Molecular Analysis of Heavy Metal Uptake and Accumulation Using a Metal Hyperaccumulating Plant Species

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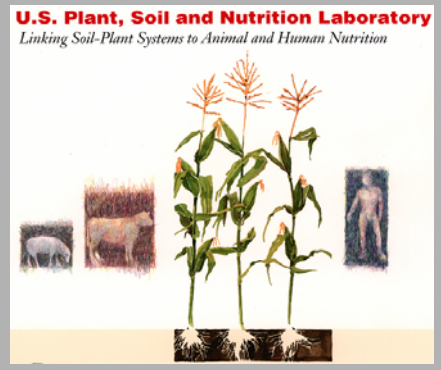
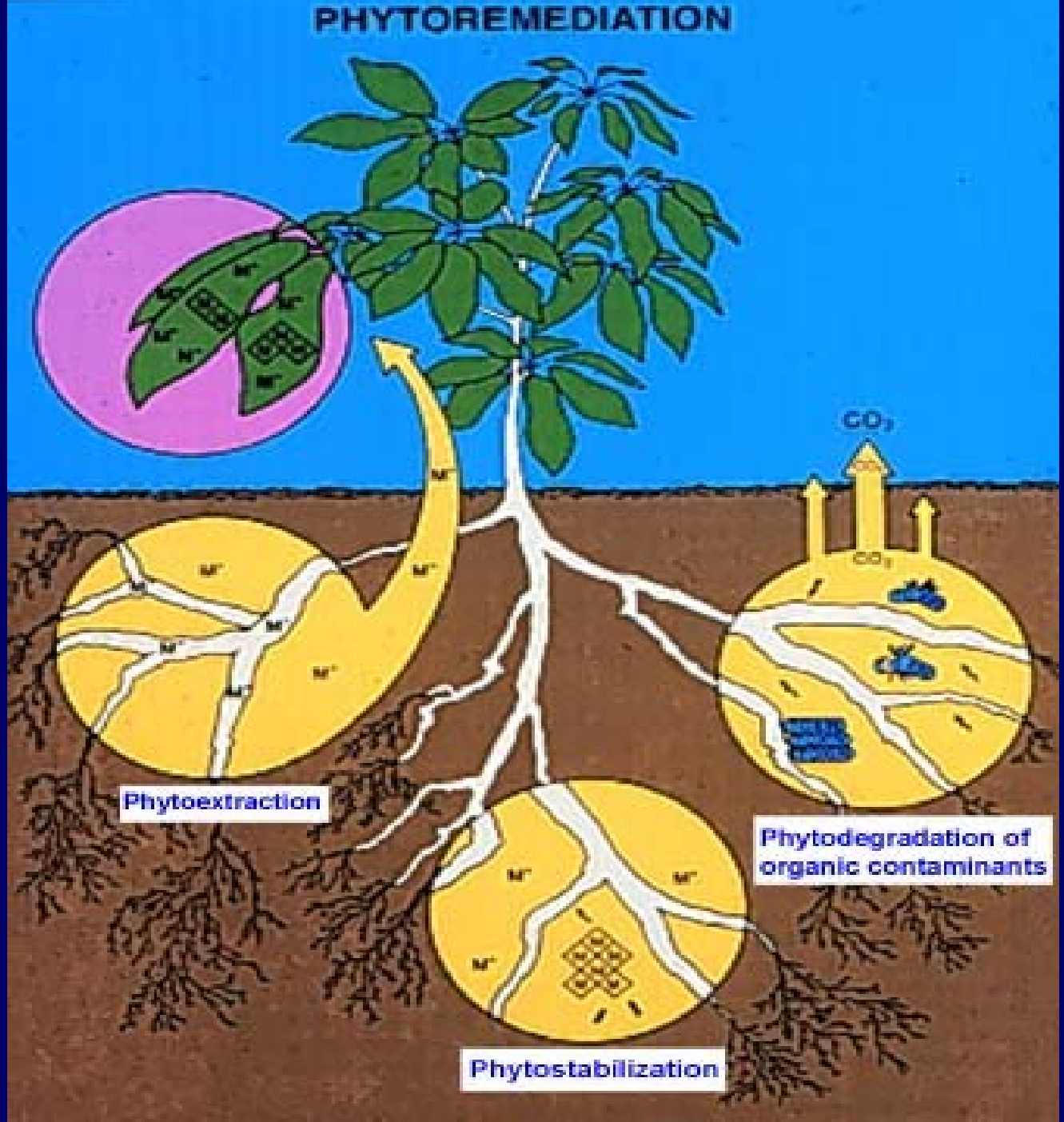



PHYTOREMEDIATION
CO_2
CO_2
Phytoextraction
Phytodegradation of organic contaminants
Phytostabilization

Thlaspi Caerulescens
A Zn/Cd Hyperaccumulator

This species in the Brassicaceae has been the focus of botanical research for over a century because of its ability to colonize metalliferous soils.

