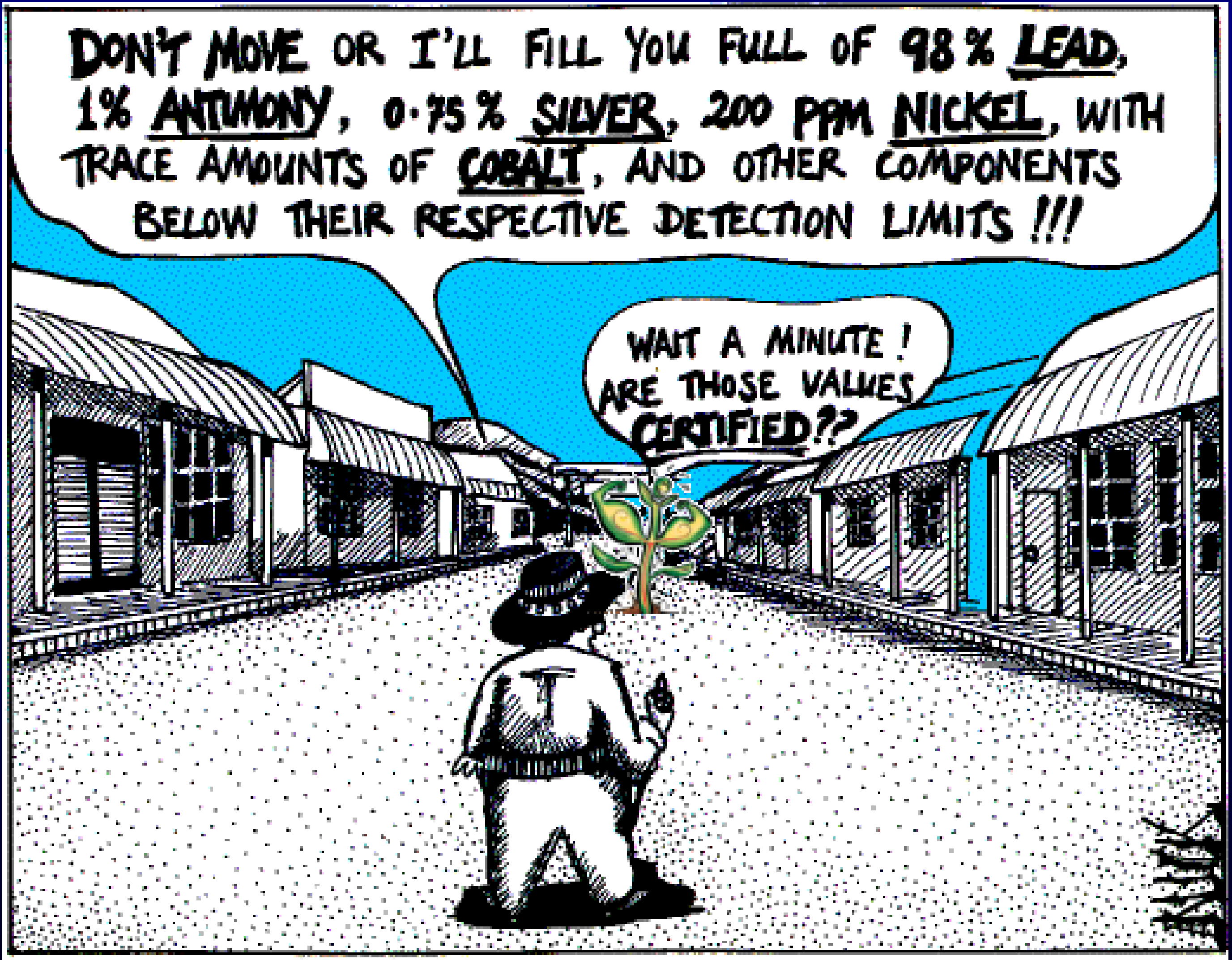
Trace metals as micronutrients

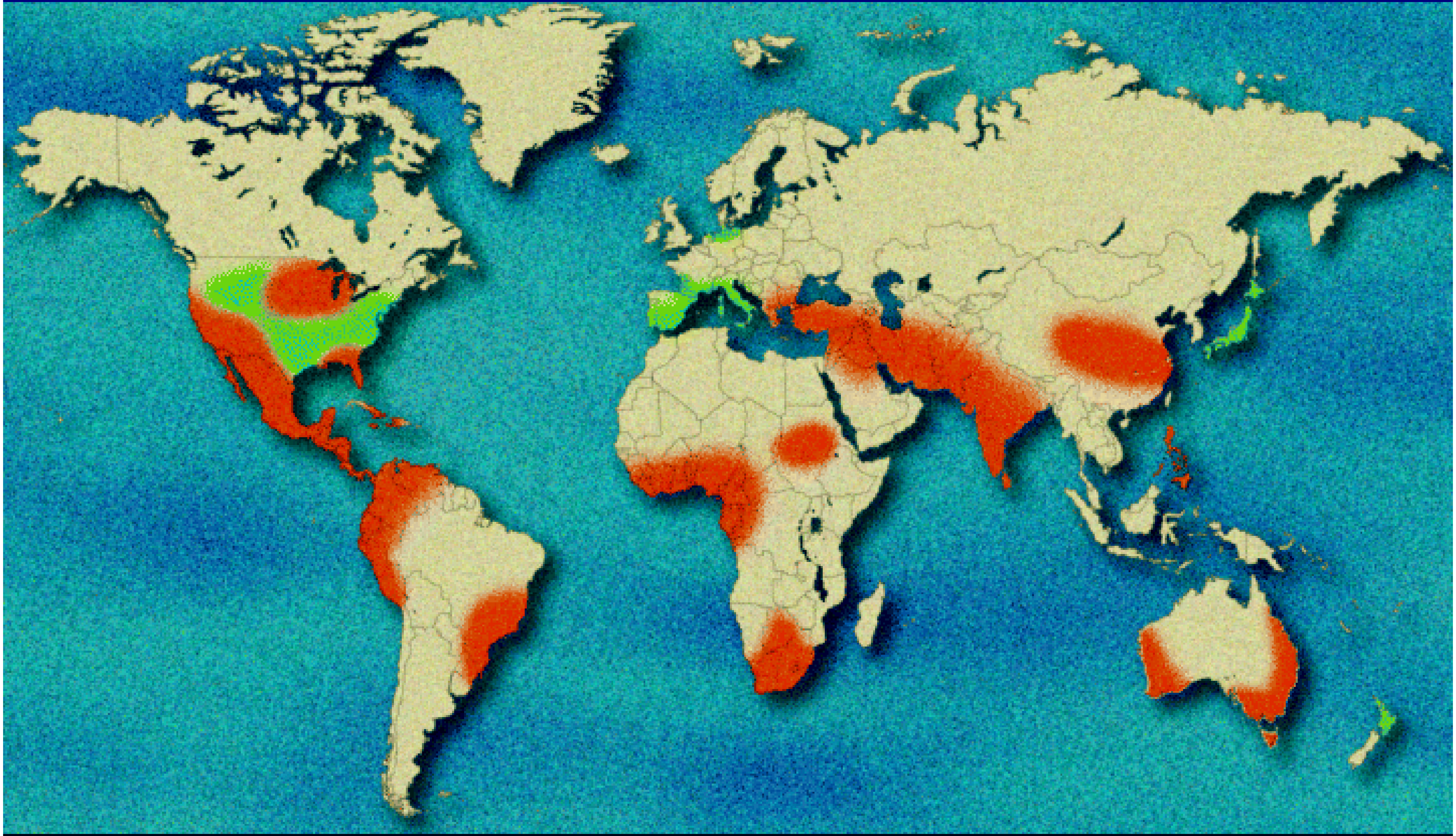


Metal uptake in the Wild West

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

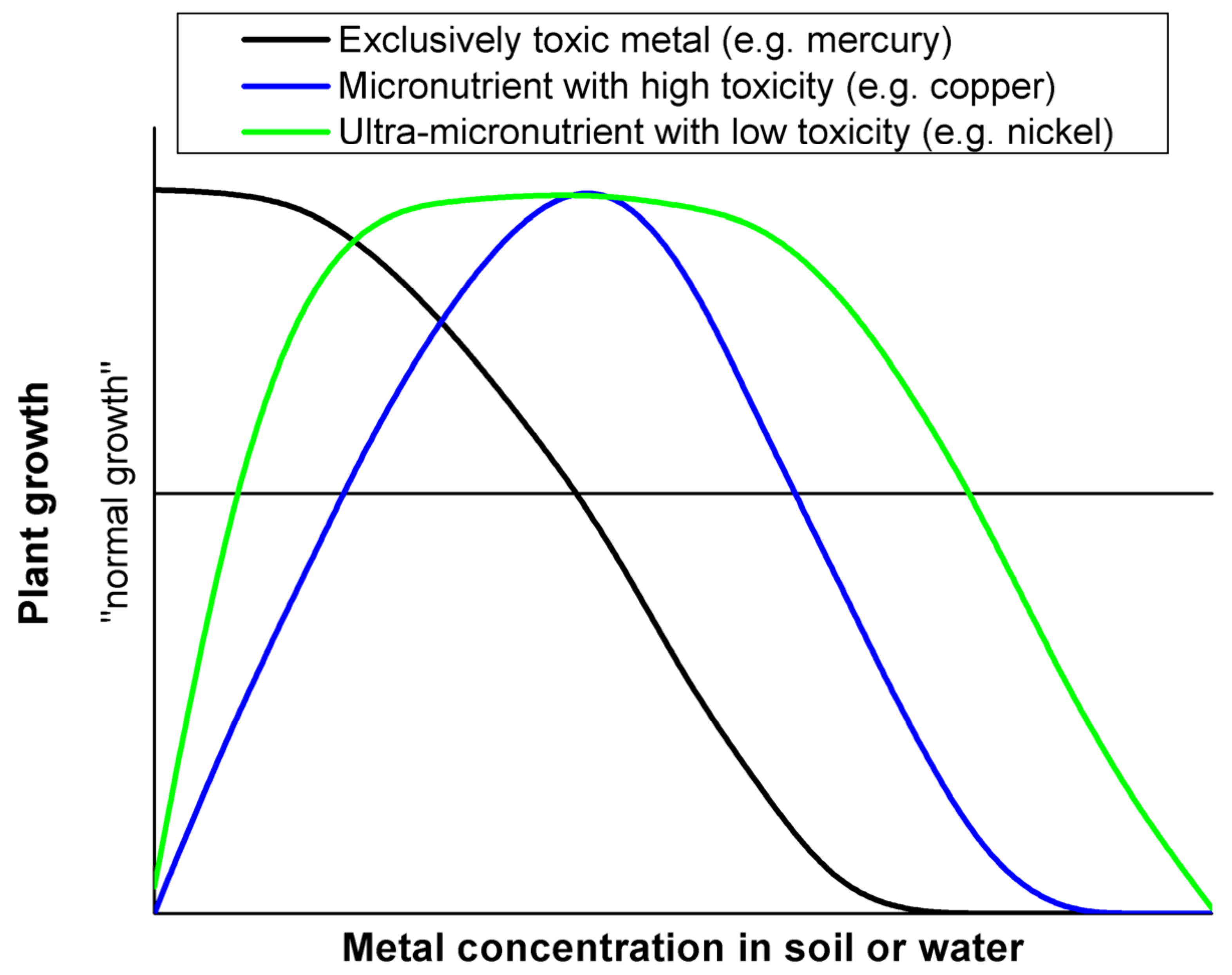
Heavy metal deficiency as a global problem of agriculture

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.

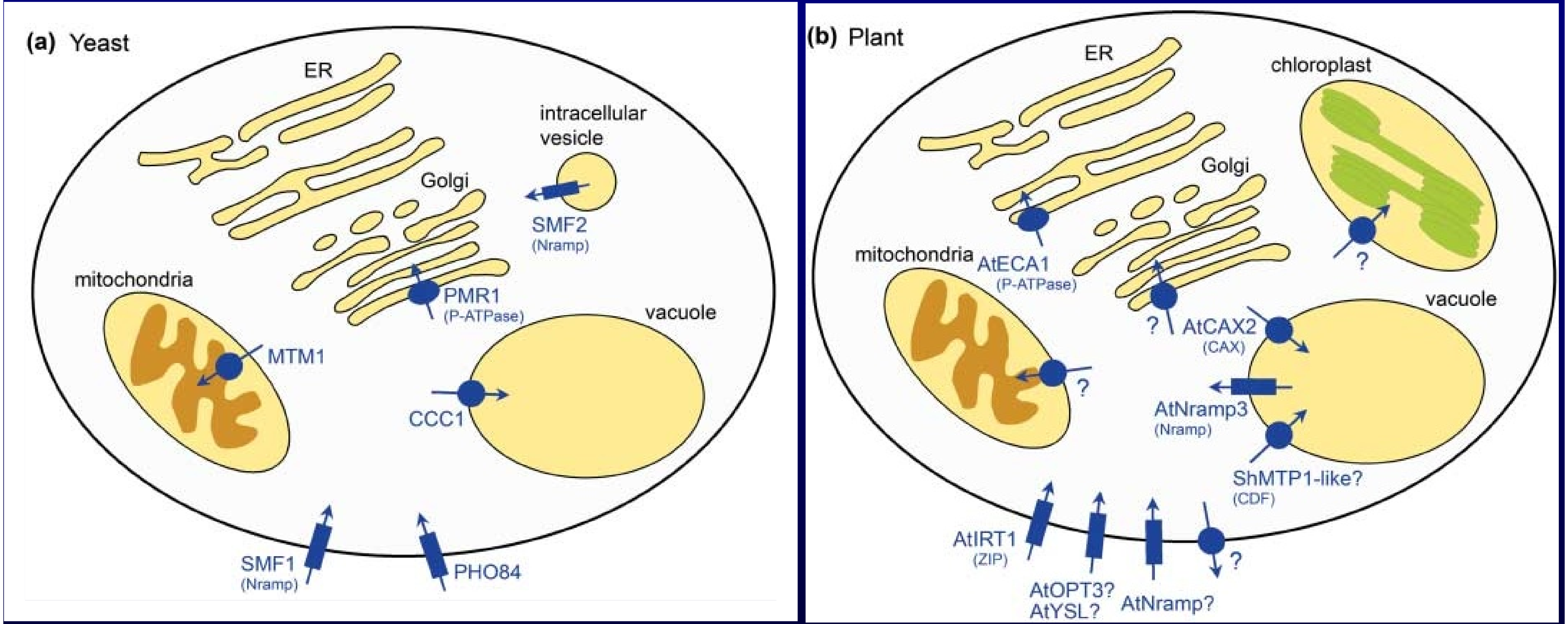
Dose-response principle for heavy metals



Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62

Mechanisms of metal uptake in Eucaryotes: Main families of metal transport proteins

example: manganese transport in yeast and plants

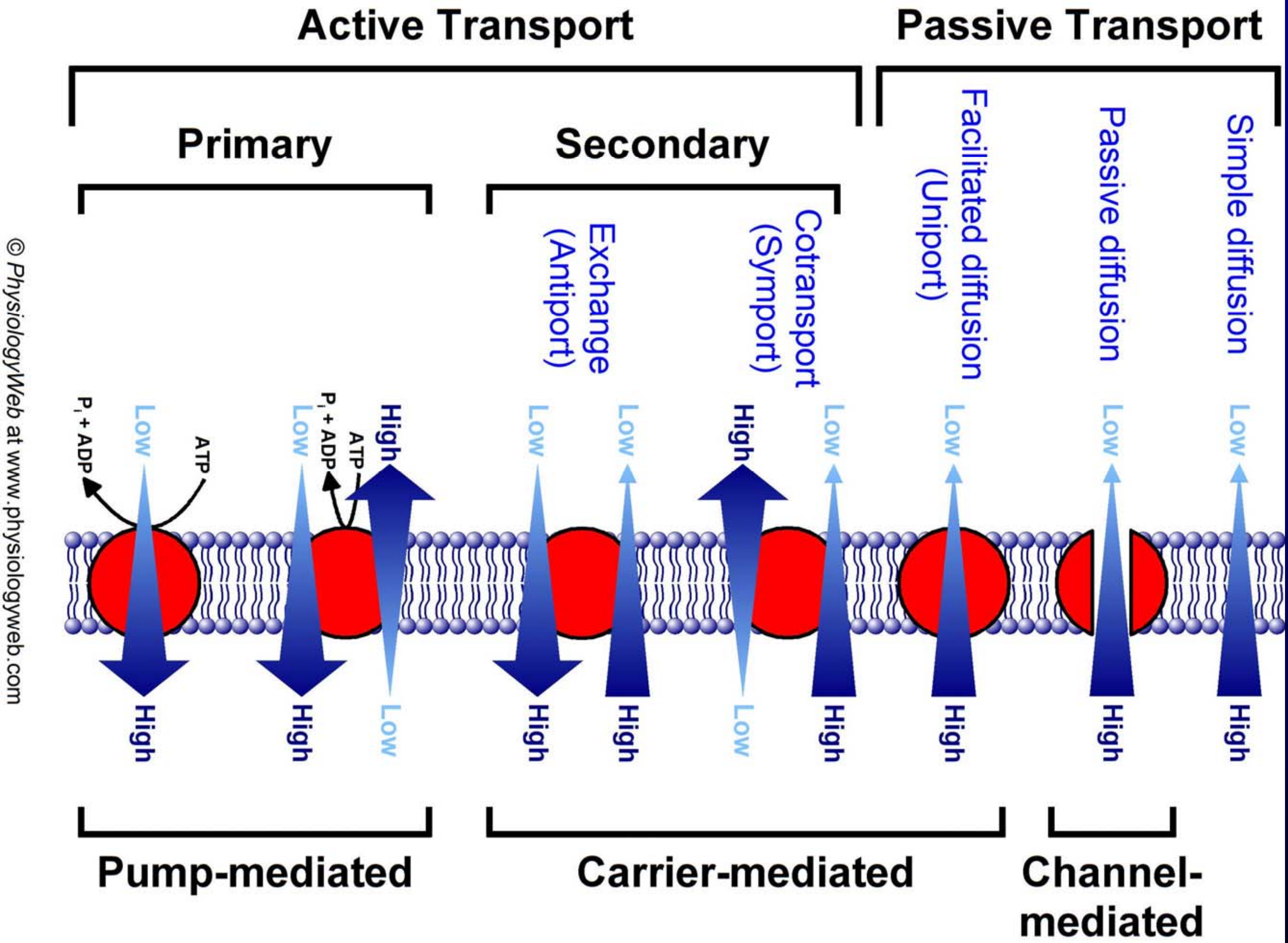


From: Pittman JK, 2005, NewPhytol167, 733-742

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 (**Nramp**-transporters)

Energetics and variants of metal transport



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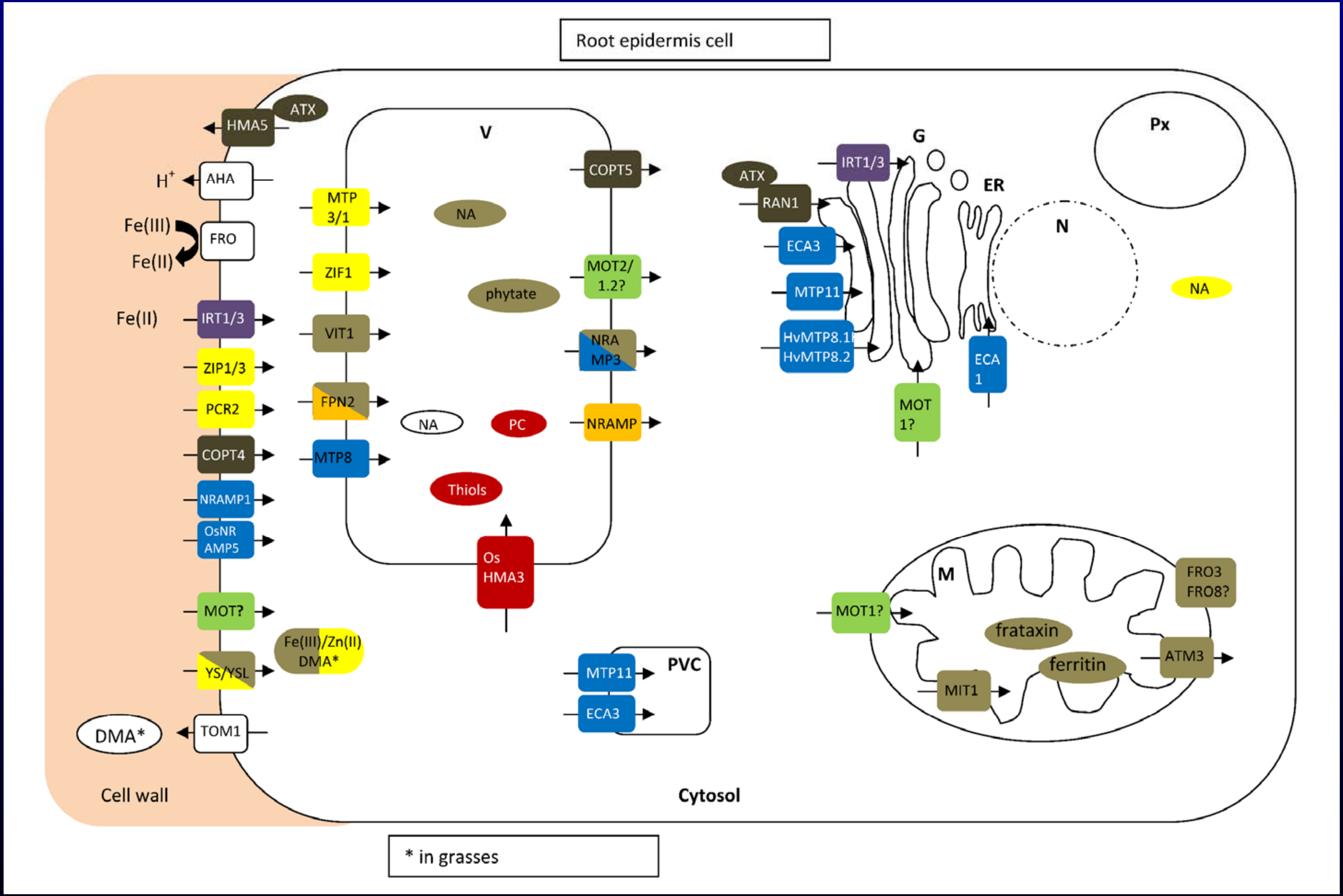
$$\Delta G = n_{Ionen} * R * T * \ln (c_{inside} / c_{outside}) + 3F (\varphi_{outside} - \varphi_{inside})$$