Growth Medium	[Zn] of Medium (ppm)	Shoot [Zn] (ppm)	Phytotoxicity Symptoms
Soil	1800	52000	Severe
Soil	178	18000	None
Hydroponics	206	26000	None
Hydroponics	6.5	6200	None

T. caerulescens is a small, slow growing plant – Thus it's utility is as an interesting model system for understanding how plants accumulate and tolerate high levels of metals

SUMMARY
Physiology of Zinc Hyperaccumulation

A number of Zn transport sites are altered in *T. caerulescens*

- Stimulated Zn^{2+} influx across the root-cell PM
- Reduced Zn sequestration in the root-cell vacuole
- Stimulated Zn transport into the xylem (?)
- Stimulated Zn^{2+} influx into leaf mesophyll cells

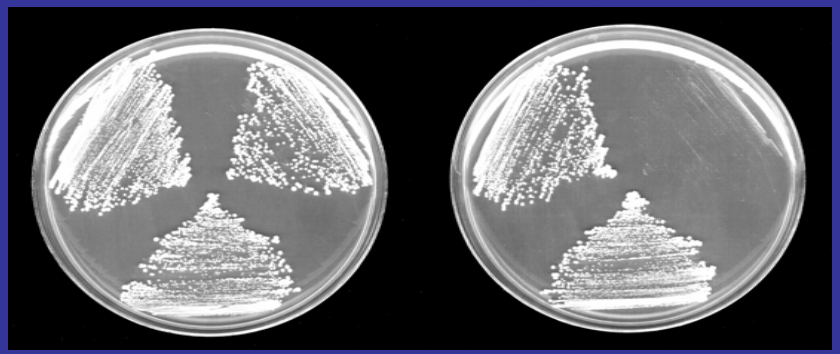
Plant Zn^{2+} Transport Gene was Cloned by Complementation

T. caerulescens cDNA library

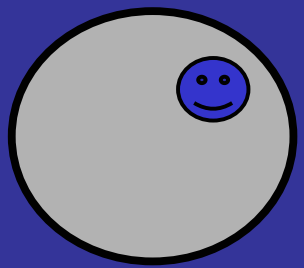
transform into

zhy3, yeast zinc transporter gene mutant

Screen on low zinc medium

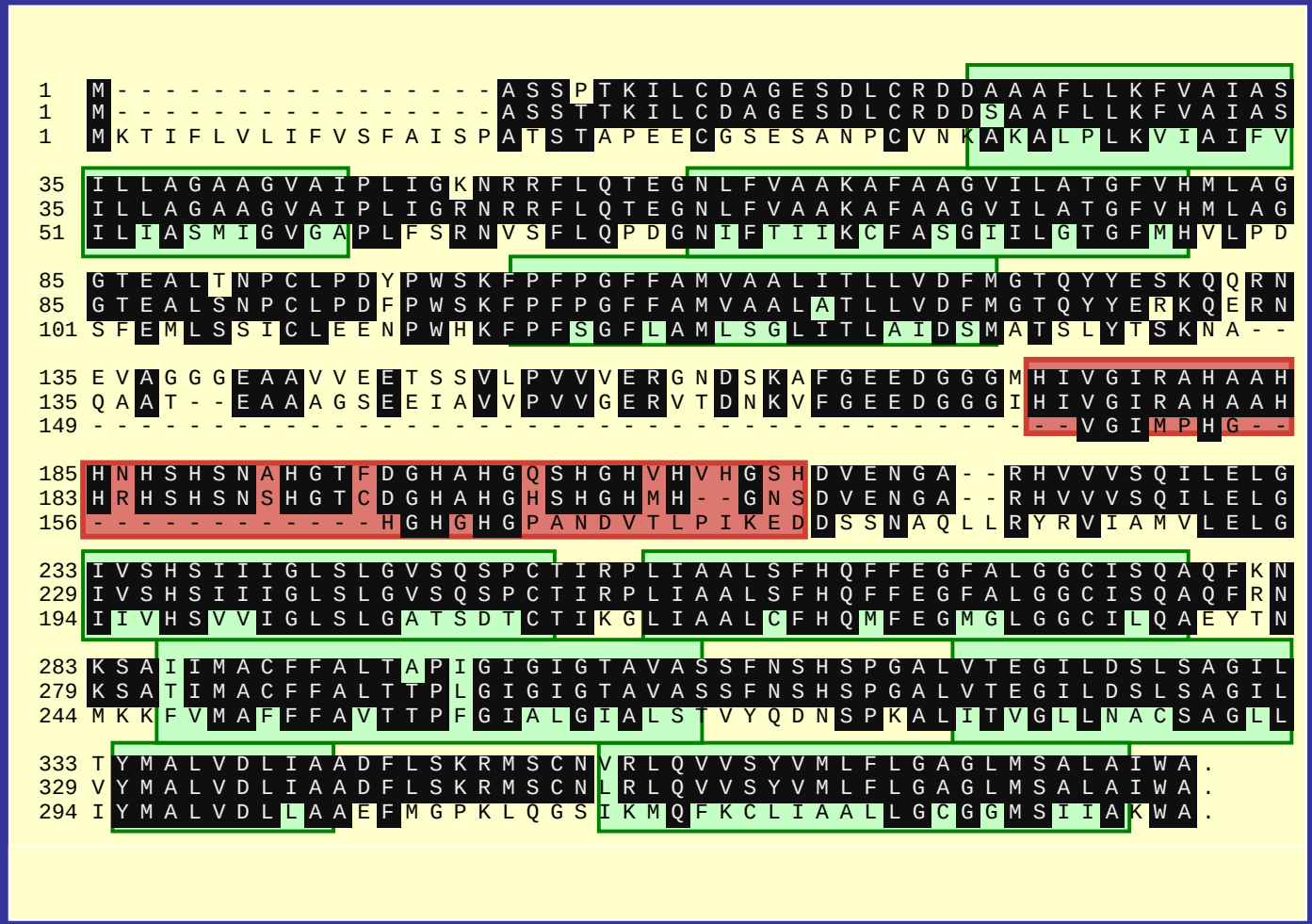


High Zn Low Zn



ZNT1 containing colony

ZNT1 is a Member of the ZIP Family of Micronutrient Transporters



Strategies for Characterizing Members of Ion Transporter Gene Families

- Tissue and cell specific localization of gene expression (Northerns, in situ mRNA localization, promoter-reporter gene constructs)
- Membrane localization of transport protein (via YFP-protein fusions)