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

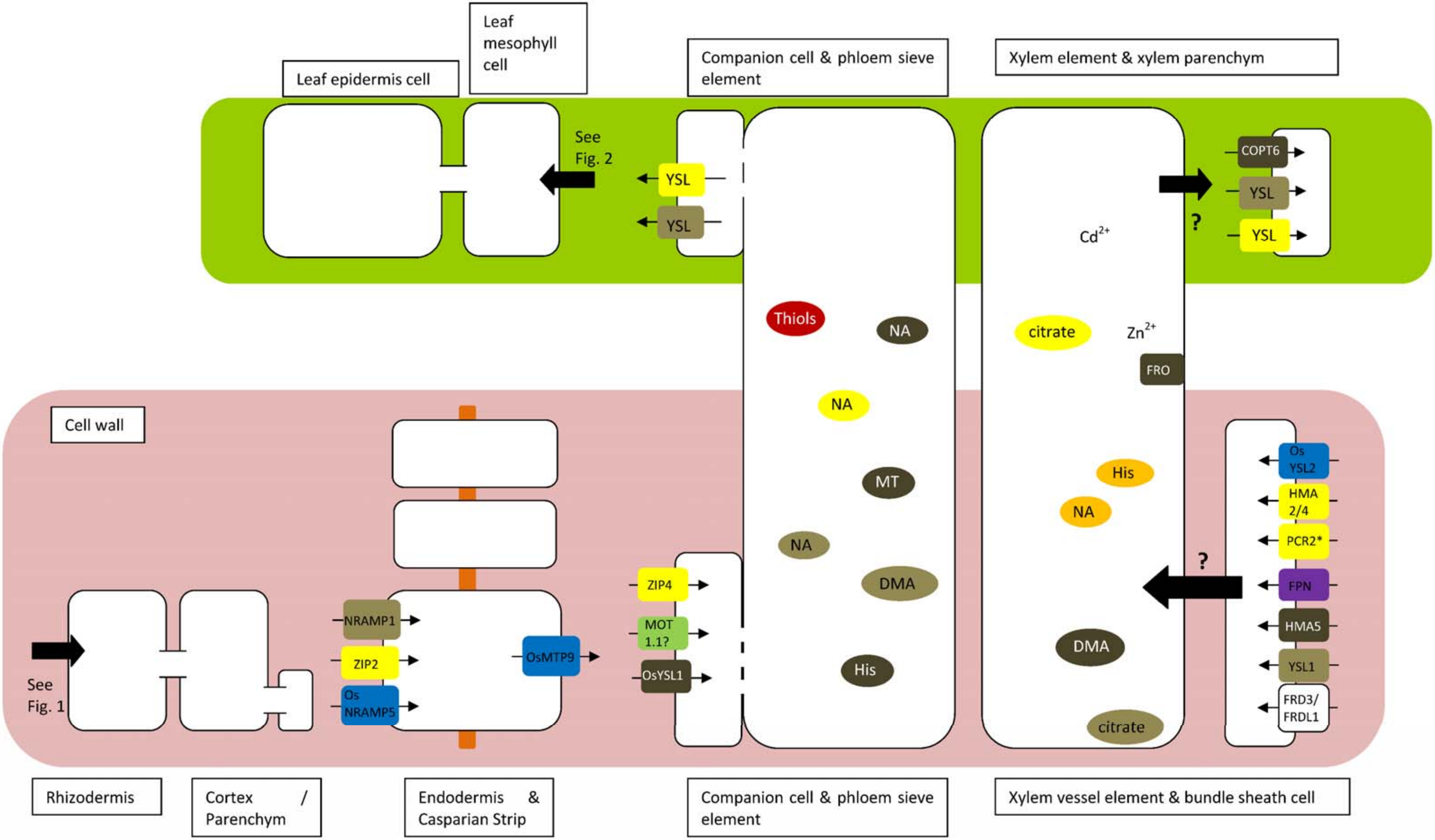
Mechanisms of metal uptake in plants: Different transport steps require different transporters

1) Root uptake and intracellular distribution



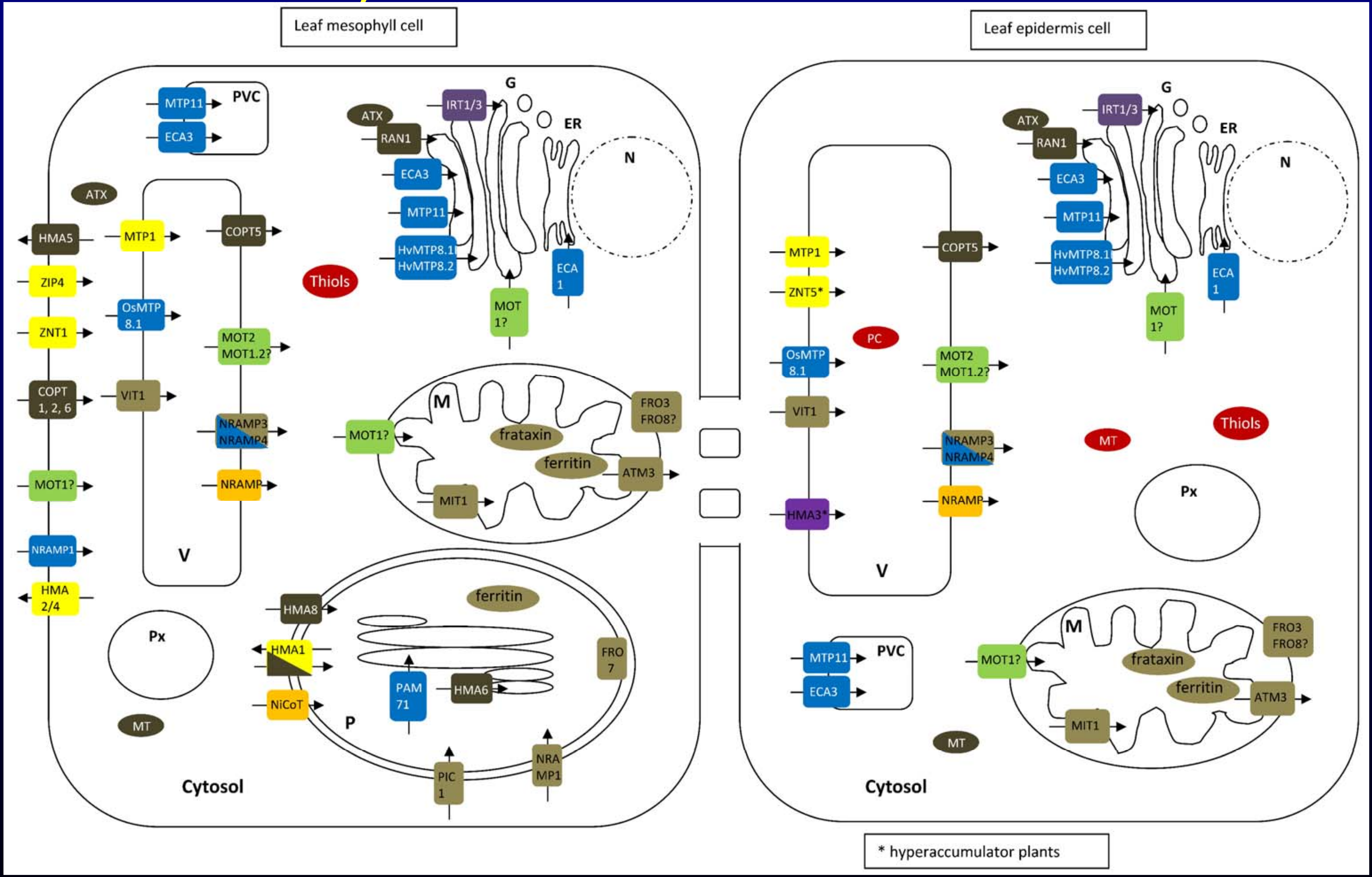
From: Andresen E, Peiter E, Küpper H (2018) Journal of Experimental Botany 69, 909-954

Mechanisms of metal uptake in plants: Different transport steps require different transporters 2) Translocation. Root-to-shoot: Xylem, shoot-to-root: phloem



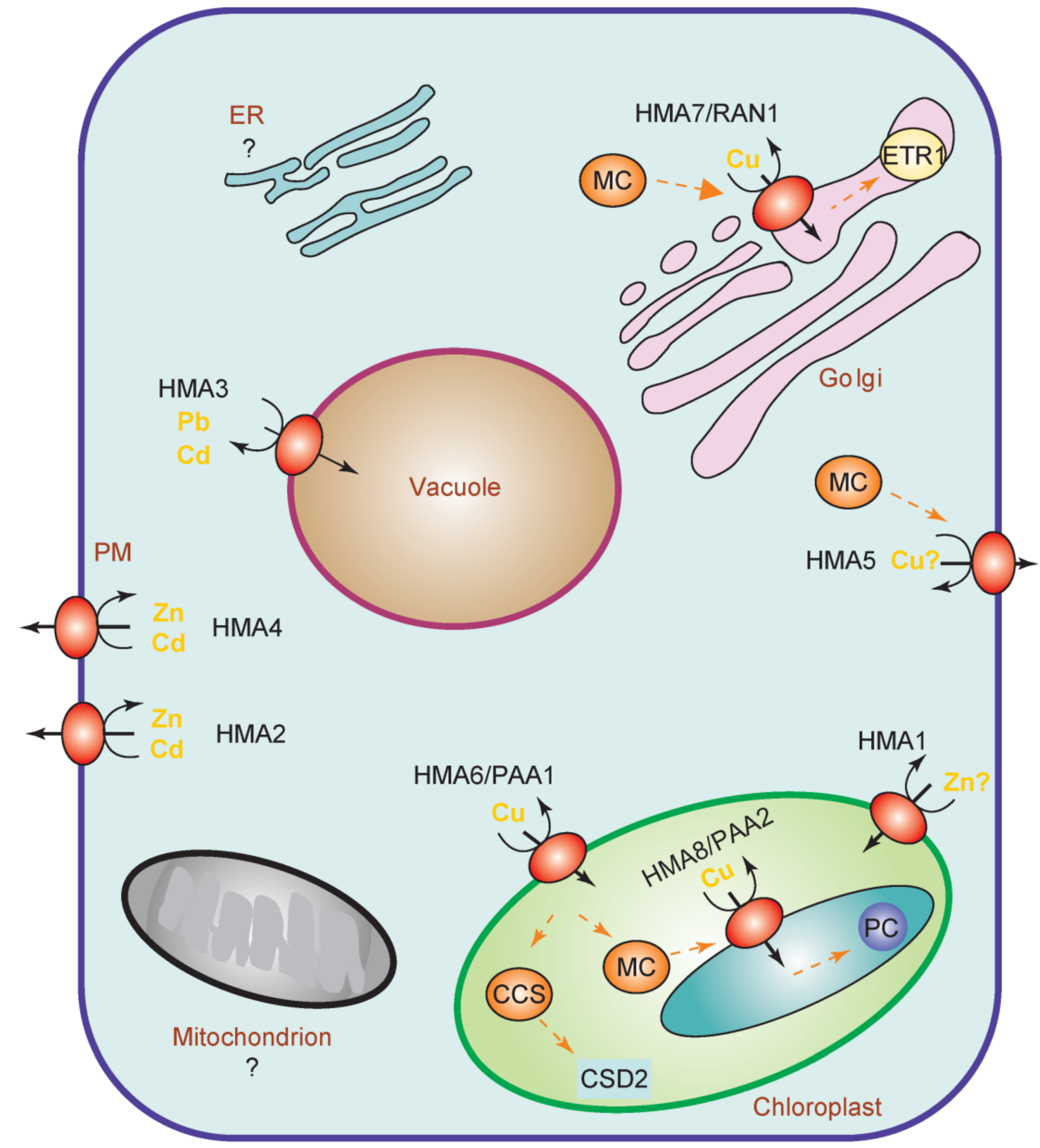
From: Andresen E, Peiter E, Küpper H (2018) Journal of Experimental Botany 69, 909-954

Mechanisms of metal uptake in plants: Different transport steps require different transporters
2) Distribution in shoot cells



From: Andresen E, Peiter E, Küpper H (2018) Journal of Experimental Botany 69, 909-954

Mechanisms of metal uptake+compartmentation in plants (I)
CPx-type (=P_{1B}-type) ATPases

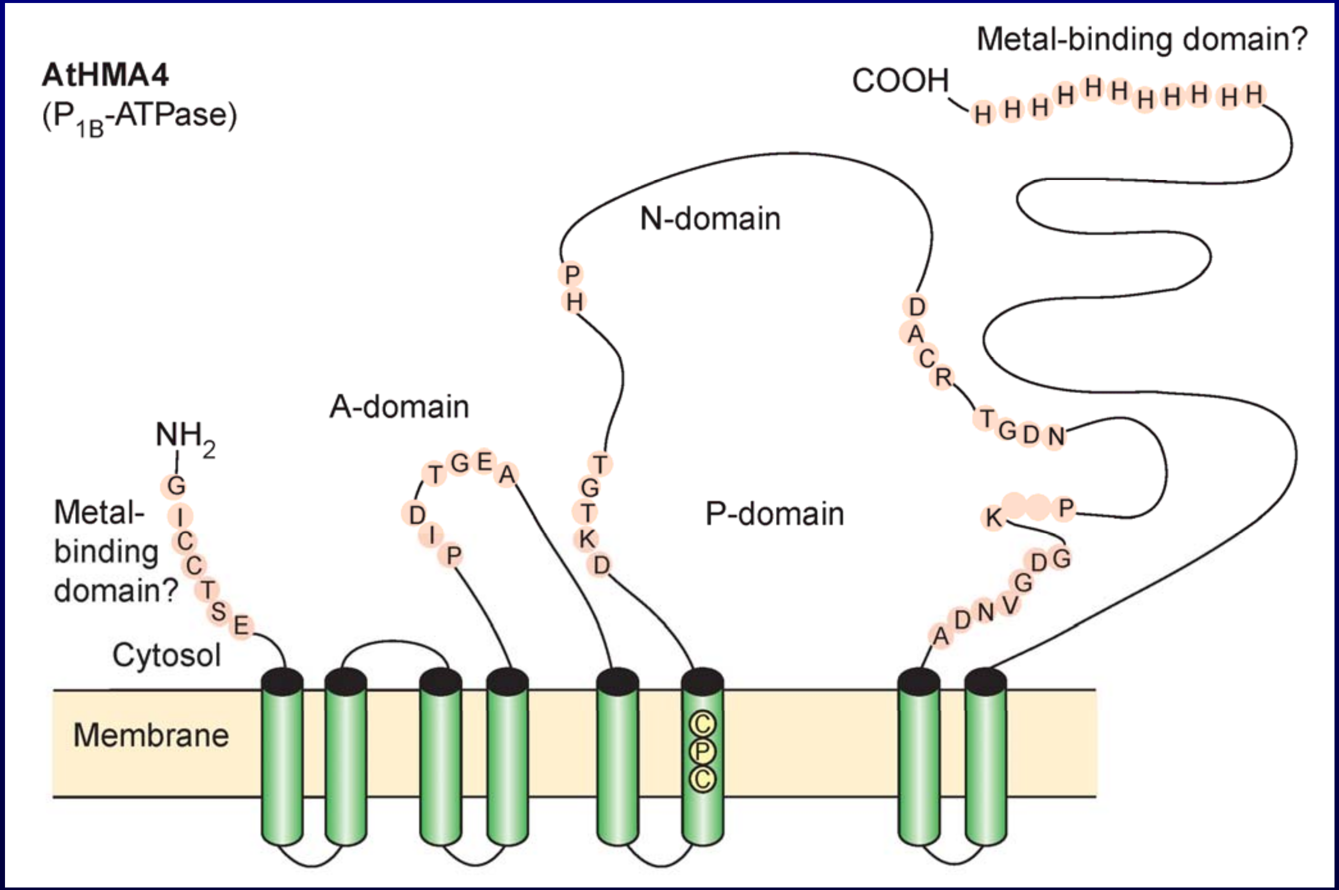


Functions (concluded mostly from differential expression studies)

- 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

From: Williams LE, Mills RF, 2005, TrendsPlantSci10, 491-502

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

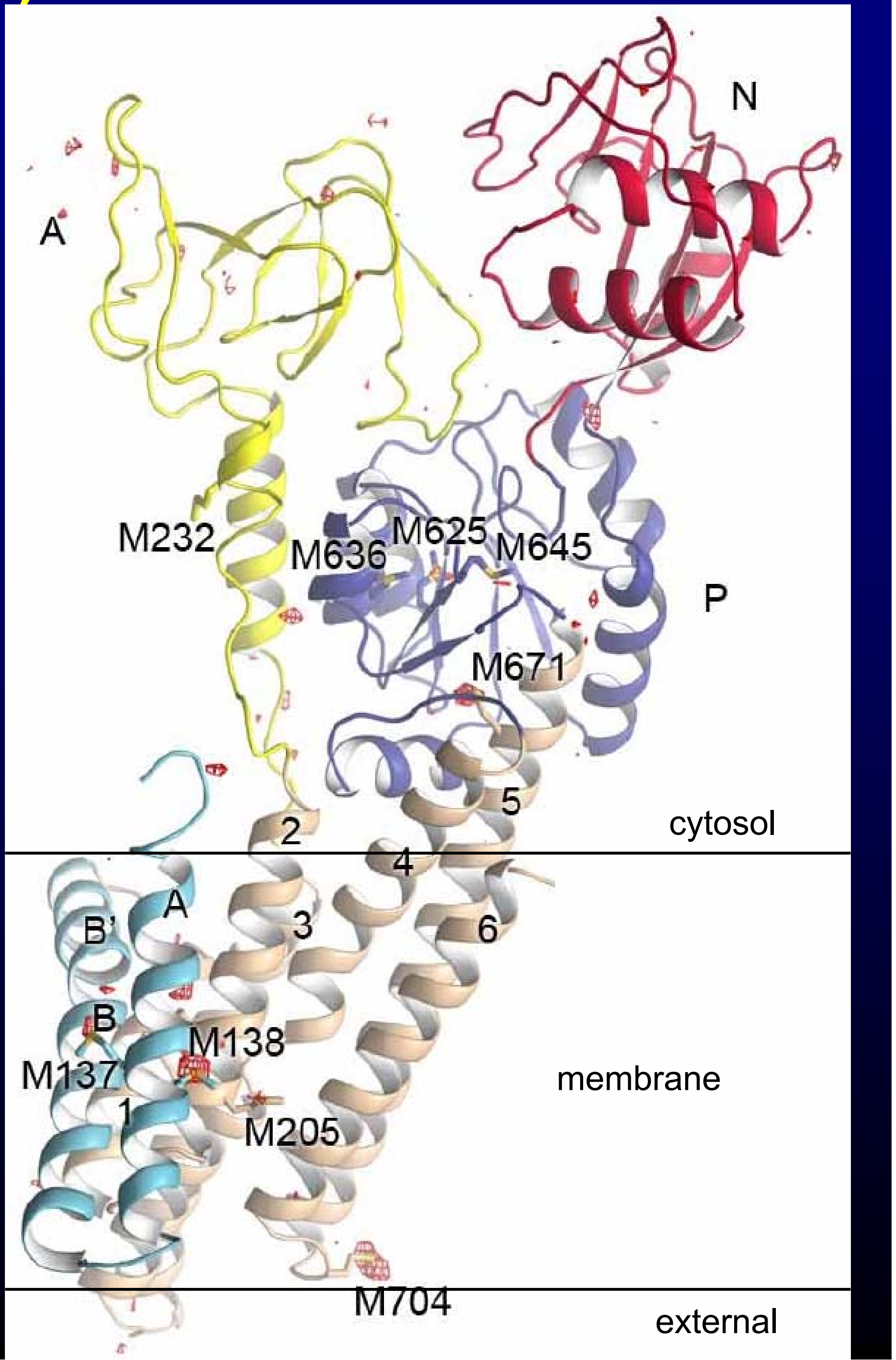
- CPx-motif in 6th transmembrane helix (→ Name!)
- Variable number of transmembrane helices
- MANY histidines and cysteines in sequence (→ e.g. 58 Cys in TcHMA4)
- Metal binding domain at N-terminus (in cytosol)
- Histidine repeat at C-terminus (in cytosol)

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 Zn^{2+}, not a larger complex
- high affinity Zn-binding site contains two cysteins



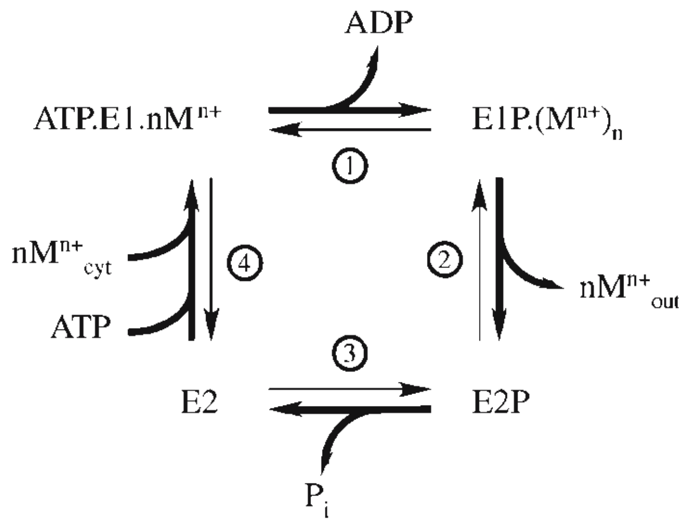
From: Wang K et al, 2014, Structure and mechanism of Zn-transporting P-type ATPases. Nature 514, 518-