- Investigate transport properties using heterologous systems (yeast, oocytes, mammalian cell lines) and via knockout mutants (currently developing stable transformation system)

ZIP Transporters in *Thlaspi caerulescens*

Thlaspi caerulescens	*Arabidopsis* homolog	% Identity	Length of *Thlaspi* clone
ZNT1	*ZIP4*	88	Full Length
ZNT2	*ZIP2*	88	Missing 28 bp at 5' end
ZNT3	*ZIP3*	88	Missing 200 bp at 5' end
ZNT4	*IRT3*	88	Full Length
ZNT5	*ZIP5*	88	Full Length

We are currently screening for ZIP1, 7 and 9 homologs in *Thlaspi*

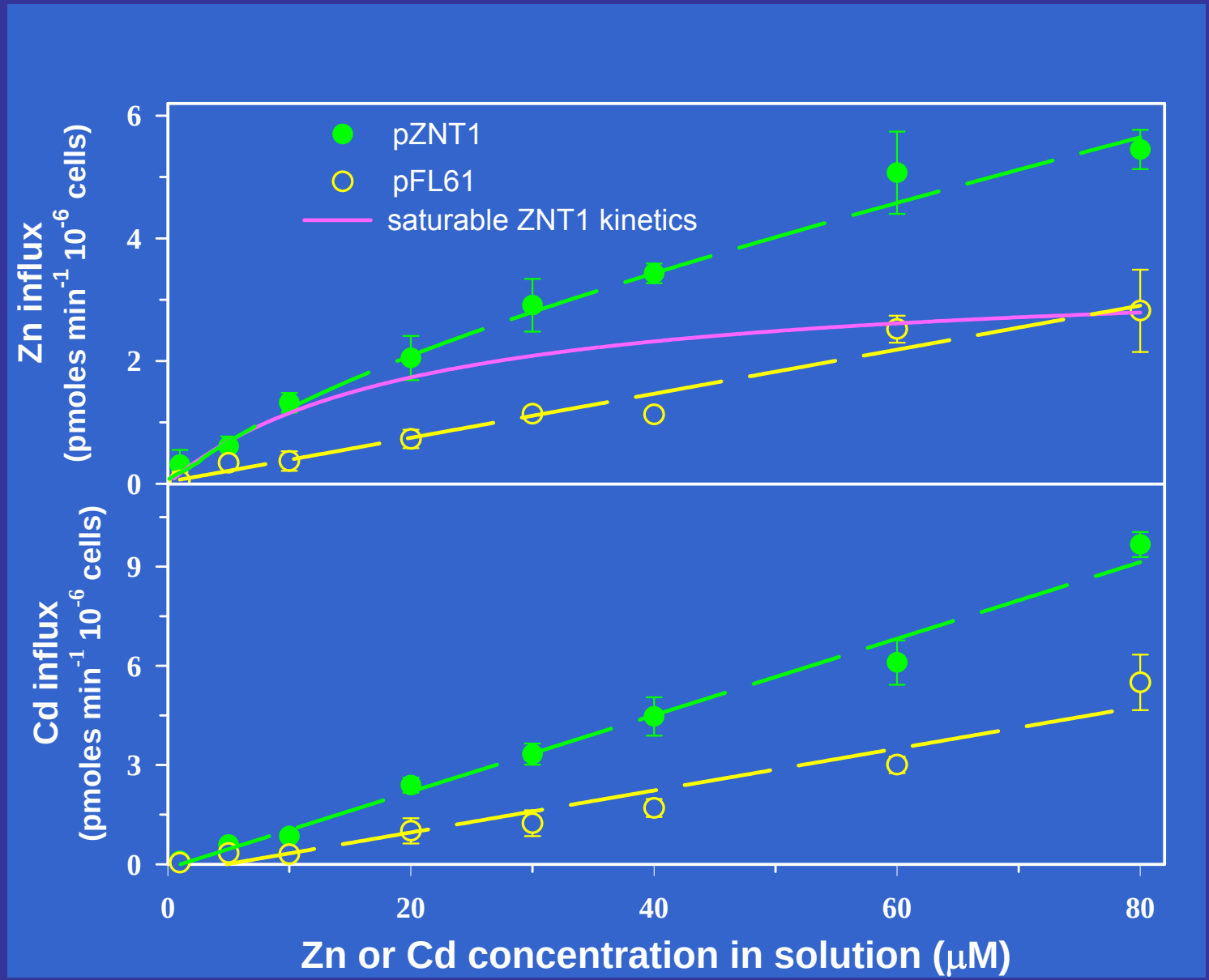
A Number of Other Micronutrient Transporter Gene Families Have Recently Been Identified in Plants