Mechanisms of metal uptake+compartmentation in plants (I)

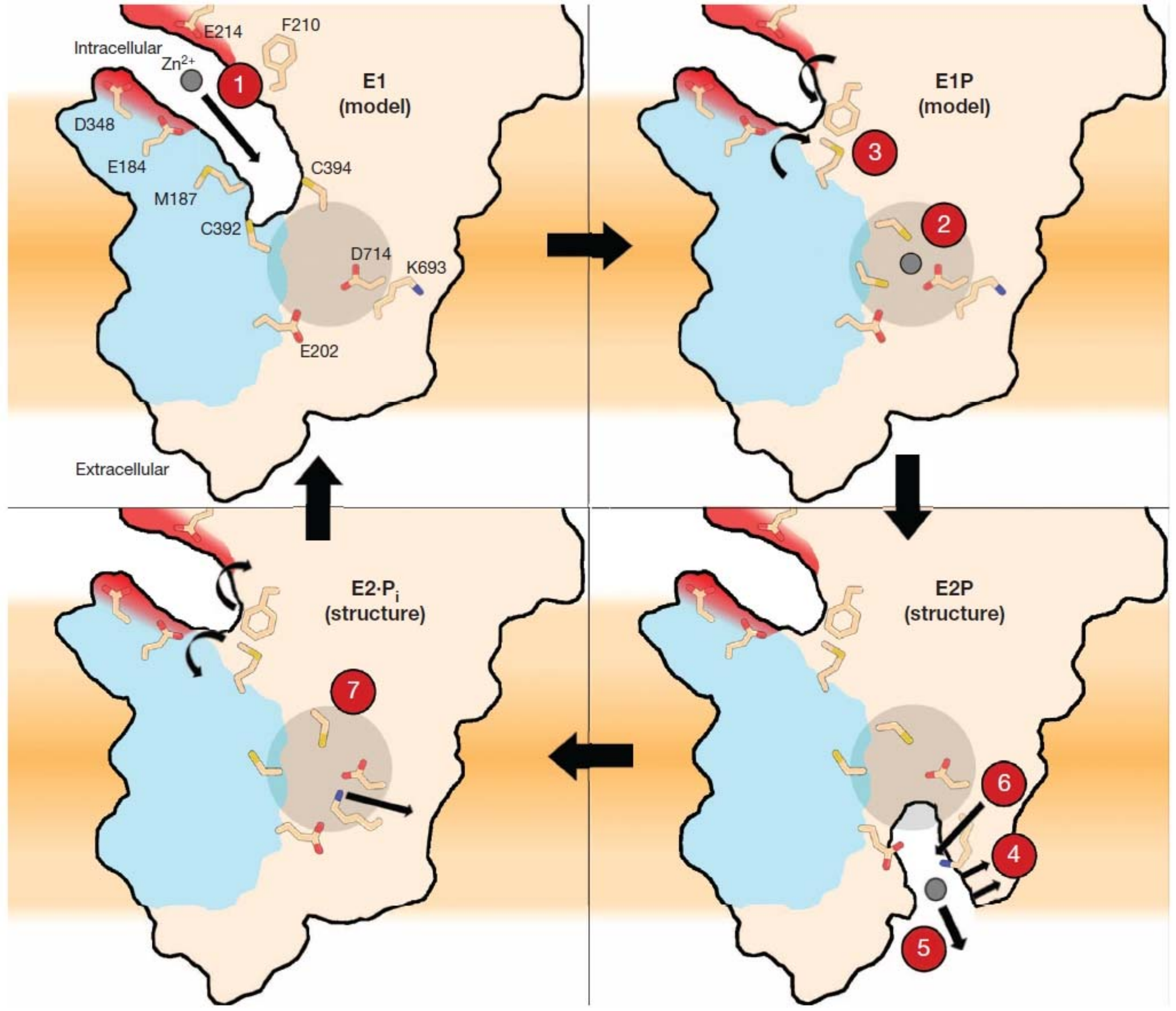
CPx-type (=P_{1B}-type) ATPases

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 Zn^{2+}
- pore closes after ATP hydrolysis



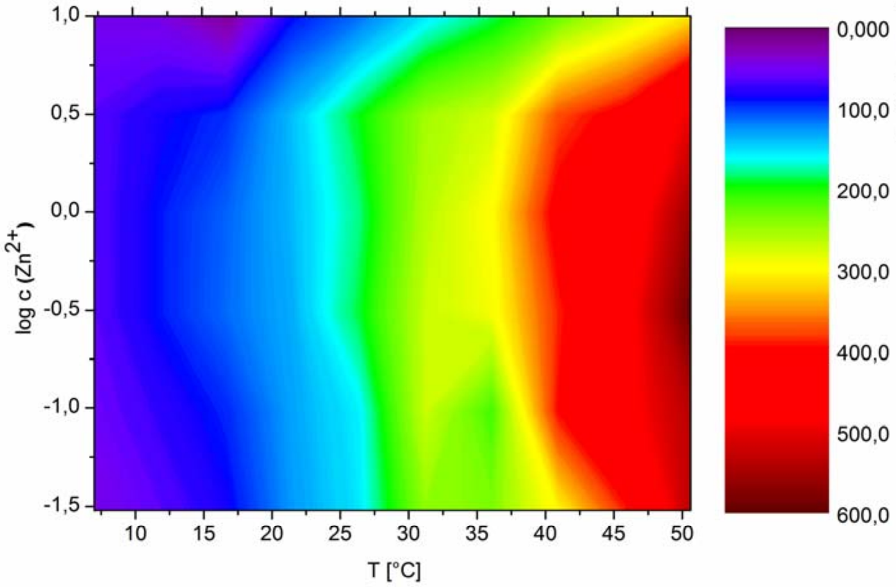
From: Argüello JM et al., 2007, Biometals, DOI 10.1007/s10534-006-9055-6

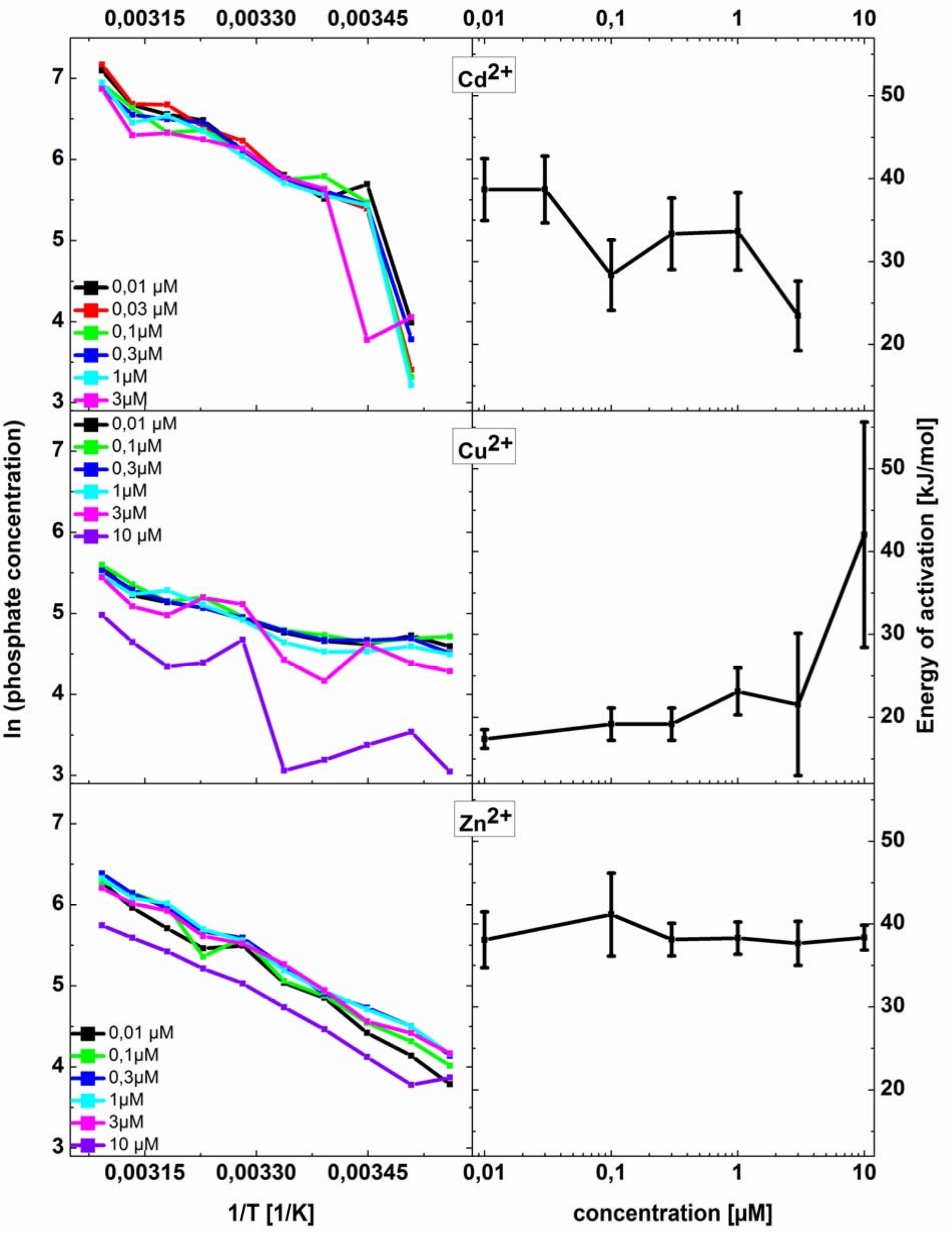


From: Wang K et al, 2014, Structure and mechanism of Zn-transporting P-type ATPases. Nature 514, 518-

Activation and substrate inhibition of TcHMA4

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

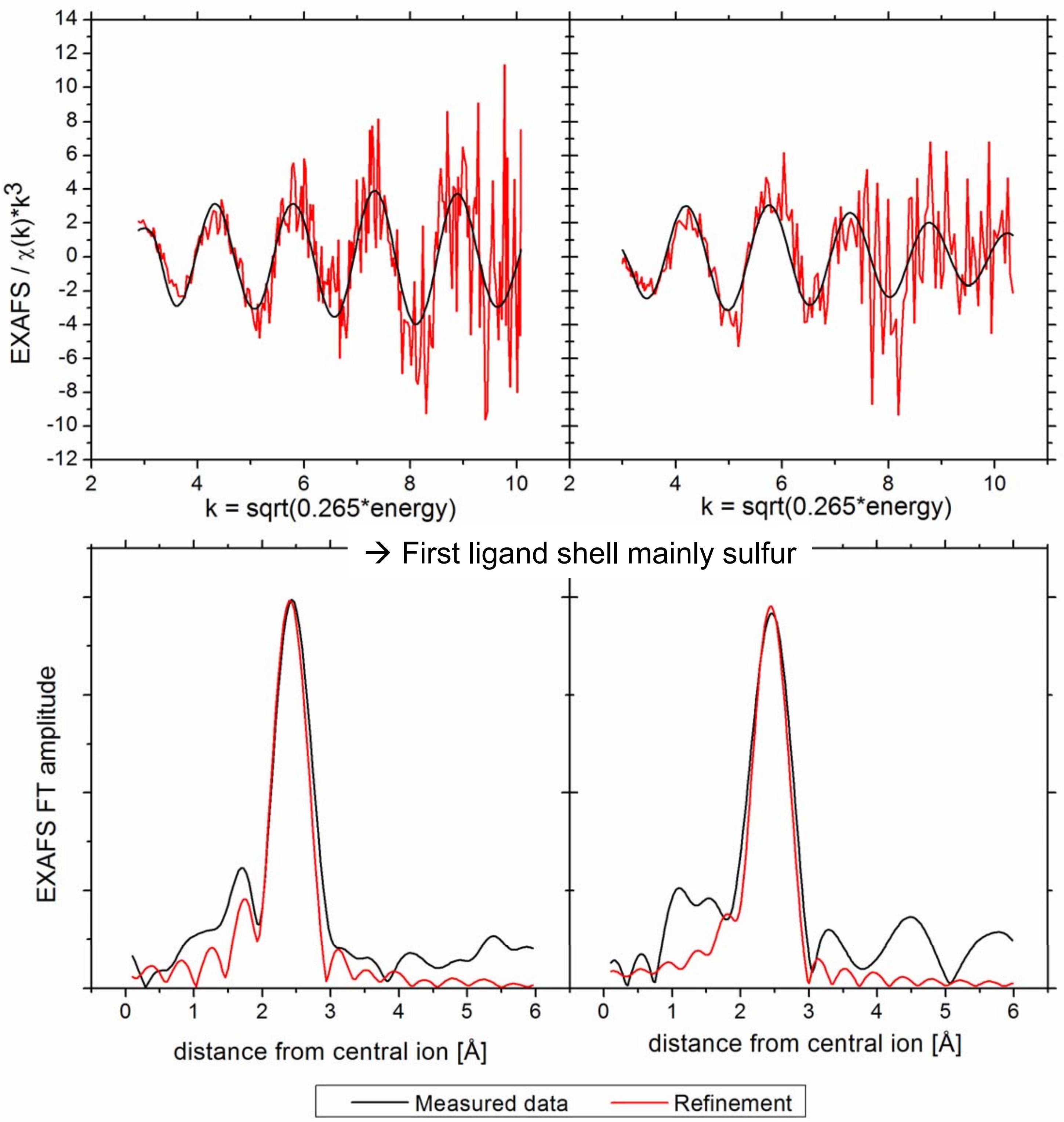




Leitenmaier B, Witt A, Witzke A, Stemke A, Meyer-Klaucke W, Kroneck PMH, Küpper H (2011) Biochimica et Biophysica Acta (Biomembranes) 1808, 2591-2599

EXAFS-analysis of TcHMA4

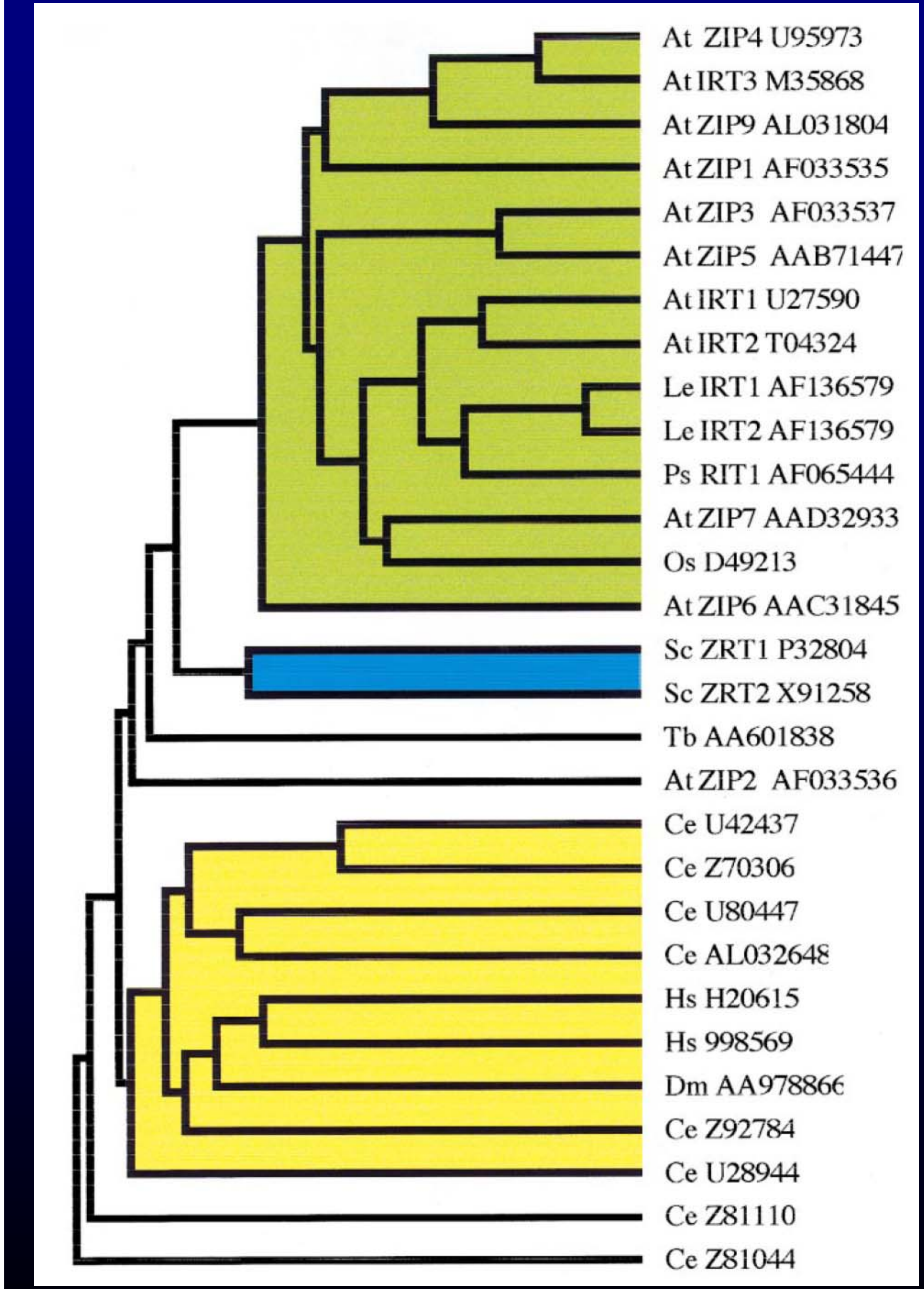
- at low Cd concentrations, the first ligand shell in this ATPase consists mainly of S (thiol groups from some of the 58 cysteines in the sequence)



→ First ligand shell mainly sulfur

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

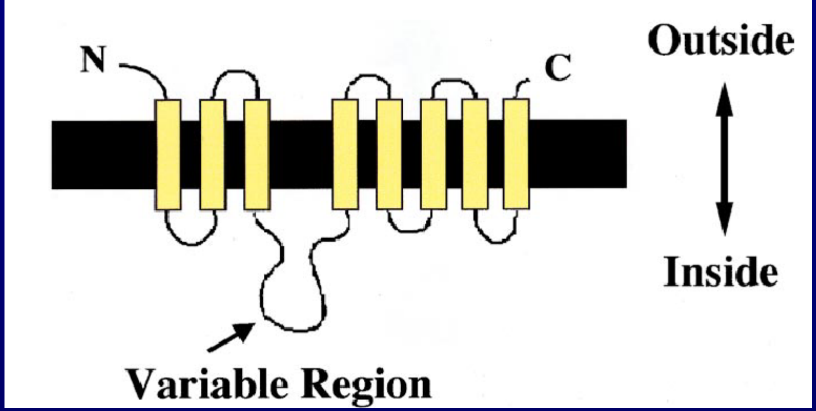


Likely Functions (deduced from expression studies)

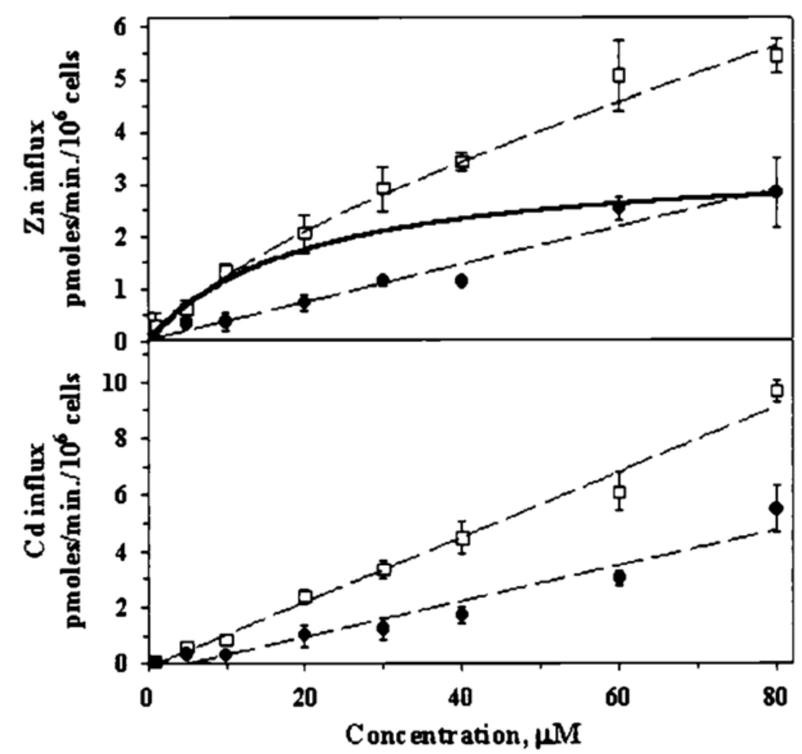
- uptake of metals into cells over the cytoplasmatic membrane
- abundant in all eucaryotes, incl. humans, plants and fungi

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



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

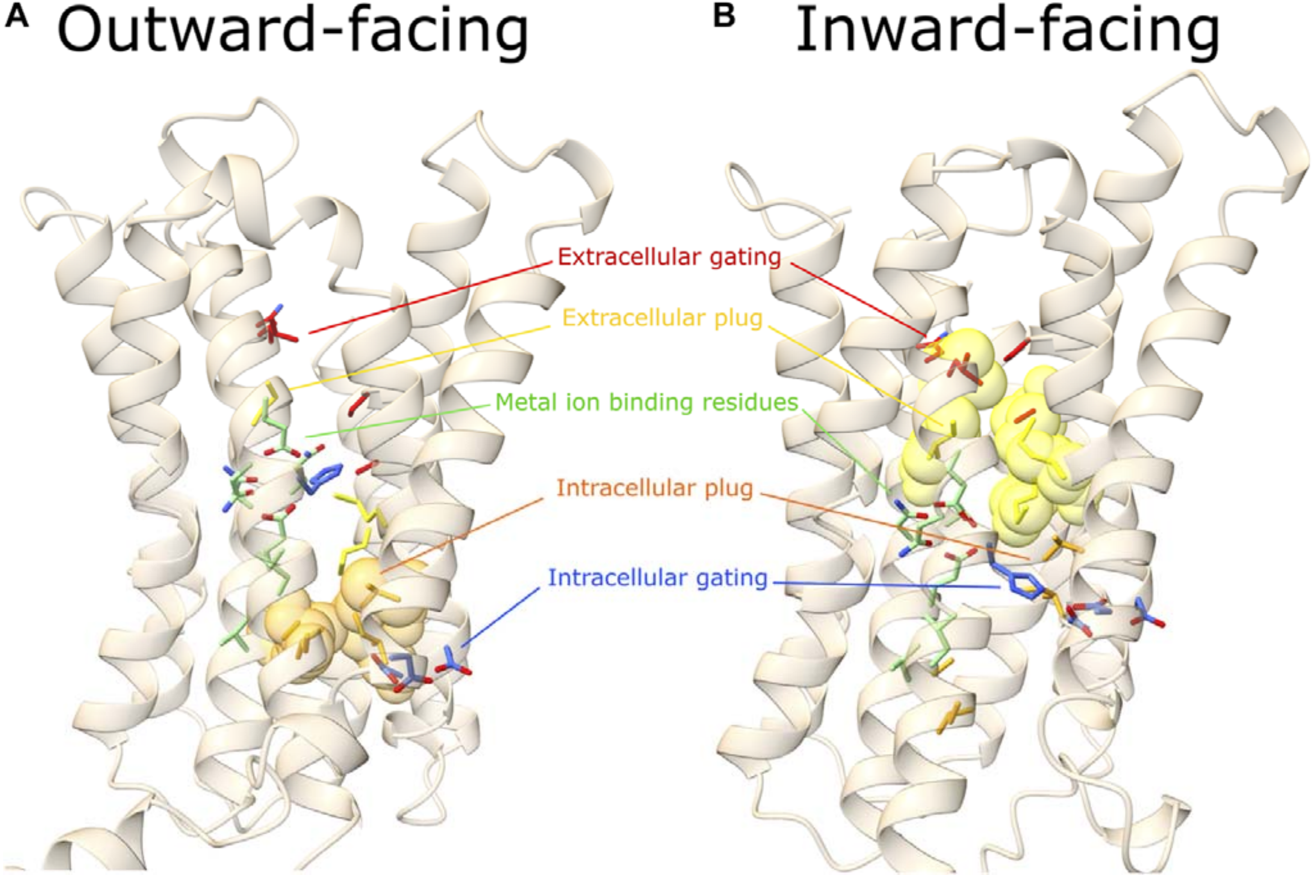
Functions suggested by expression studies)

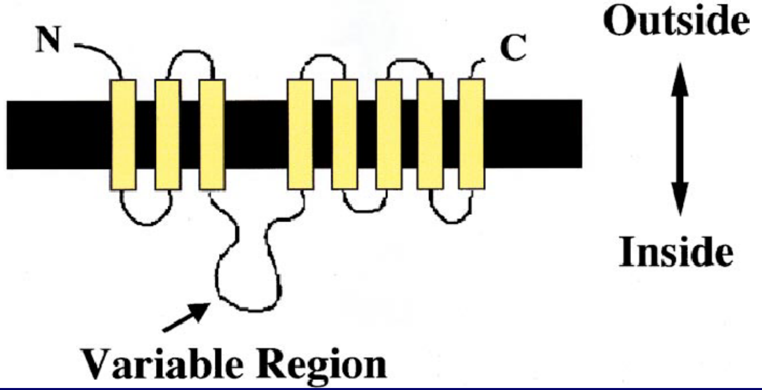
- uptake of metals into cells over the cytoplasmatic membrane
- abundant in all eucaryotes, incl. humans, plants and fungi

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) ZIP-transporters





Plant ZIPs – general structure
From:Guerinot ML, 2000, BBA 1465, 190-8

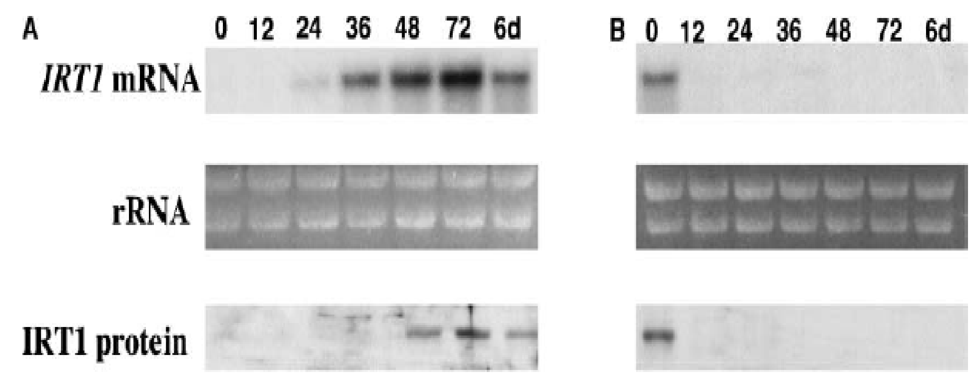
Structure of a human ZIP transporter as predicted by AlphaFold
From: Pasquadibisceglie A, Leccese A and Polticelli F (2022) Front. Chem. 10:1004815. doi: 10.3389/fchem.2022.1004815

Structure predicted by sequence

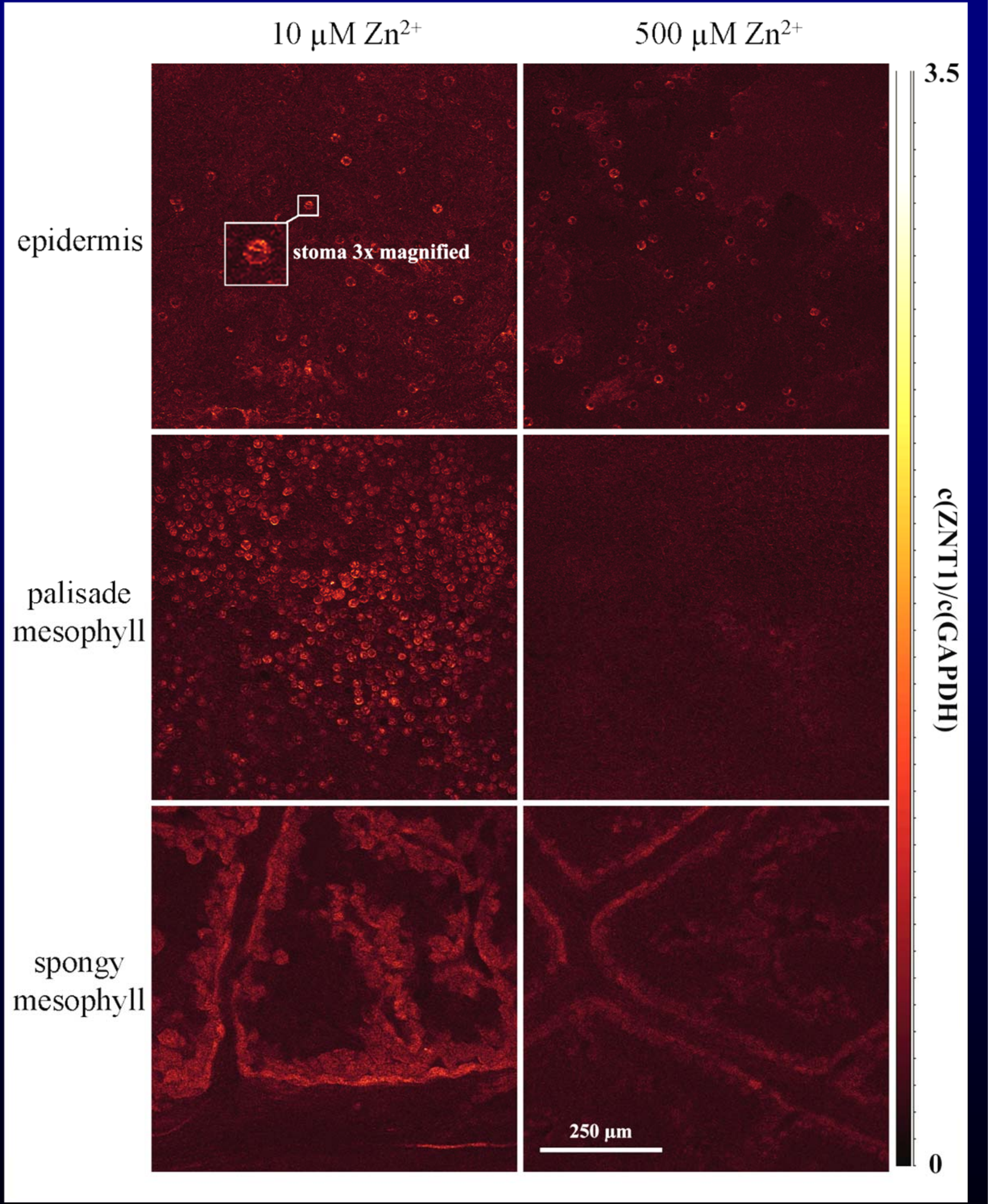
- usually 8 transmembrane helices, one long variable region, predicted to be in the cytoplasm
- 309-476 amino acids
- still no complete 3D structure available – only predictions e.g. via AlphaFold
- Mechanism according to predicted structure: change between “outward-facind” vs. “inward-facing” conformation of central channel: “elevator-type transport mechanism”

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

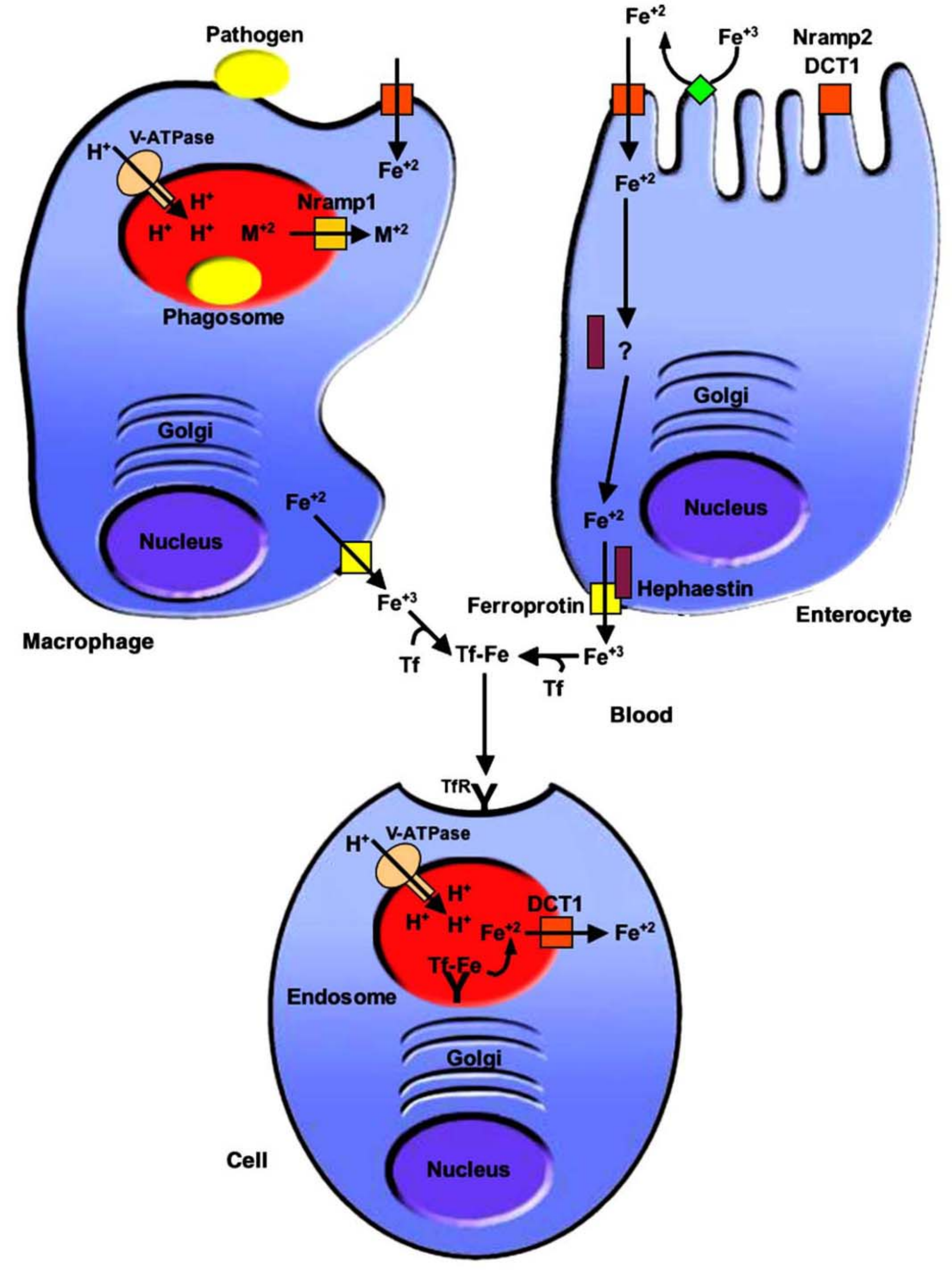


Expression pattern

- 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)

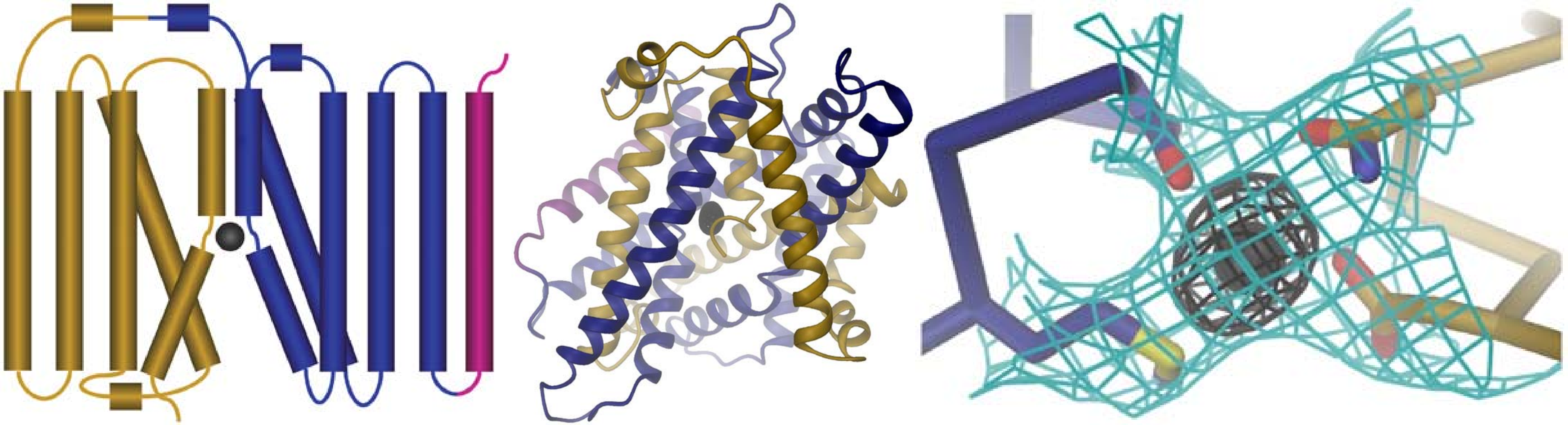


Likely Functions (deduced from expression studies)

- ➢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

From: Nevo Y, Nelson N, 2006, BBA 1763, 609-620

Mechanisms of metal uptake in plants (III)
Natural resistance associated macrophage proteins (Nramps)

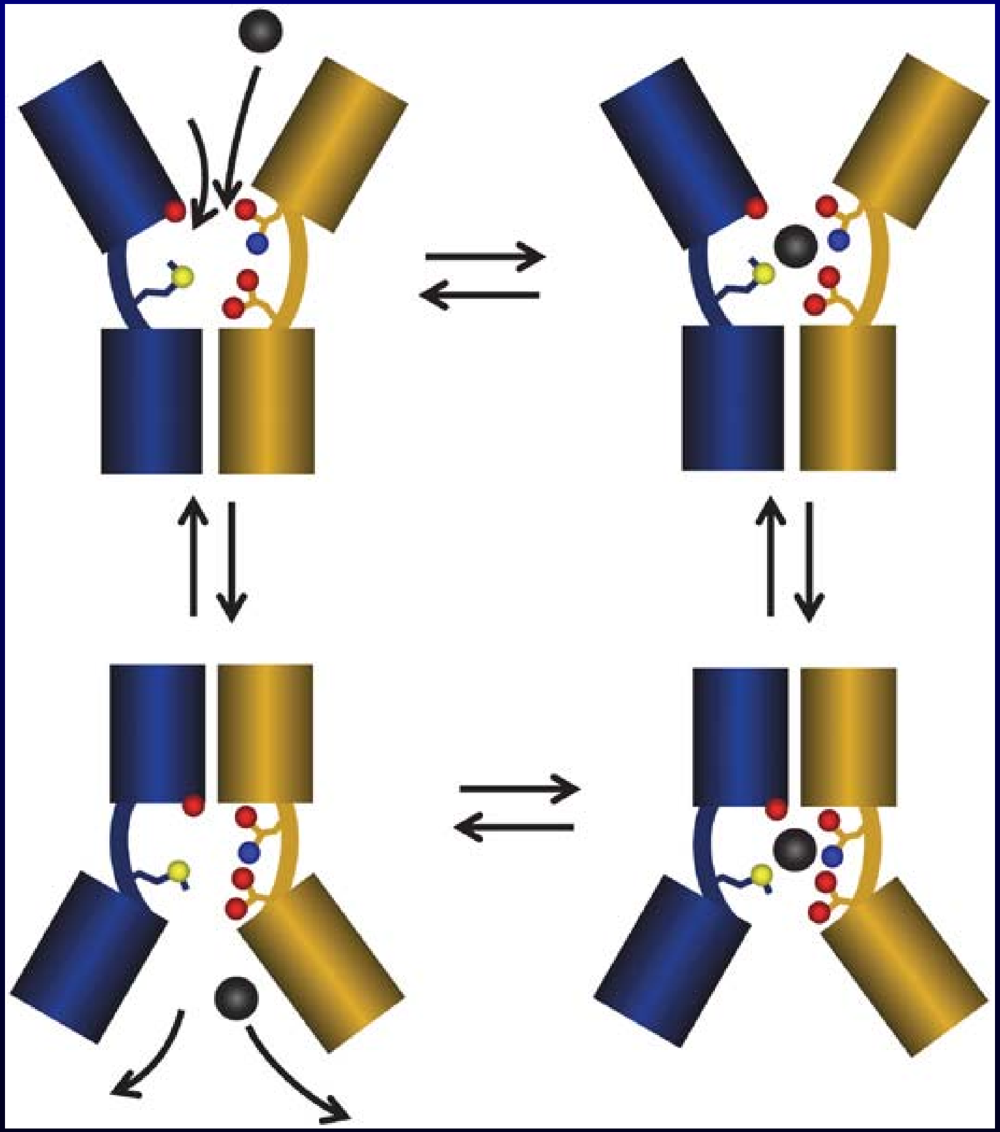


From: Ehrnstorfer IA, Geertsma ER, Pardon E, Steyaert J, Dutzler R. Nat Struct Mol Biol. 21, 990-6

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)

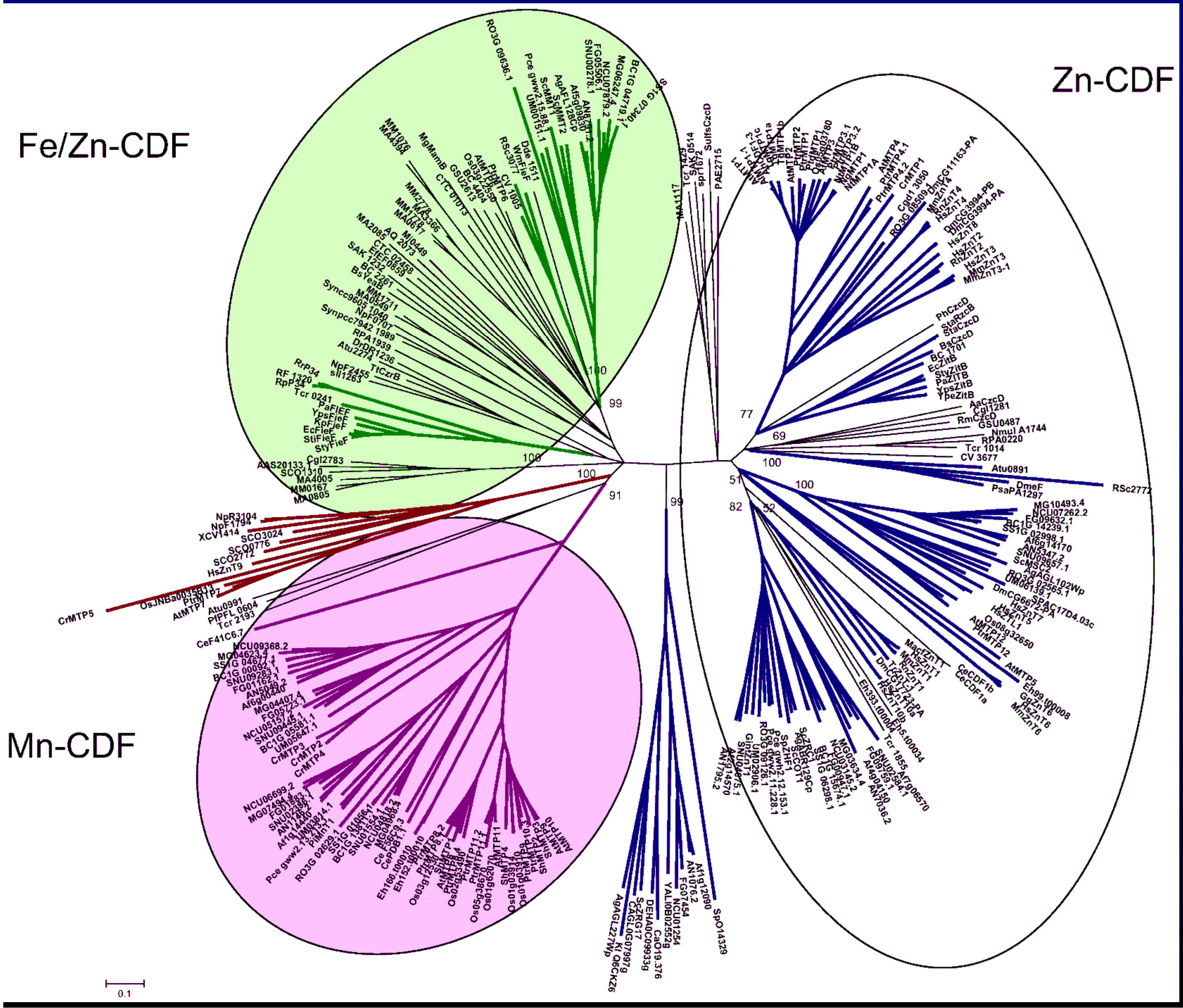


From: Ehrnstorfer IA, Geertsma ER, Pardon E, Steyaert J, Dutzler R. 2014. Nat Struct Mol Biol. 21, 990-6

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.