- Nramp family of broad specificity micronutrient transporters
- CAX family of vacuolar membrane divalent cation/H^+ transporters
- P-type heavy metal ATPases
- ZAT family of tonoplast and PM micronutrient/heavy metal transporters (members of the CDF superfamily)
- Subset of ABC transporter super family

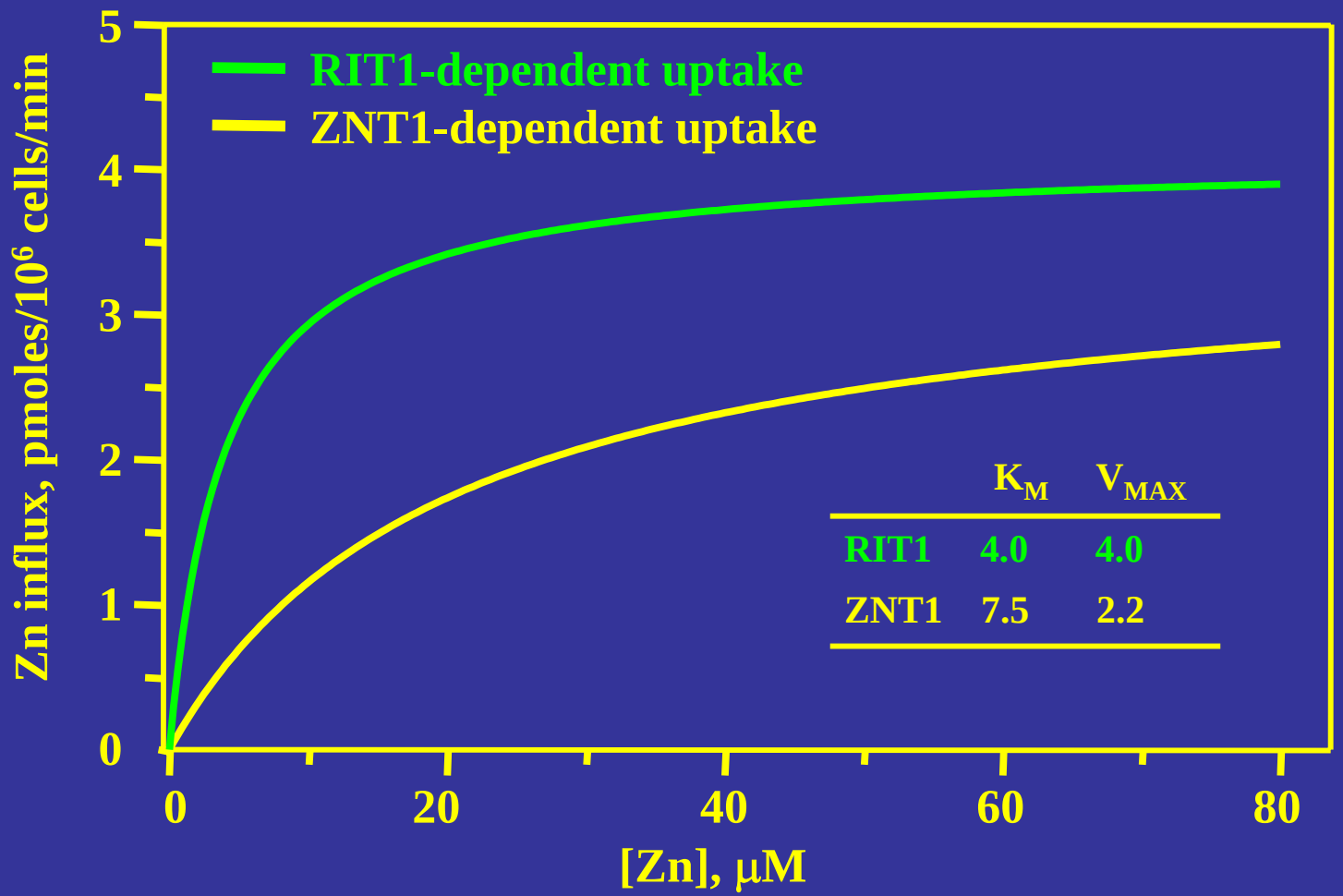
Characterization of ZNT1

Is It a “Super” Transporter?

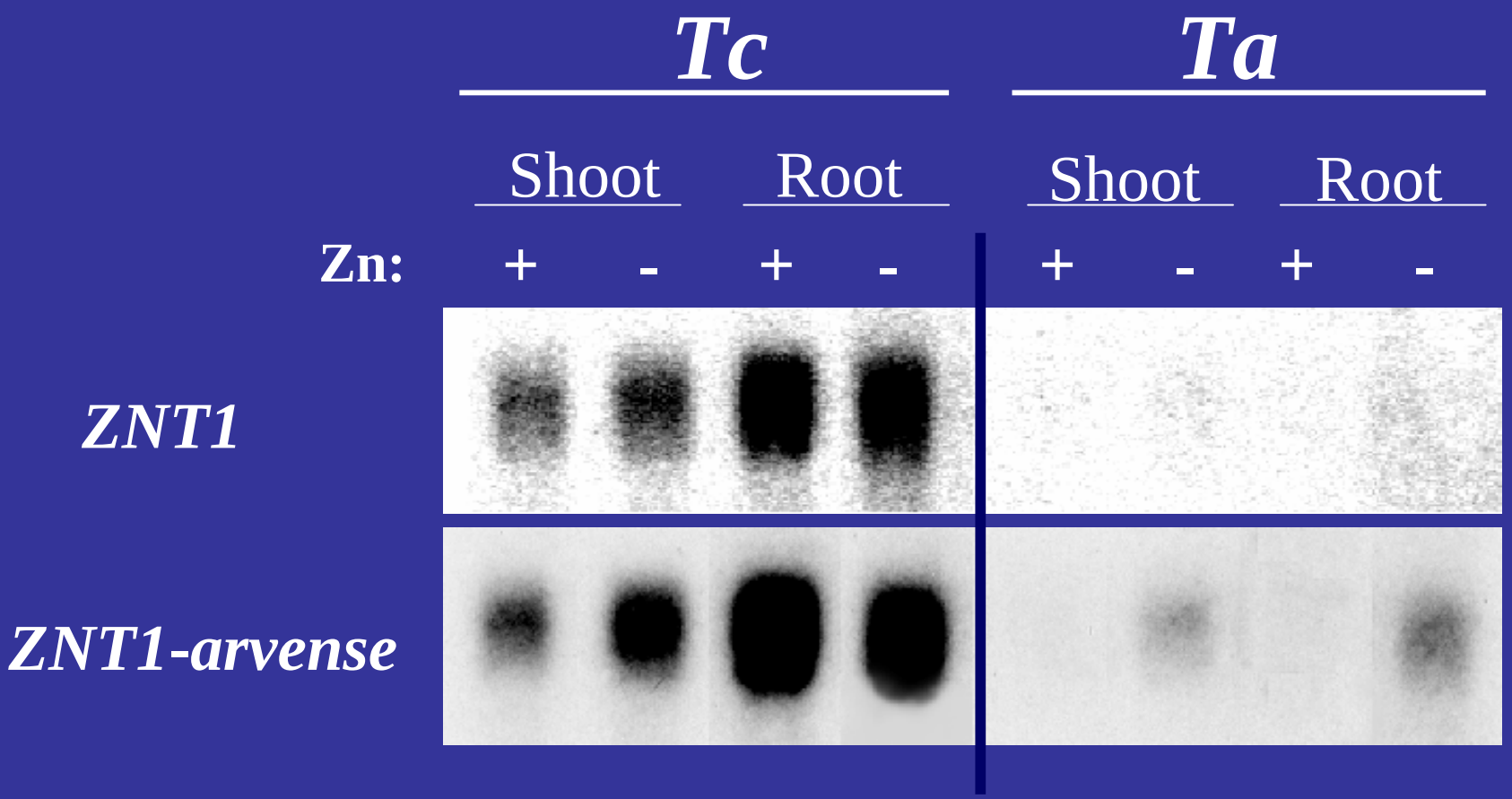
ZNT1-Mediated Zn and Cd Influx in Yeast



Mediation of Zn uptake by RIT1 and ZNT1



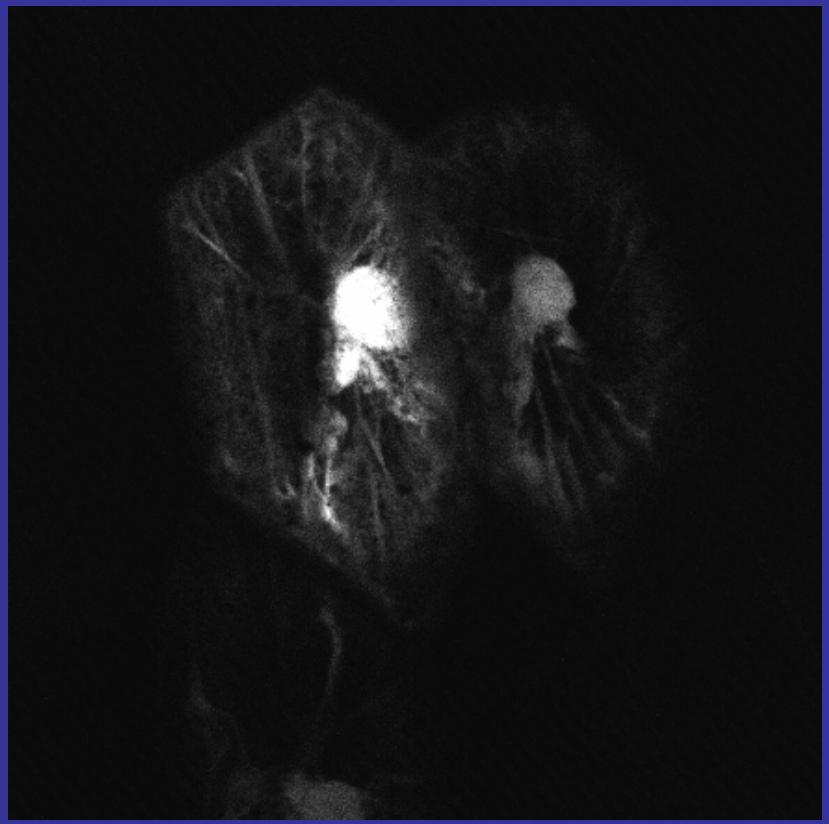
Northern Analysis of *ZNT1* Expression



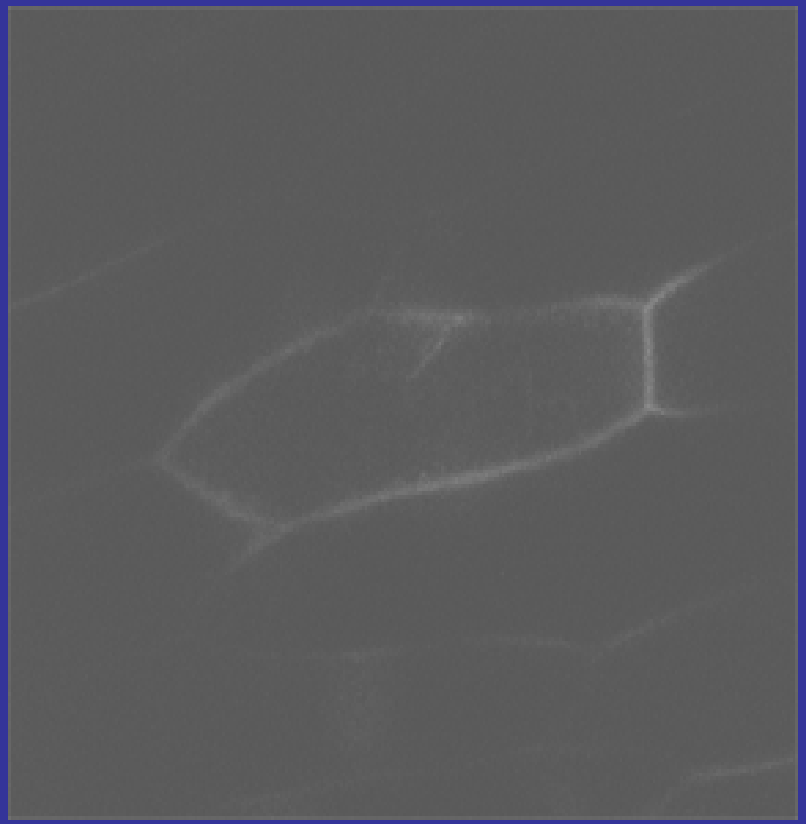
Membrane Localization of ZNT1 Using ZNT1-GFP Construct