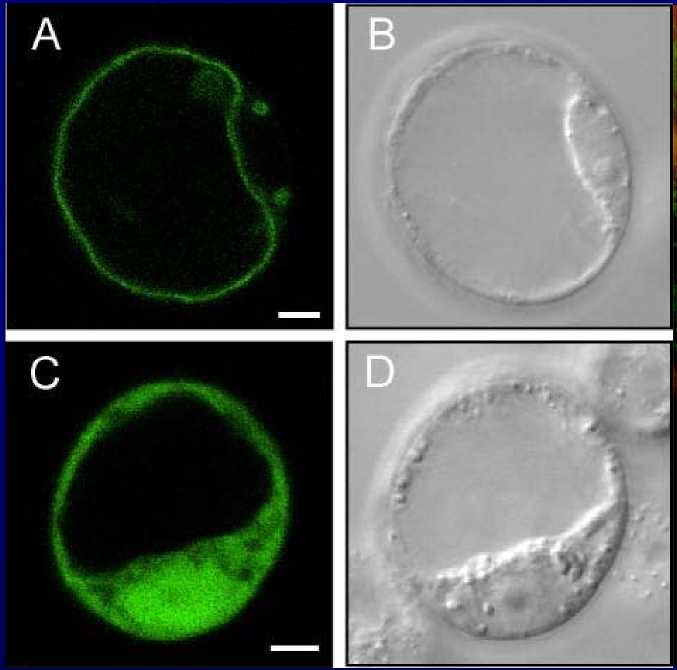
Mechanisms of metal uptake in plants (IV): Cation diffusion facilitator (CDF)-transporters



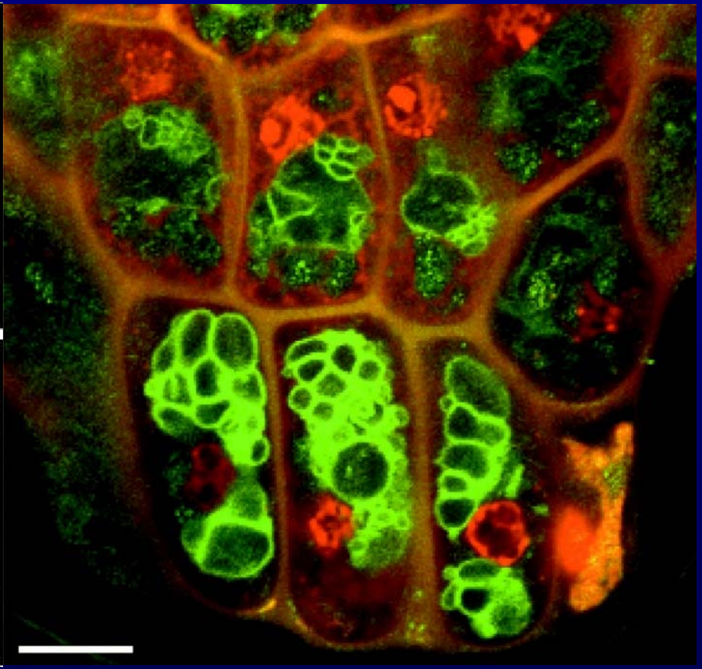
- abundant in all eucaryotes, incl. humans, plants and fungi
- different subfamilies

From: Montanini B et al., 2007, BMCGenomics 8, 107

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



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

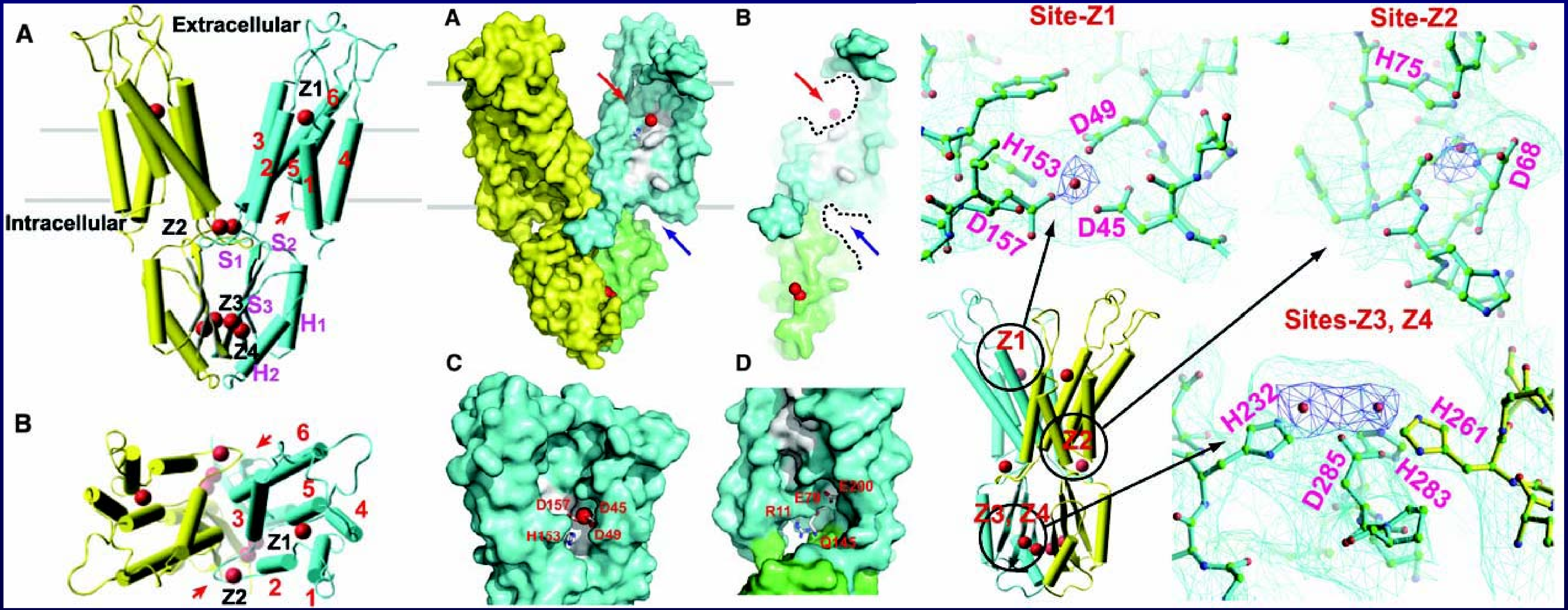


From: Blaudez D et al., 2003,
PlantCell 15, 2911-28

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

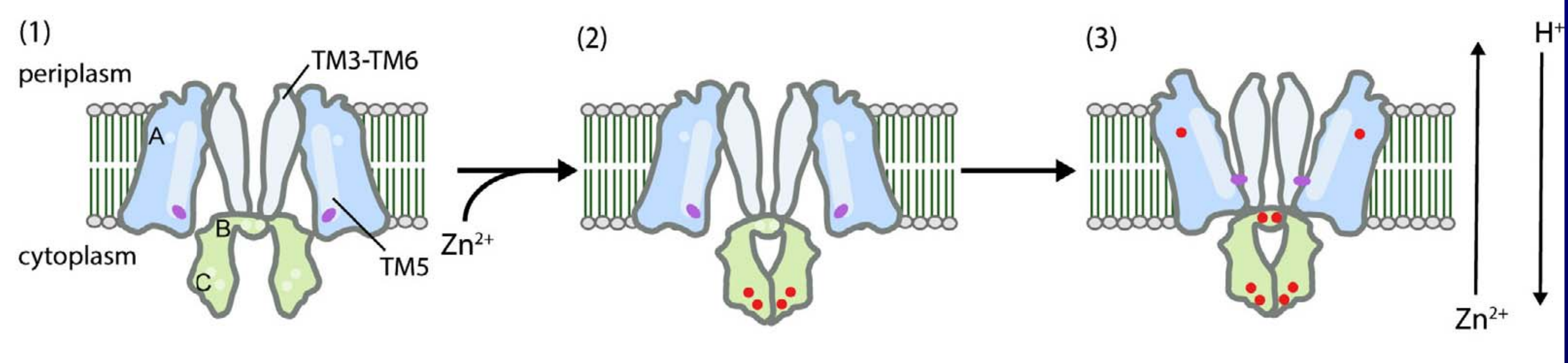


From: Lu M, Fu D. 2007. Structure of the zinc transporter YiiP. Science 317, 1746-8

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

- MANY histidines in sequence used for metal binding
- 6 transmembrance 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

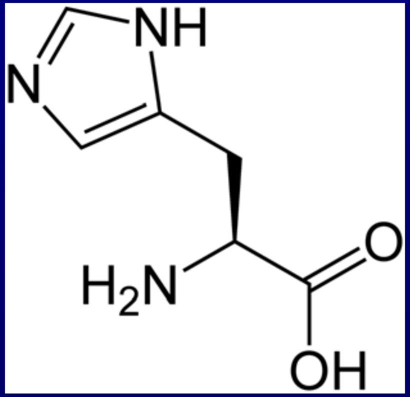


From: Kolaj-Robin O, Russell D, Hayes KA, Pembroke JT, Soulimane T. 2015. FEBS Lett. 589, 1283-95

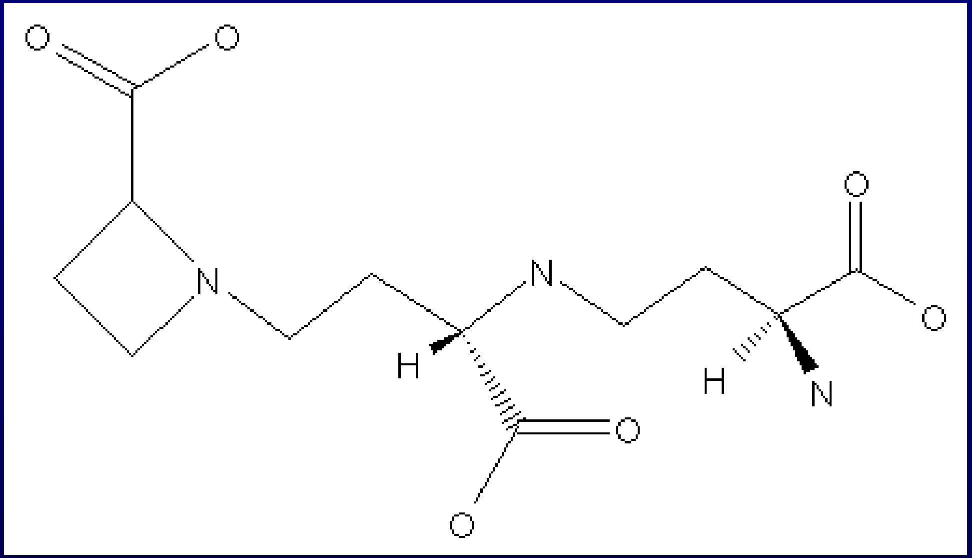
Mechanism concluded from structure and kinetic studies

- Proton-metal antiport
- The exact movements are still discussed as the only available complete crystal structure is rather low resolution (3.8A)
- 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

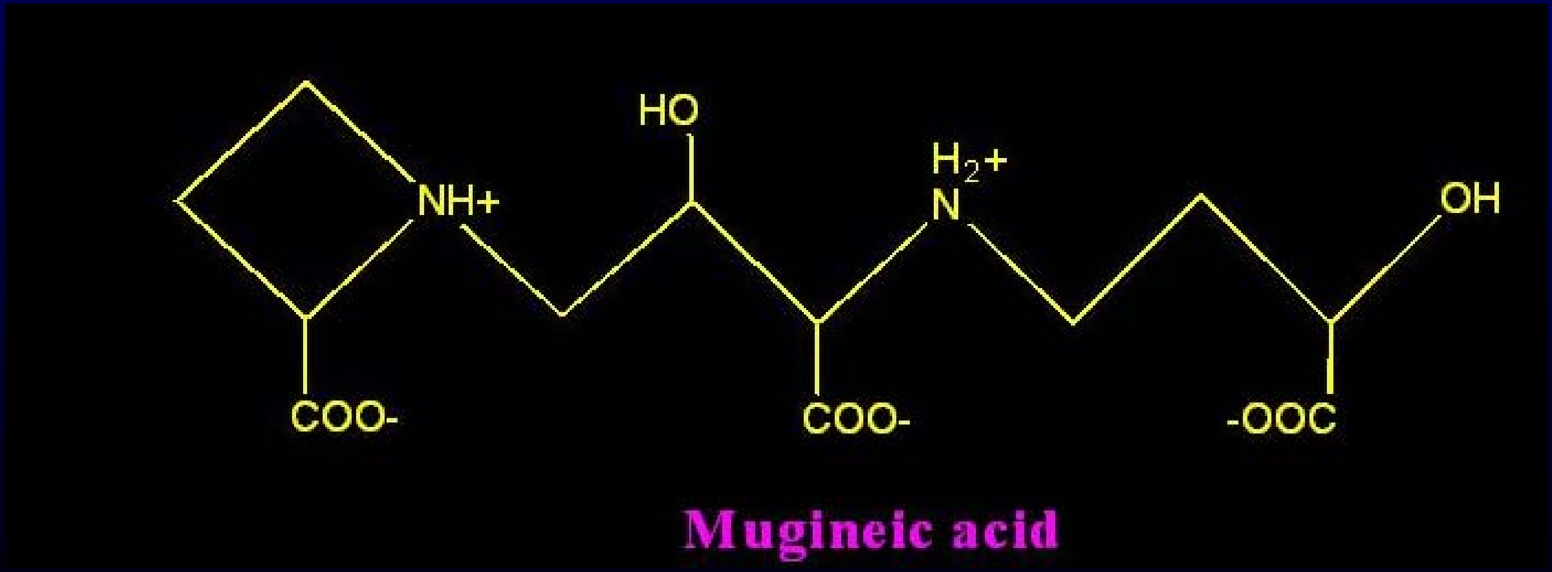
Mechanisms of metal uptake in plants (V): Long-distance transport ligands



Histidine

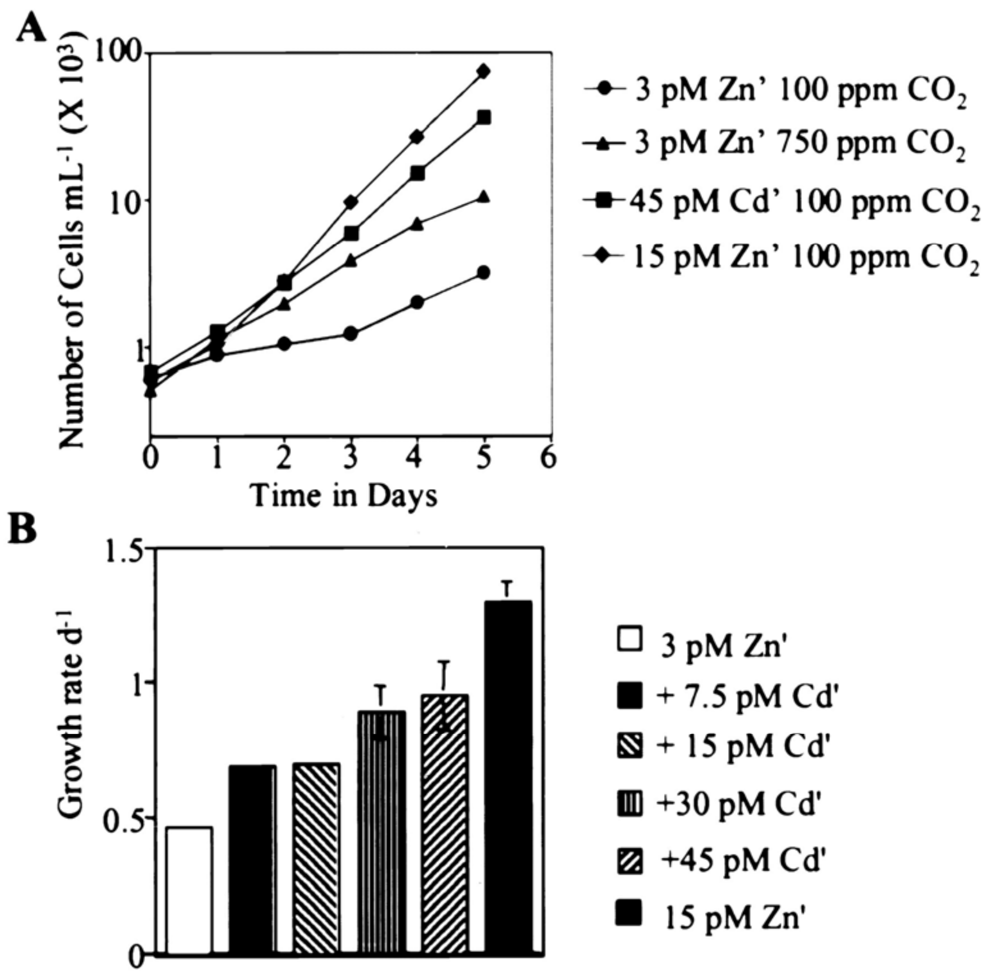


Nicotianamine



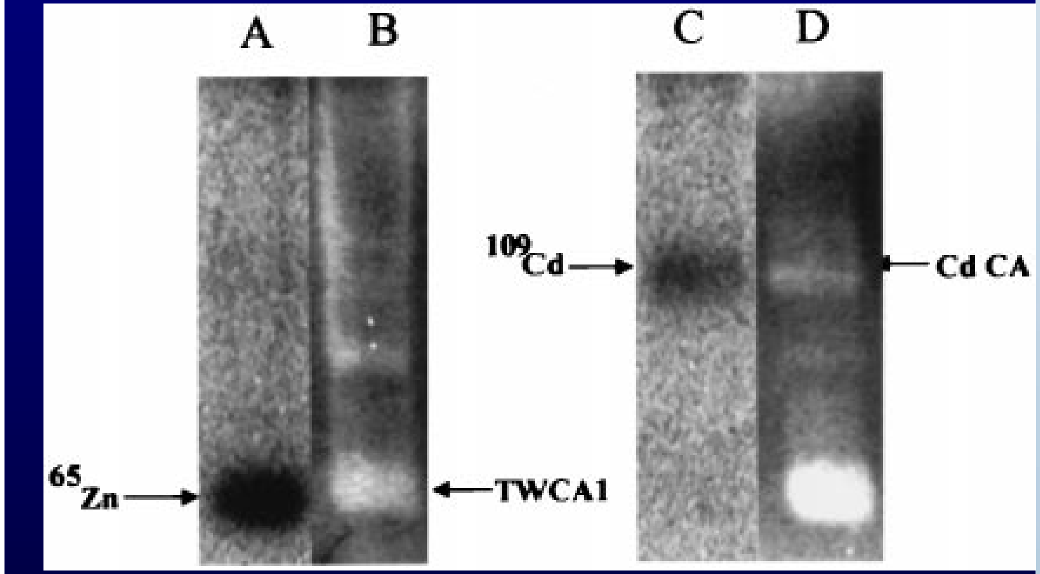
Now let's have a look at a few plant micronutrients