Transient transformation by particle bombardment into onion, *Thlaspi* and *Arabidopsis* and via PEG transformation of protoplasts

ZNT1-GFP Expression in Onion Cells



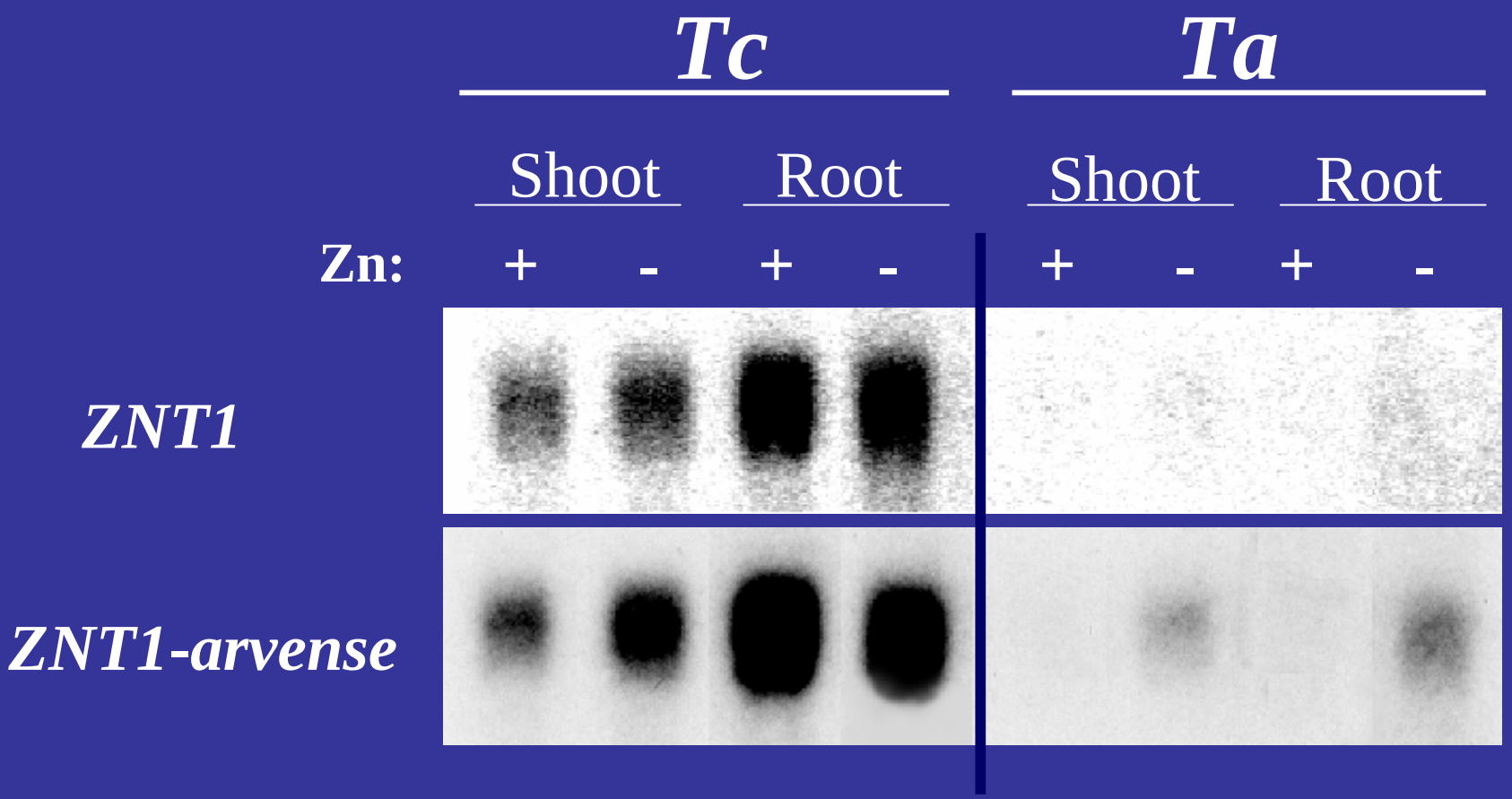
pAVA120



pZNT1-GFP

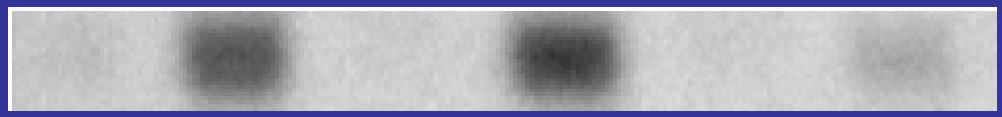
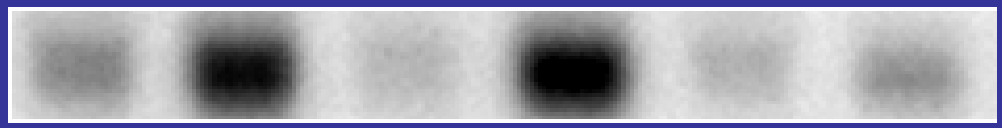
REGULATION OF HEAVY METAL GENE EXPRESSION: DOES HYPEREXPRESSION DRIVE HYPERACCUMULATION?