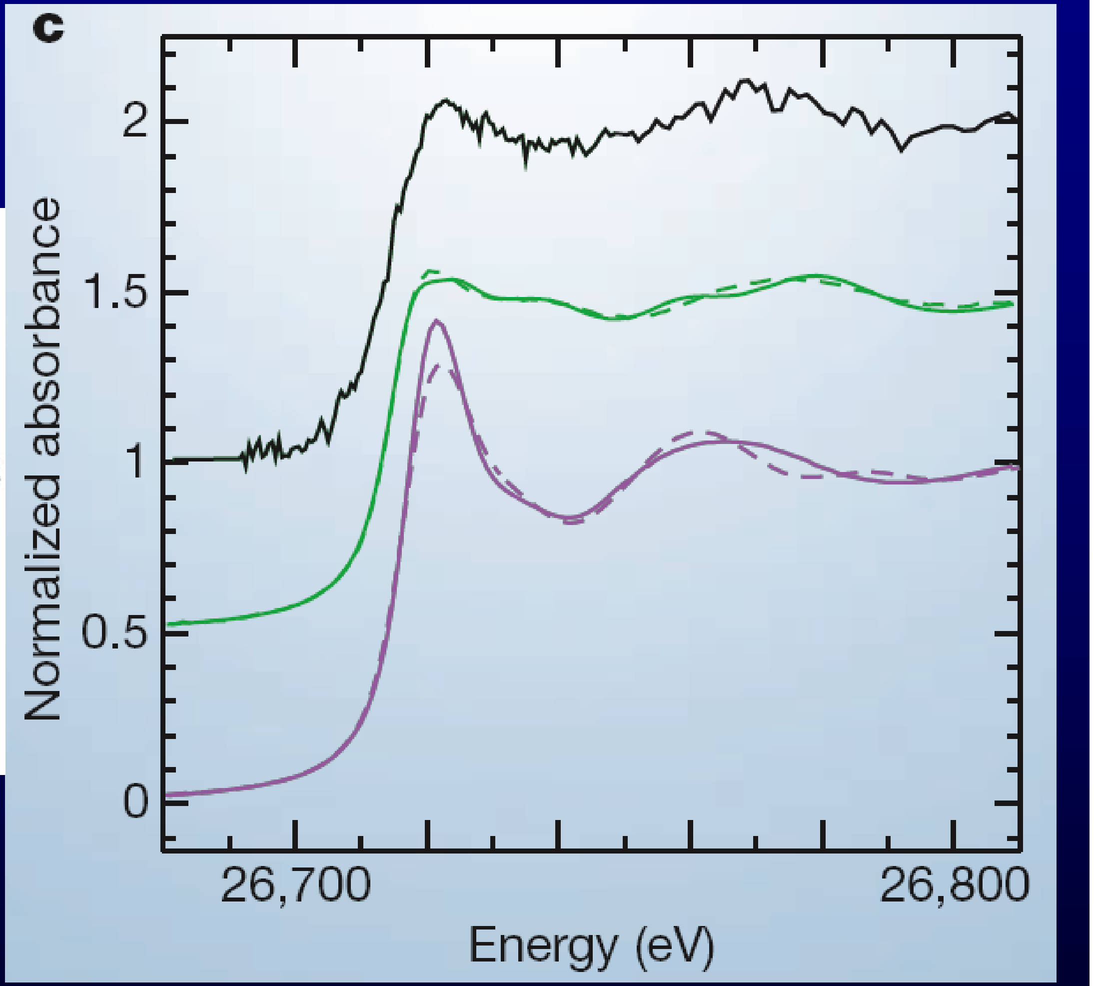
Cadmium as a micronutrient



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



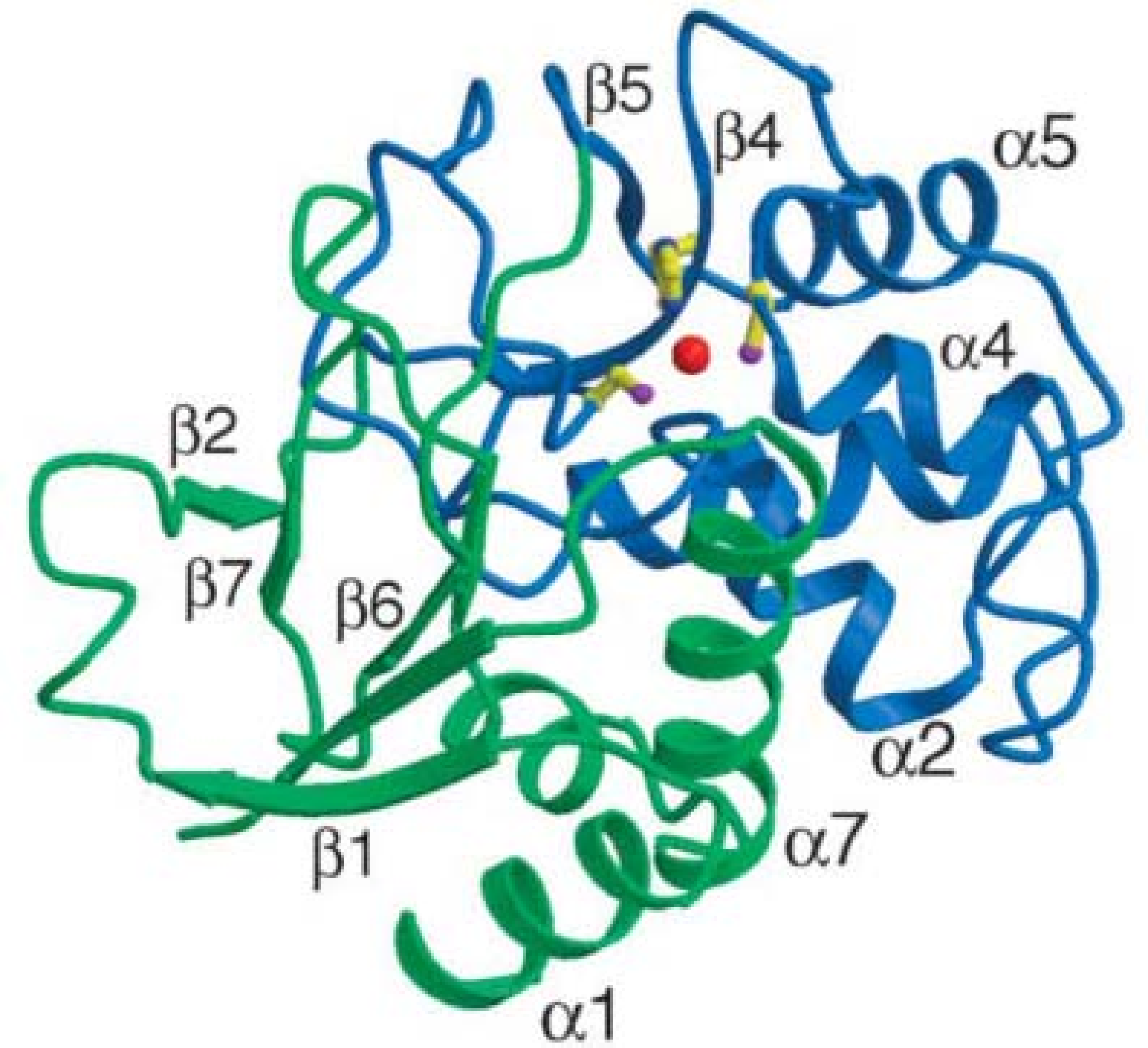


Size of the cadmium-carboanhydrase in comparison to the normal Zn-carboanhydrase (Lane and Morel, 2000, PNAS Vol. 97)

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
- Cd^{2+} is bound via three conserved amino acid residues: 2x cystein and 1x histidin, plus 1x Water (→ tetrahedral coordination). Further fixed water molecules nearby

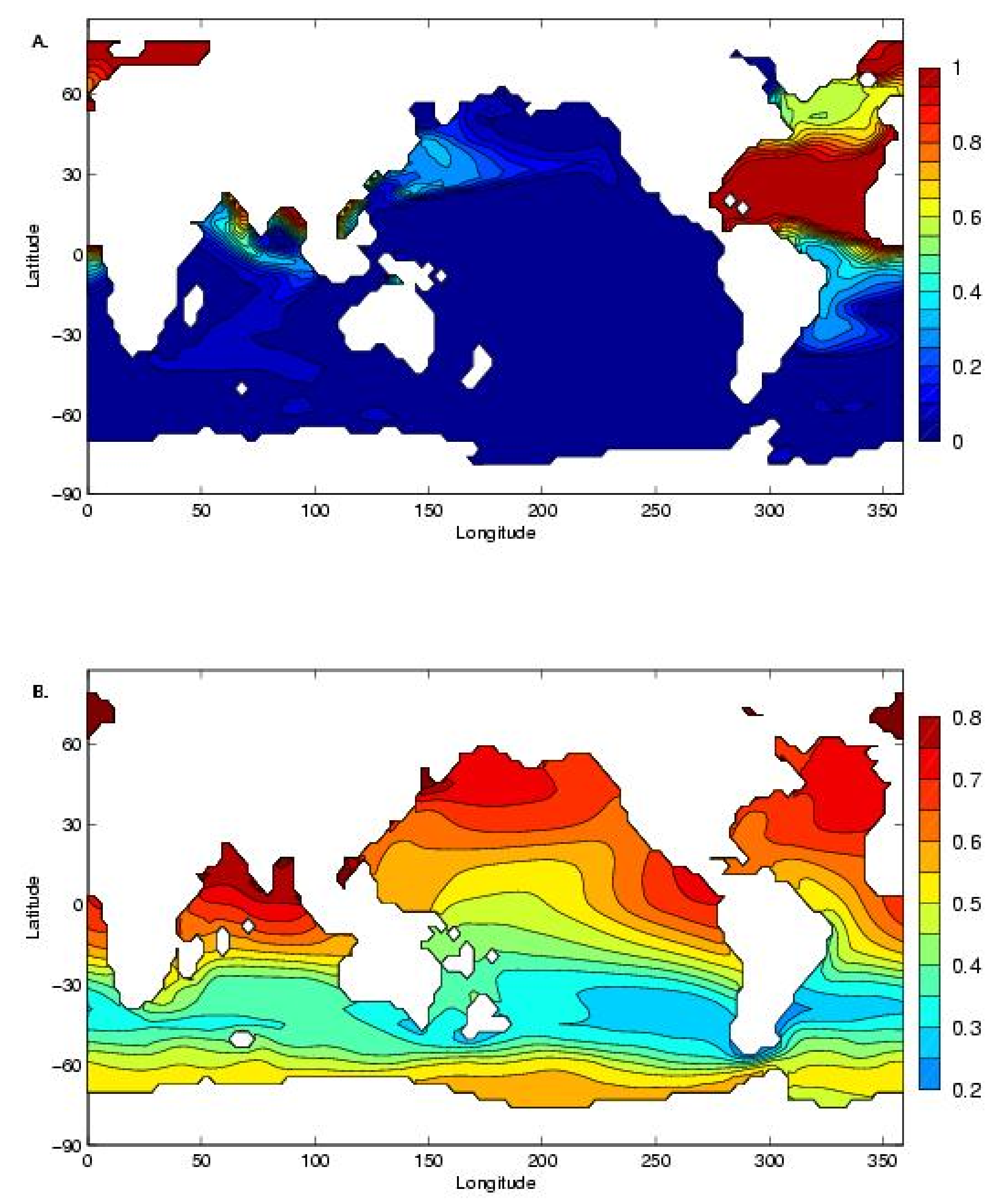
Cadmium deficiency in the Cd/Zn-hyperaccumulator *Thlaspi caerulescens*

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

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

Küpper H, Kroneck PMH (2004) MIBS 44 (Sigel et al., eds), chapter 5

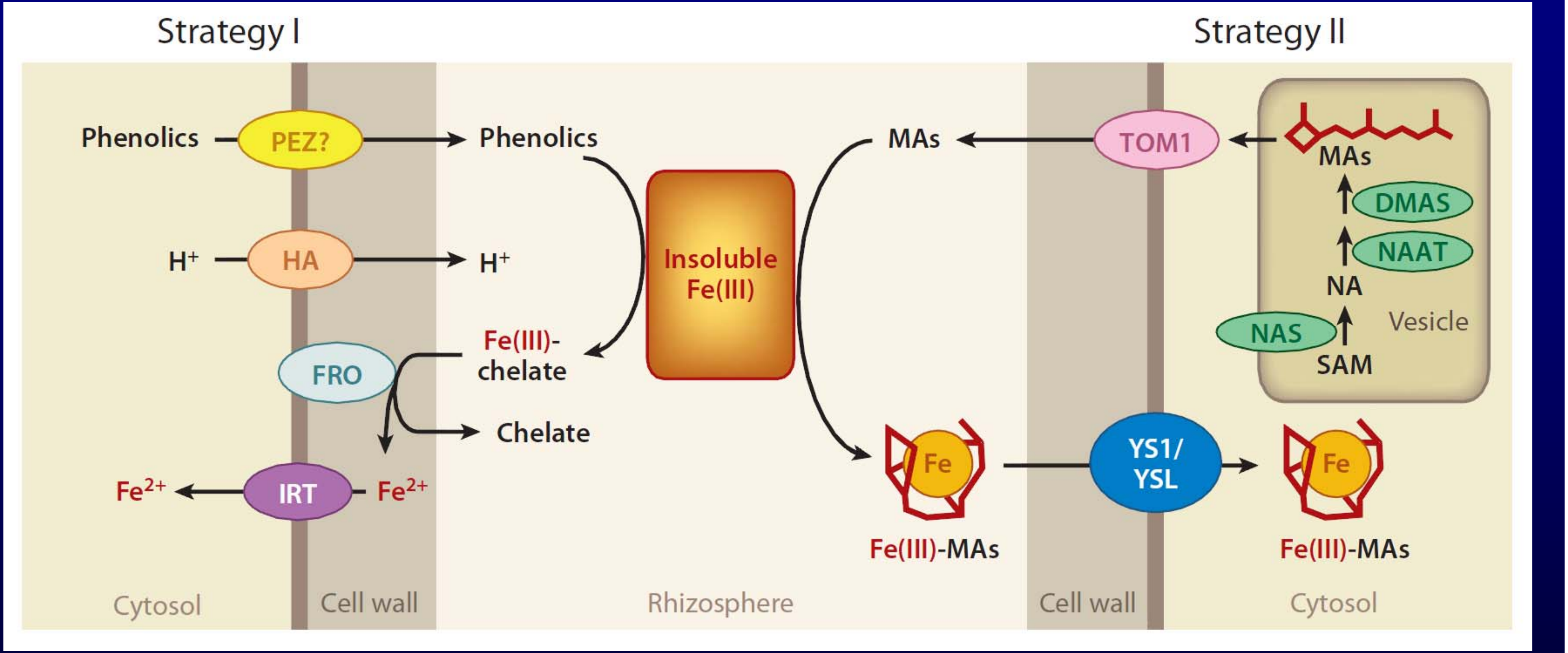
Iron



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

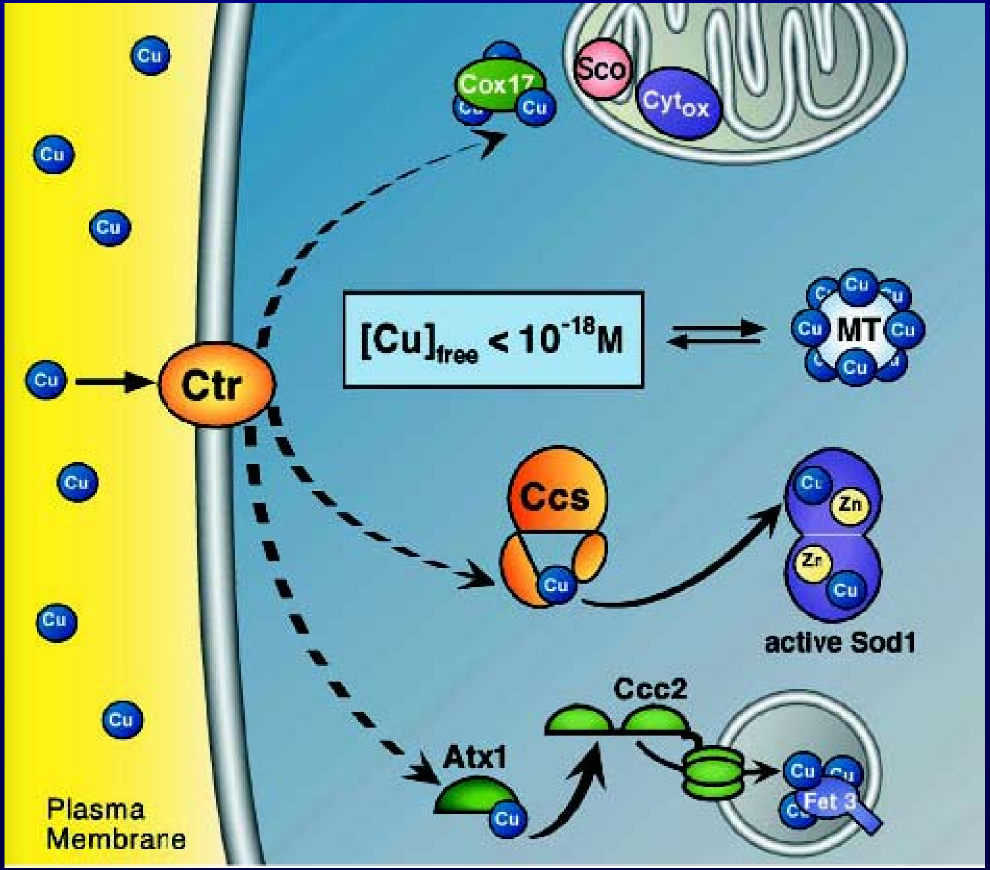


From: Kobayashi T, Nishizawa NK. 2012. Ann Rev Plant Biol 263, 131-152

Strategies of making insoluble Fe(III) bioavailable

- Strategy I (most plants): use mostly of soil acidification and iron reductase at root surface
- Strategy II (not only grasses): use of secreted iron ligand mugineic acid

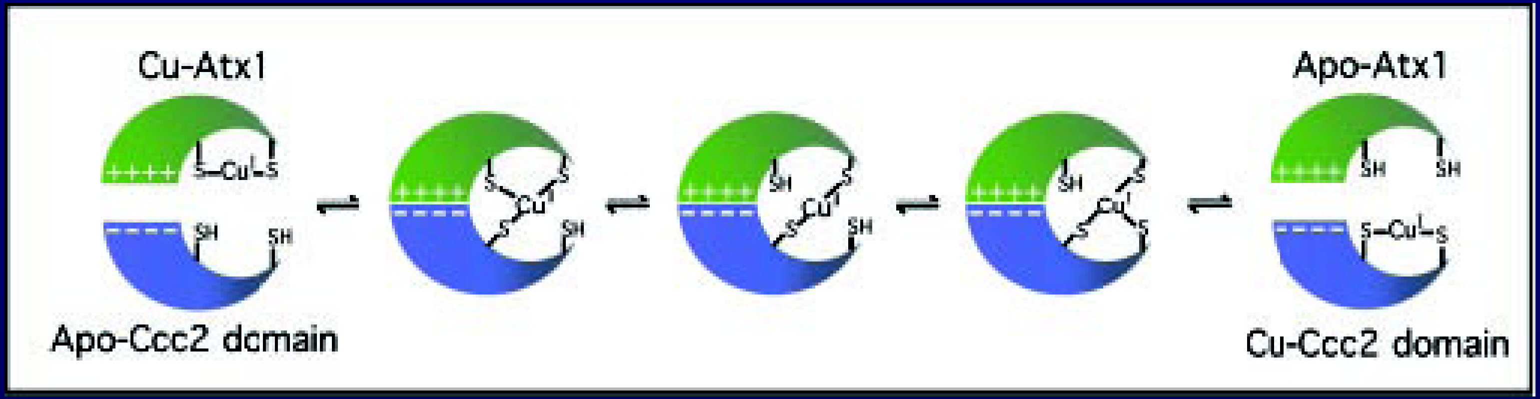
Copper delivery inside cellular compartments



From: OHalloran 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 ≈ CCH
(a) occurrence

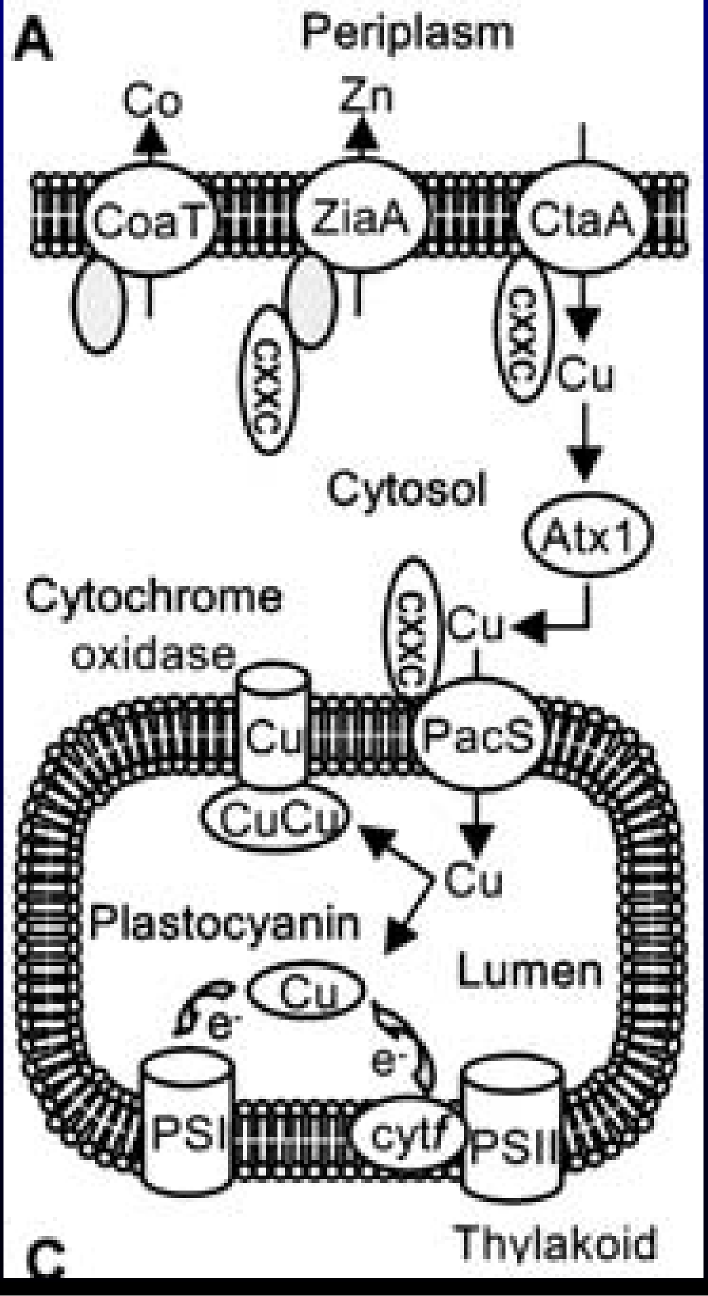


From: OHalloran TV, Culotta VC, 2000, JBC275, 25057-60

- 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

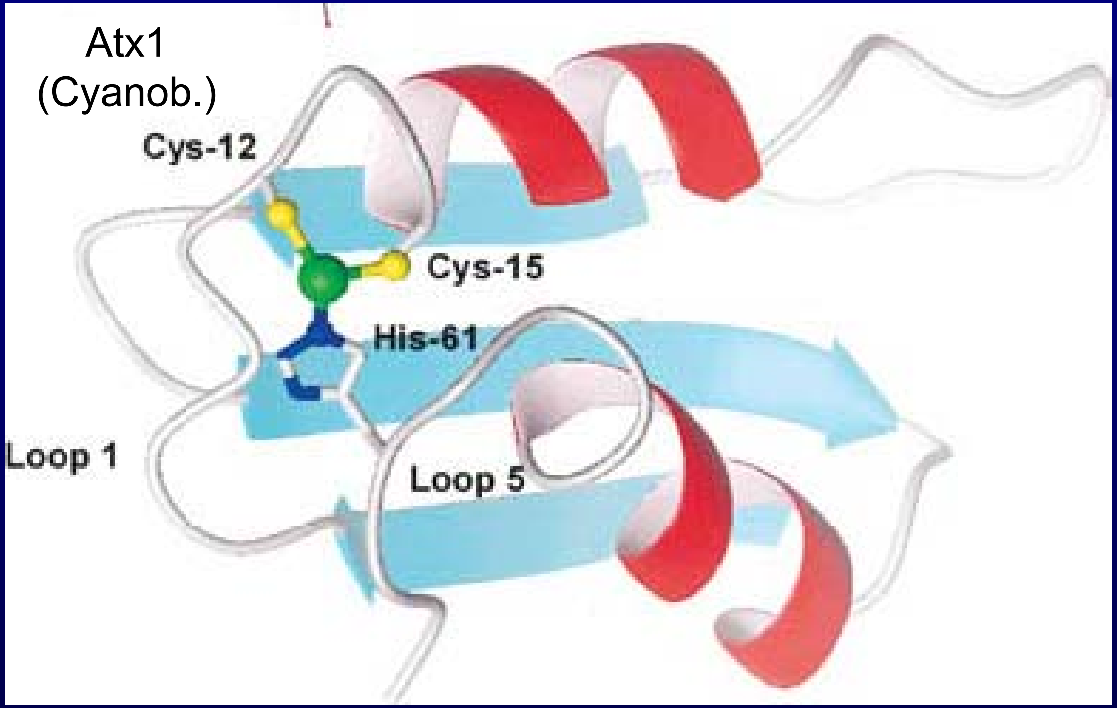
Copper delivery to the Golgi and thylakoid:
ATX1 = HAH1 = ATOX1 = CopZ
(b) function in bacteria, cyanobacteria+plants

- 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



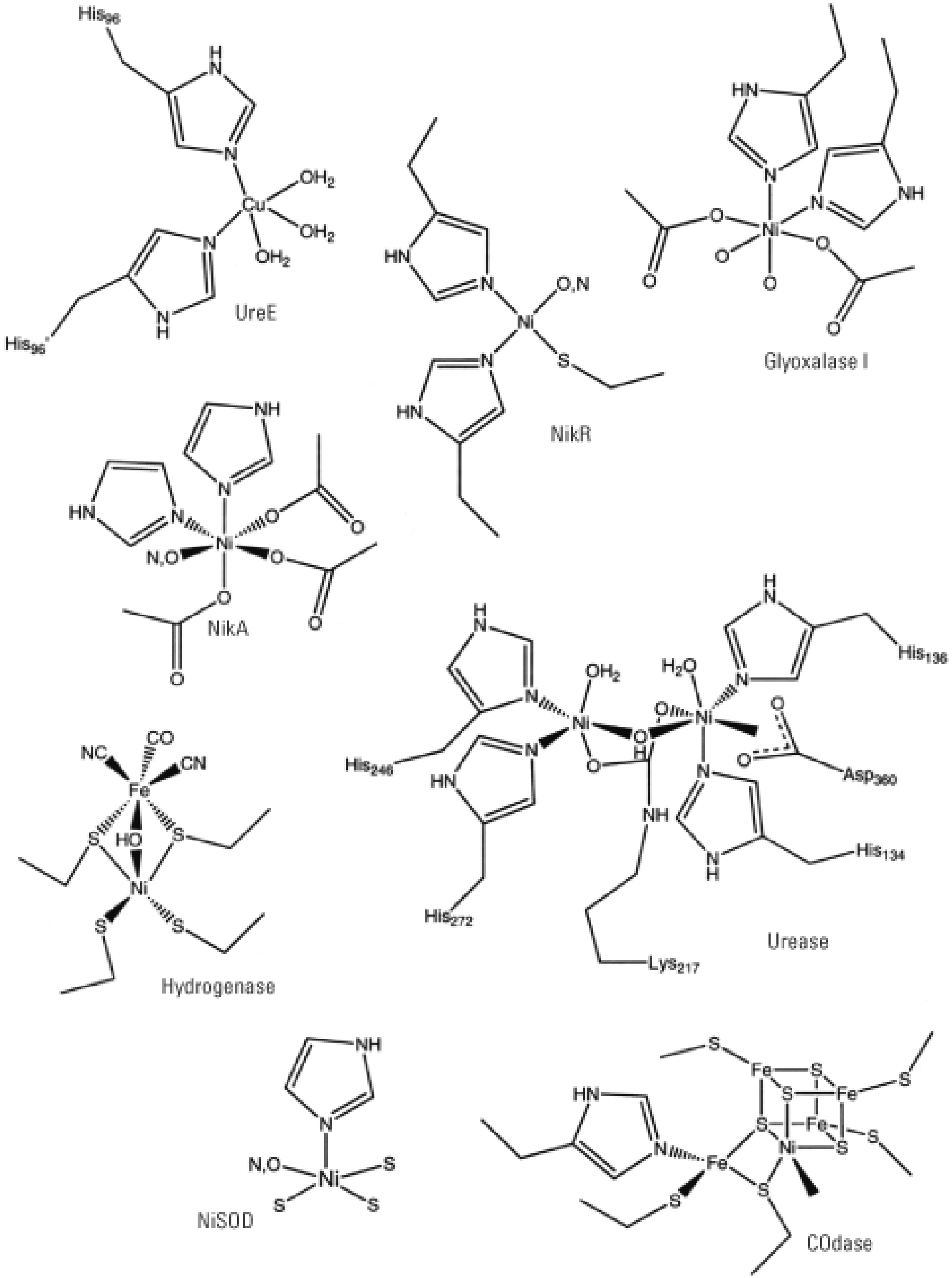
From: Borrelly GPM et al., 2004, BiochemJ378, 293-7

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



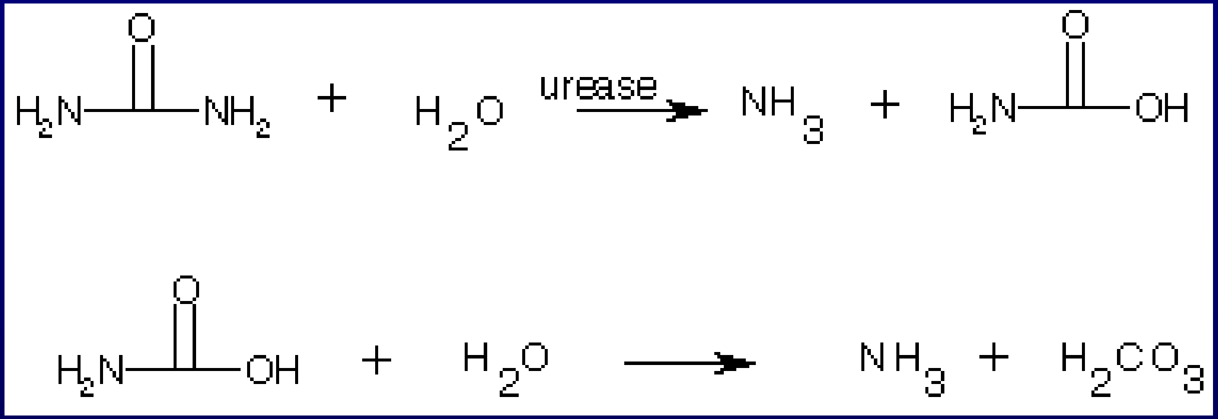
Examples of nickel complexes in proteins

Characteristics

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

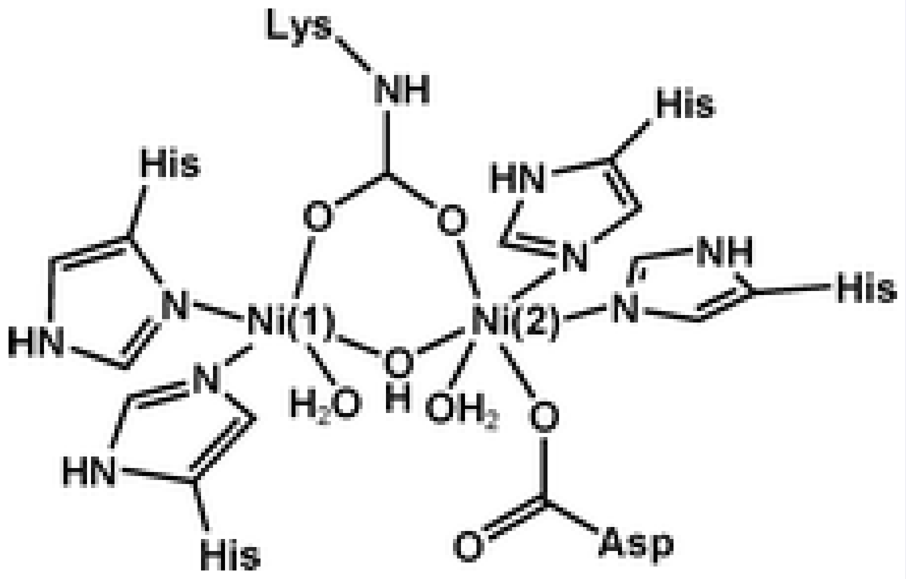
From:
Carrington PE et al, 2002, EnvHealthPers110, 705-

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

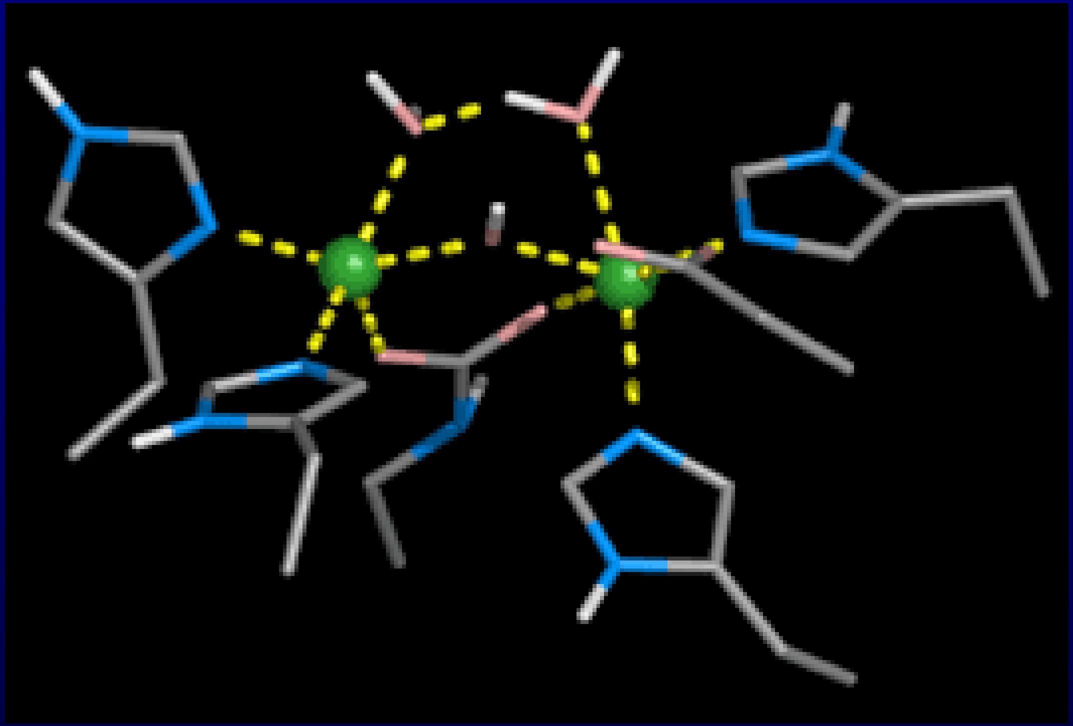


- Catalyses the decomposition of urea into carbon dioxide and ammonia
- In most organisms (plants, fungi, bacteria)
- Very important for metabolism: urea toxicity is one of the main mechanisms of damage caused by nickel deficiency in plants
- Very specific for urea as a substrate
- Rather fast: turnover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

Urease
b) Active site



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

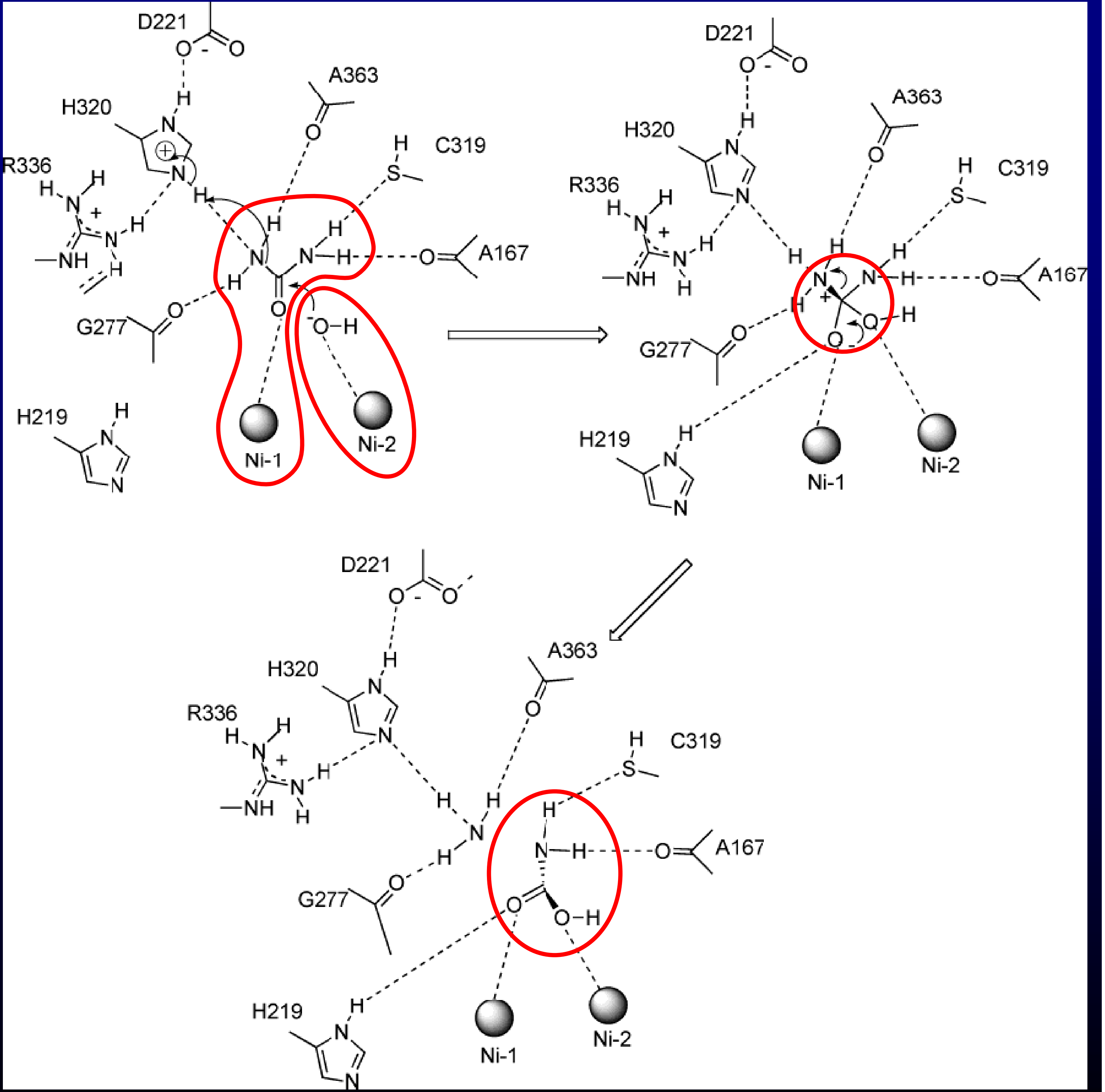


- two Ni^{2+} ions
- one Ni^{2+} bound by three fixed ligands (2 His-N and 1 Lys-O) and one water
- the other Ni^{2+} bound by four fixed ligands (2 His-N, 1 Asp-O and 1 Lys-O) and 1 water
- the two nickels are bridged by a water molecule

Urease
c) Mechanism

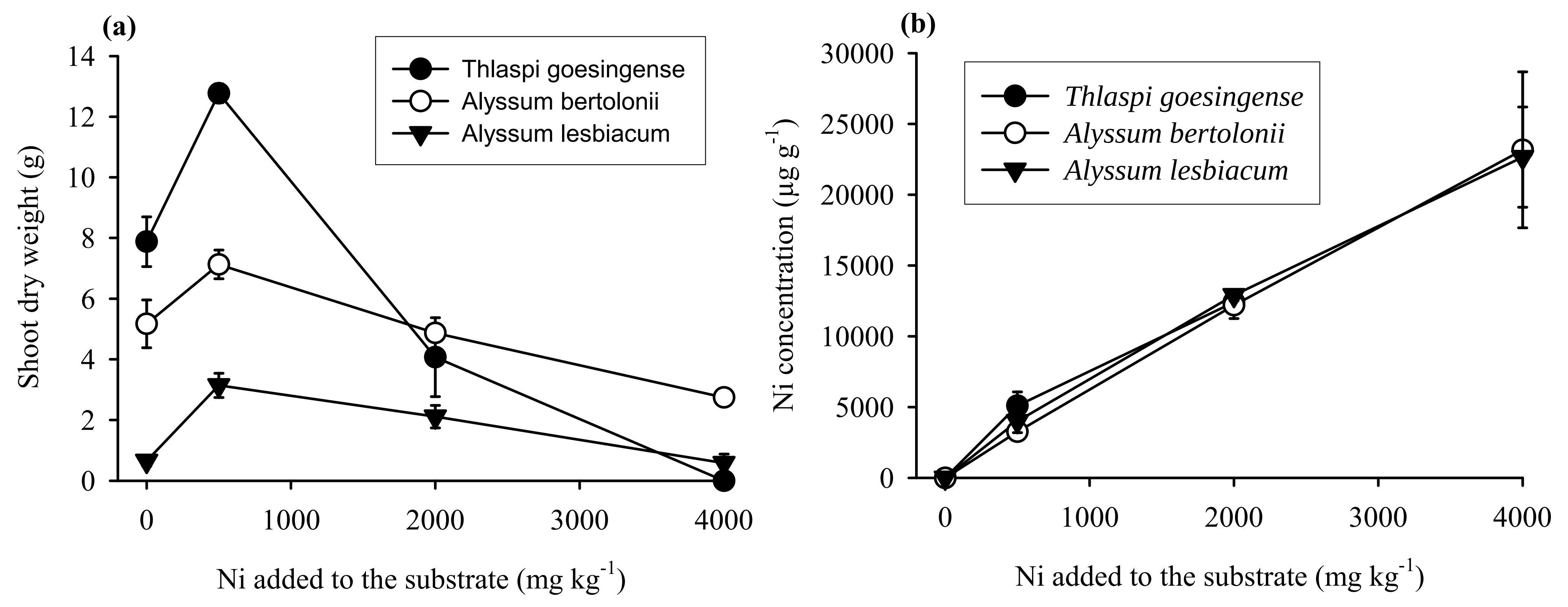
Steps

- Nickel1 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

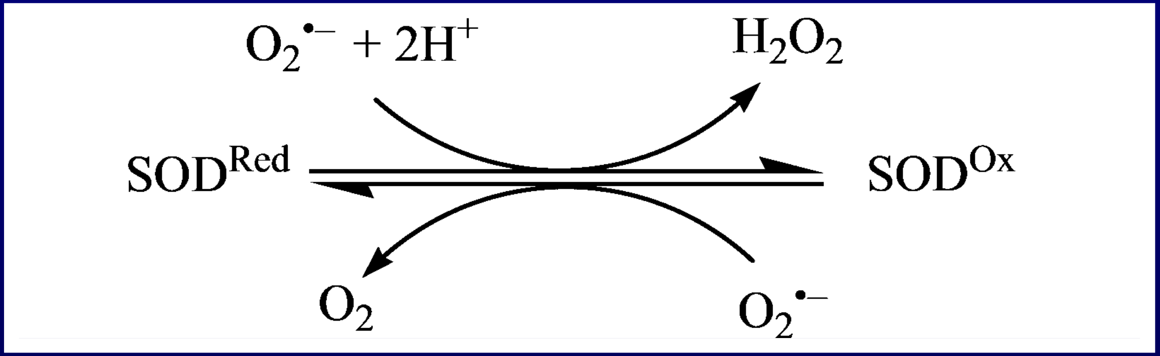
Nickel deficiency in Ni-Hyperaccumulators



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



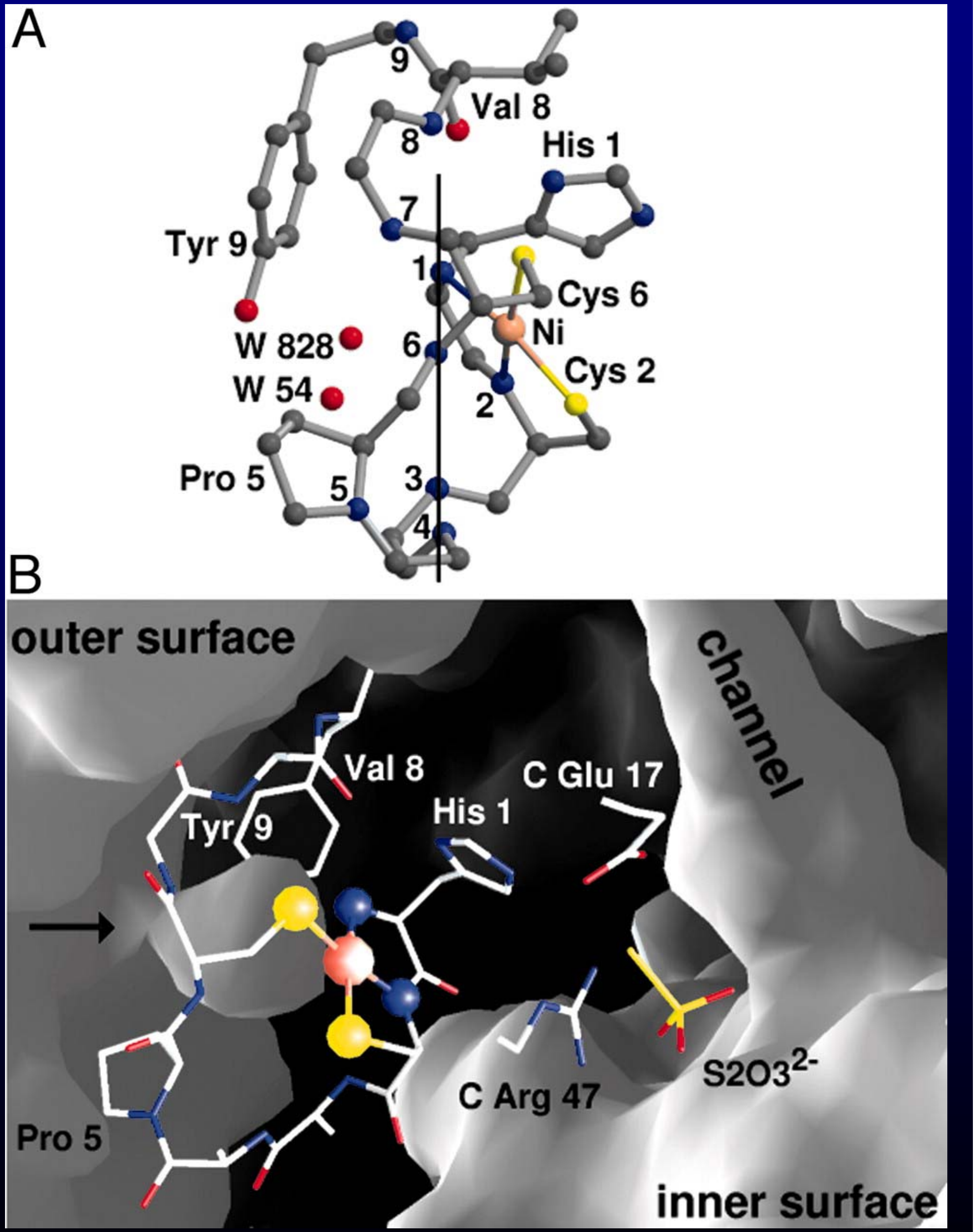
Characteristics

- Catalyses the detoxification of superoxide (O_2^-) 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!

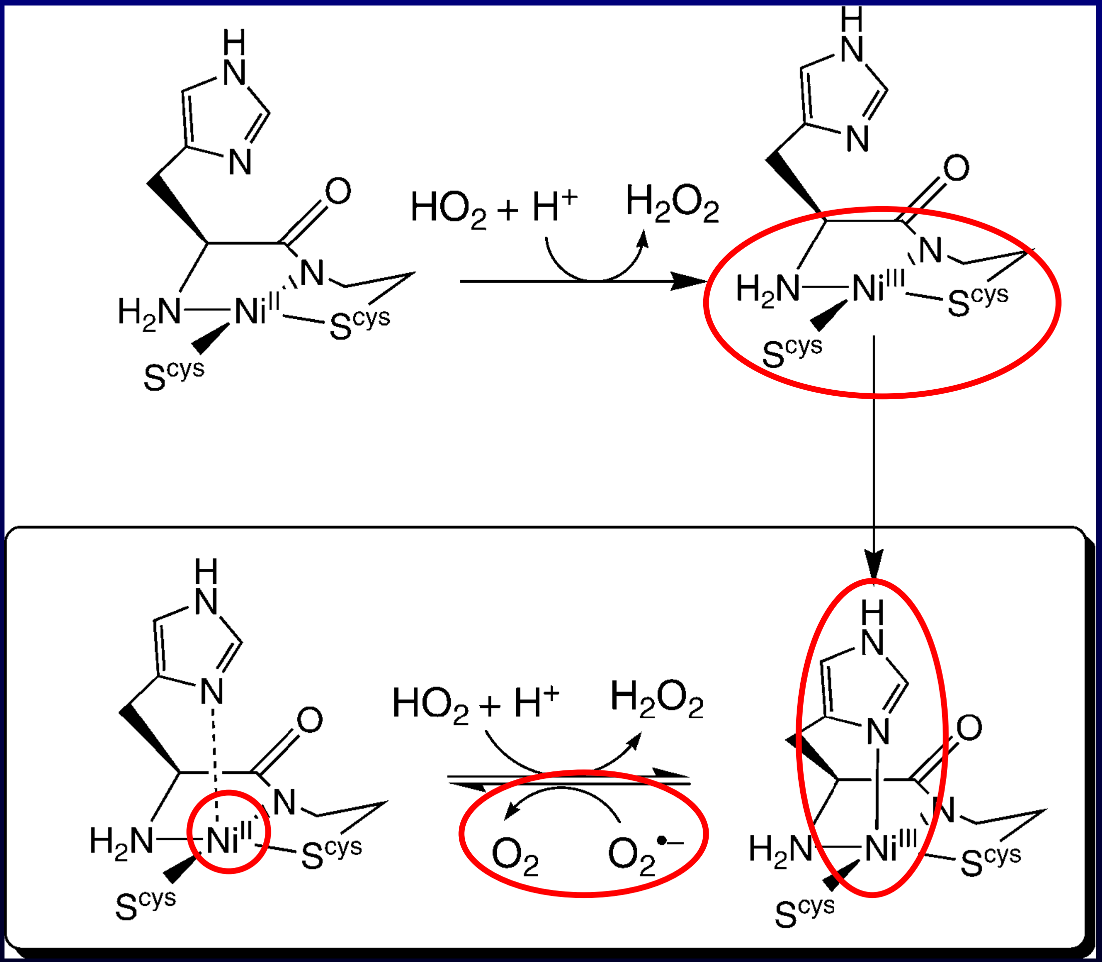


From: Wuerges J et al, 2004, PNAS101, 8569-74

2. Nickel superoxide dismutase
(d) Mechanism

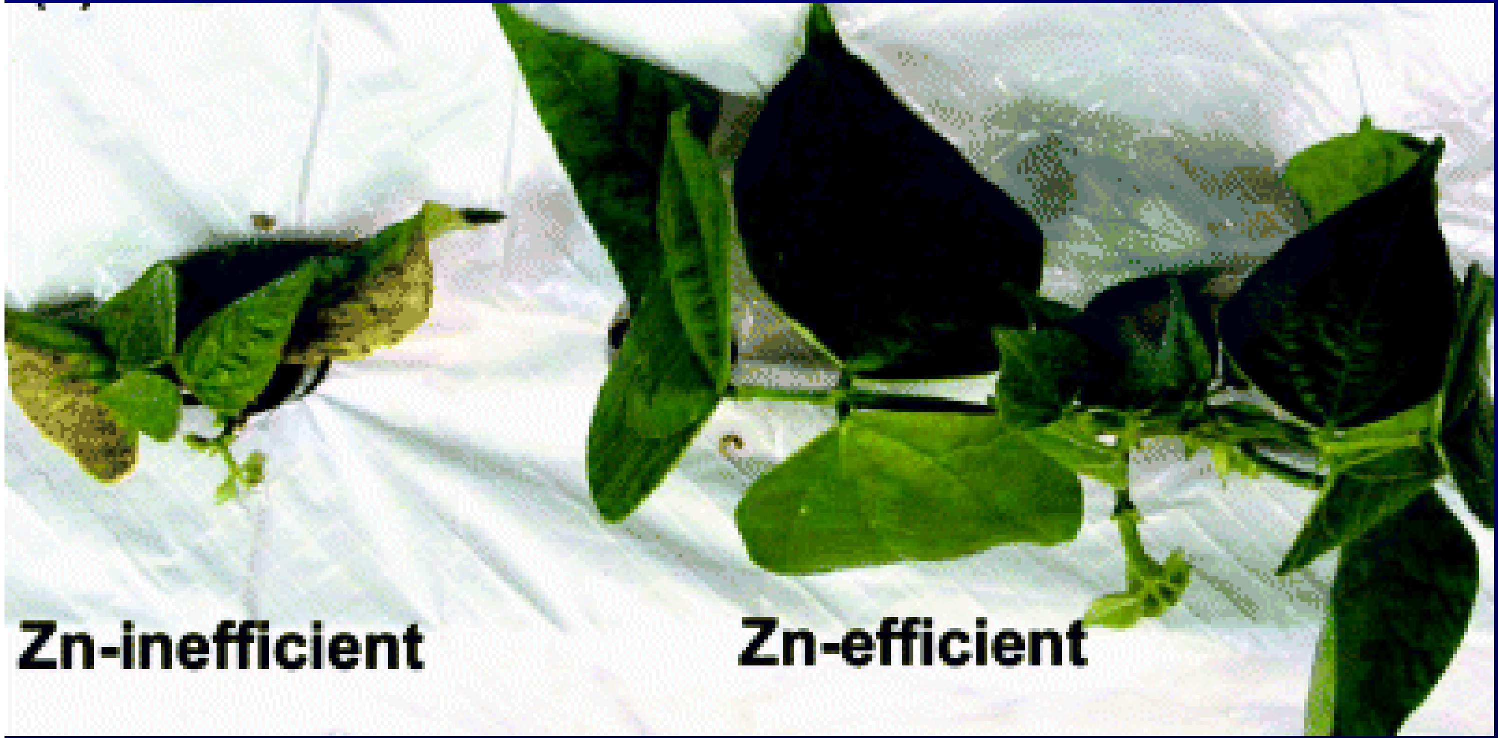
Steps and Characteristics

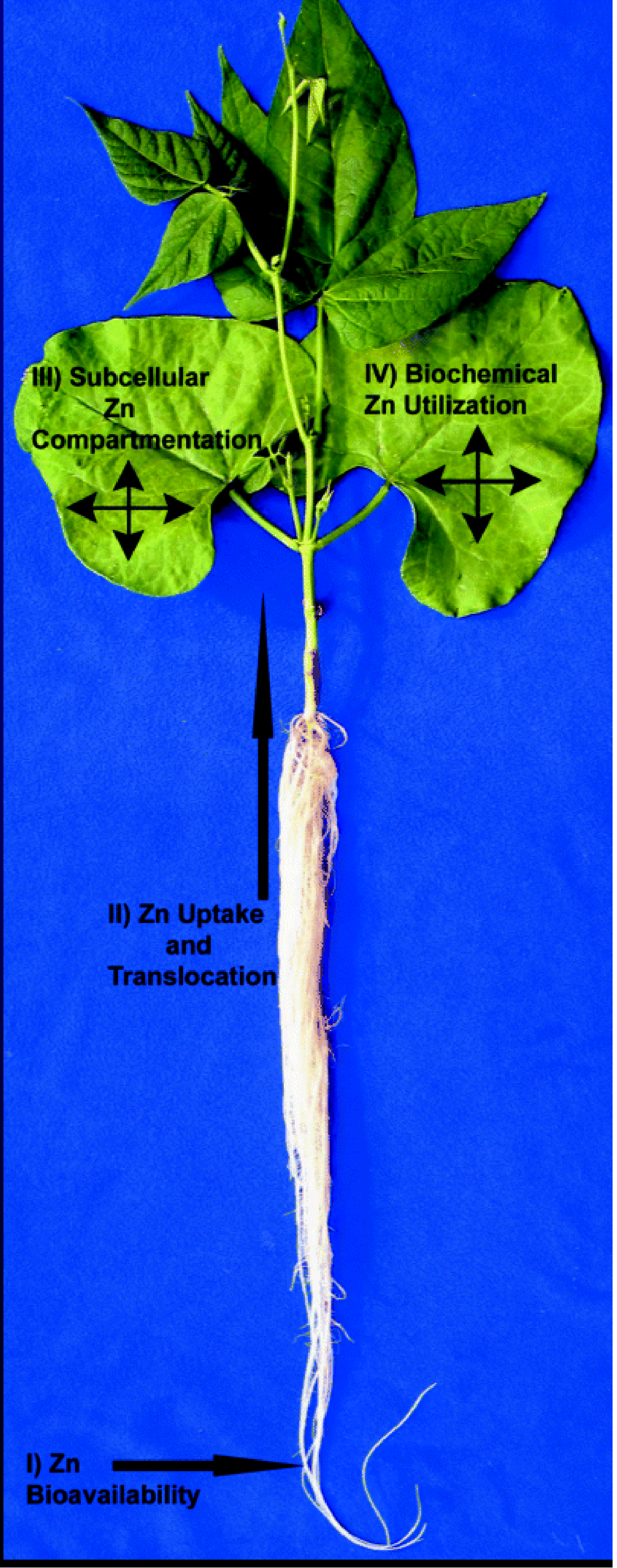
- Oxidation of the four-coordinate Ni^{2+} center to Ni^{3+} (unusual!)
- Rapid imidazole coordination. Once the imidazole is coordinated to the oxidized Ni^{3+} center it will remain ligated throughout catalysis
- Re-reduction of Ni^{3+} to Ni^{2+}
- the axial H(1) imidazole enhances the activity of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



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

Zinc efficiency



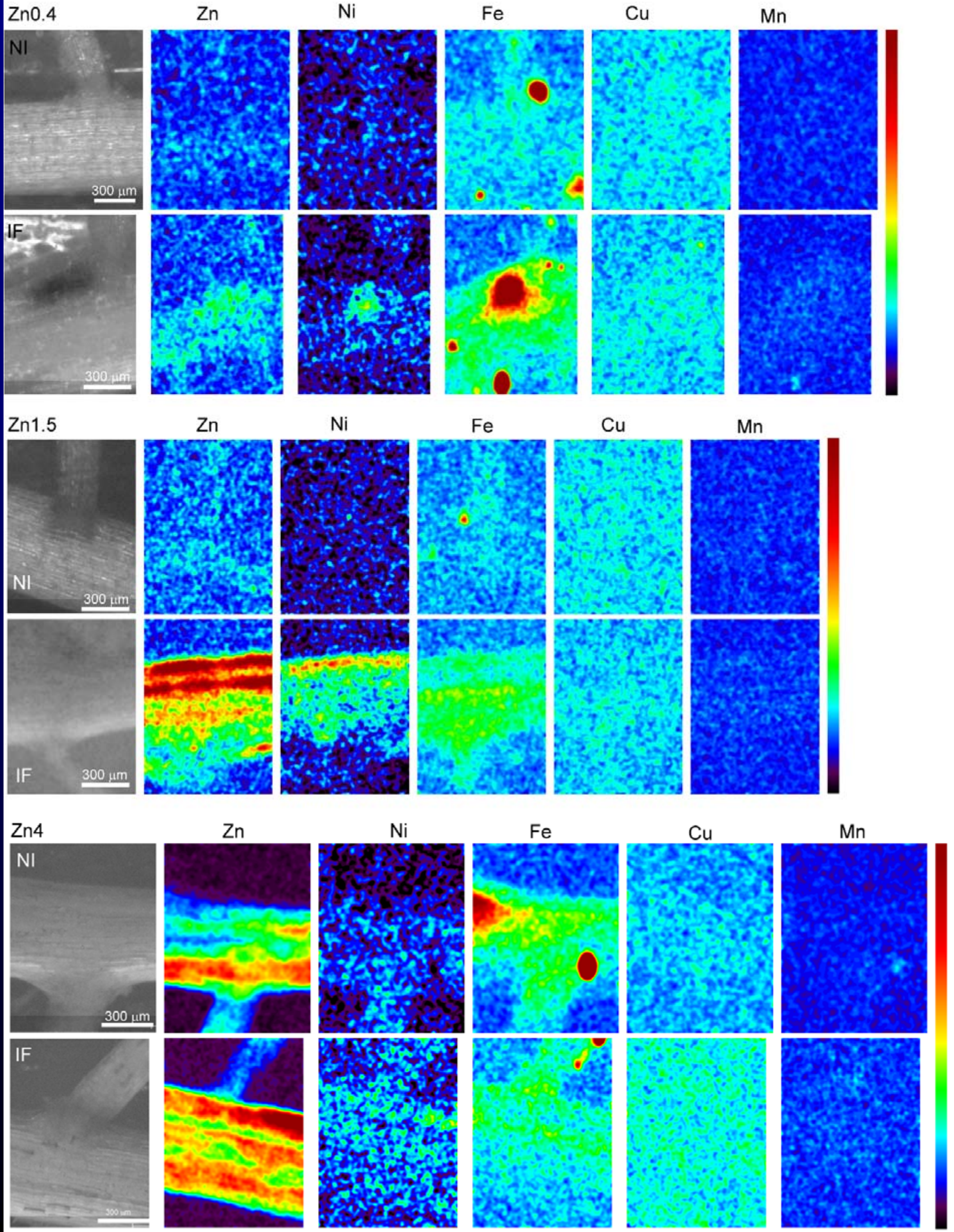


From: Hacisalihoglu G, Kochian, LV.How do some plants tolerate low levels of soil zinc?
Mechanisms of zinc efficiency in crop plants.
New Phytologist 159 (2), 341-350.

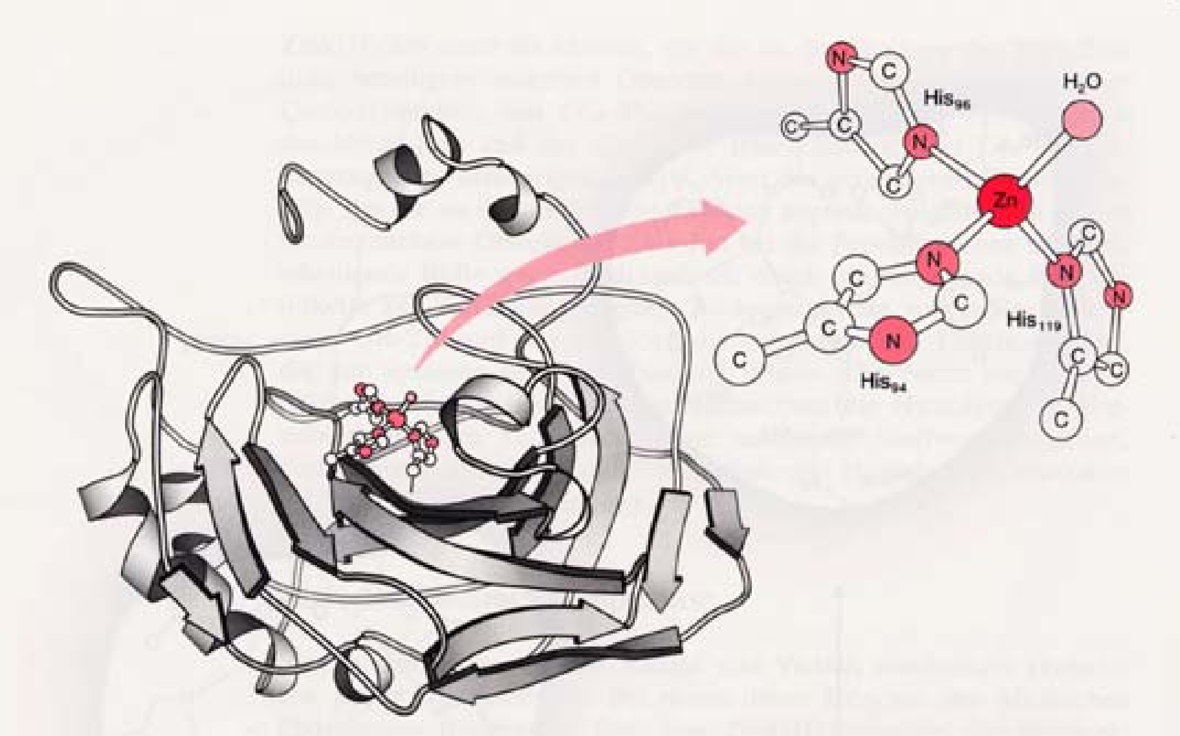
Emerging field: Role of trace metals in plant defence response to biotic stress

- Local Zn mobilization in response to pathogen Phomopsis in soybean roots revealed by µXRF 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

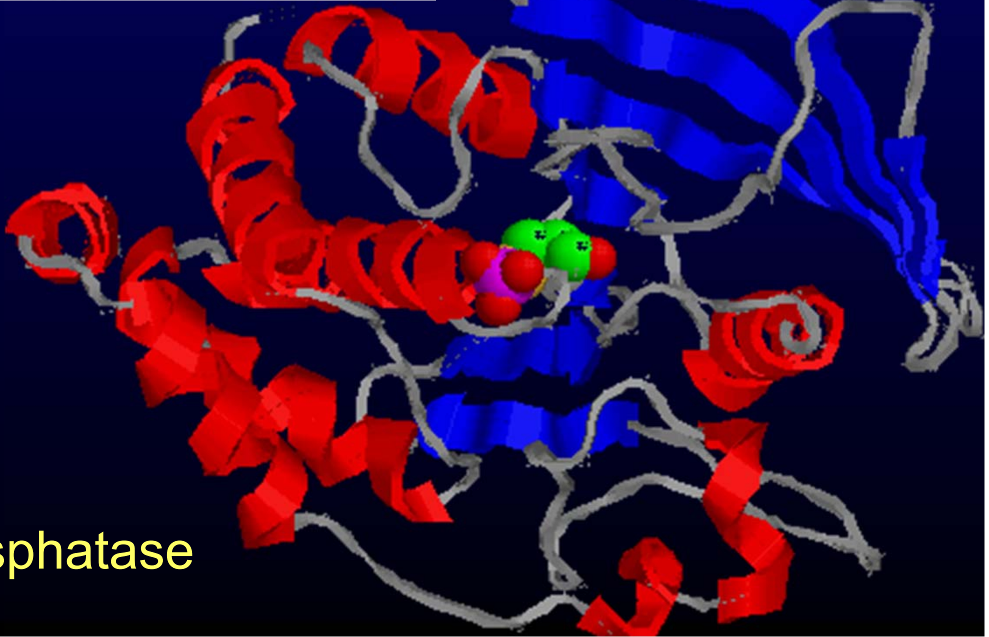


Selected important plant enzymes with zinc in their active centre



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