Northern Analysis of *ZNT1* Expression



Increasing Plant Zn Status Decreases *ZNT1* Expression

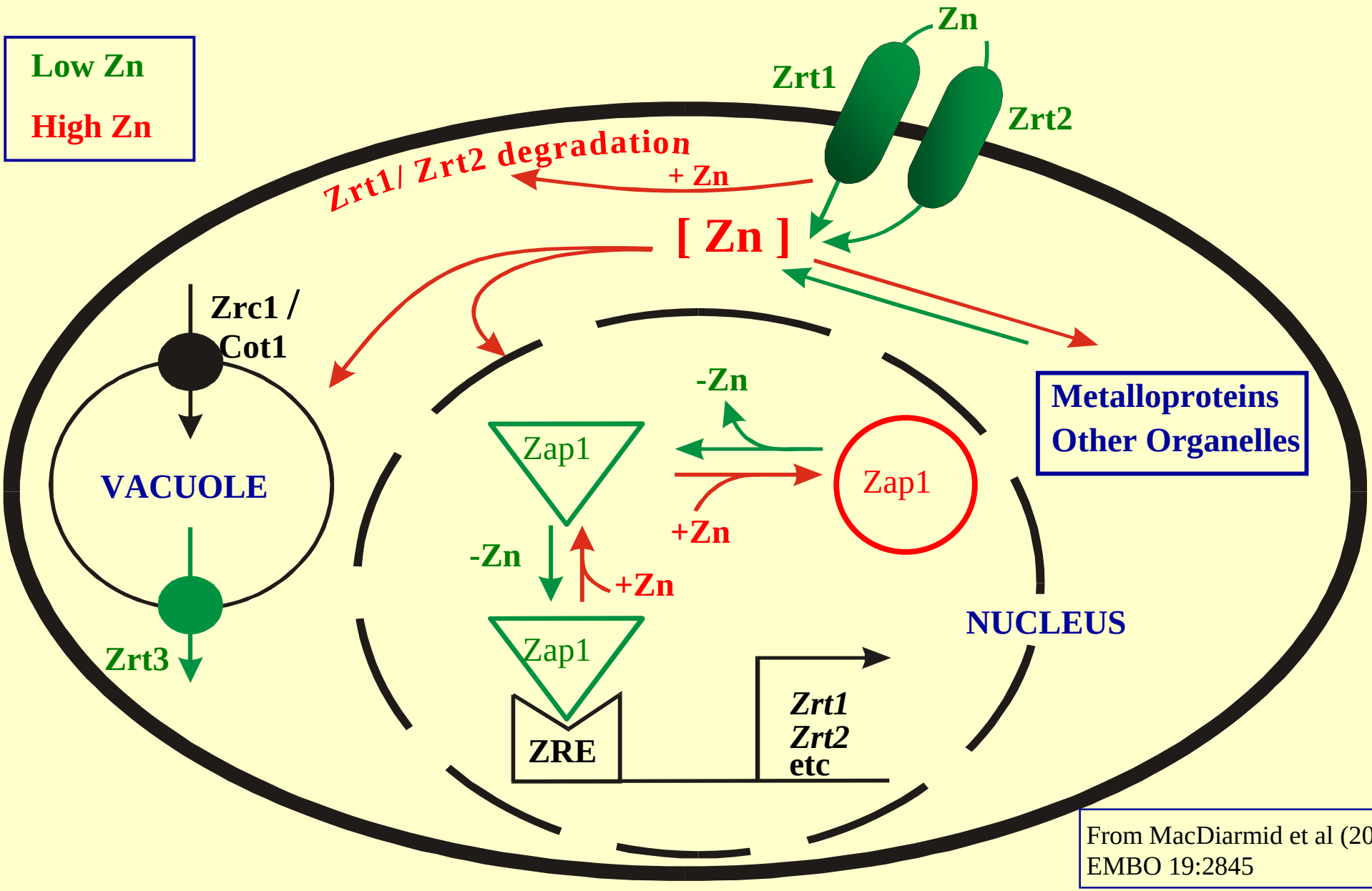
[Zn] in growth solution

	0	0	1	1	10	50	μM
***ZNT1 caer.* probe**							
***ZNT1 arvense* probe**							
	A	**C**	**A**	**C**	**A**	**C**	
K_m	2	4	2	6	2	5	
V_{max}	80	240	40	270	40	80	

Altered Regulation of ZIP Transporters in *T. caerulescens*

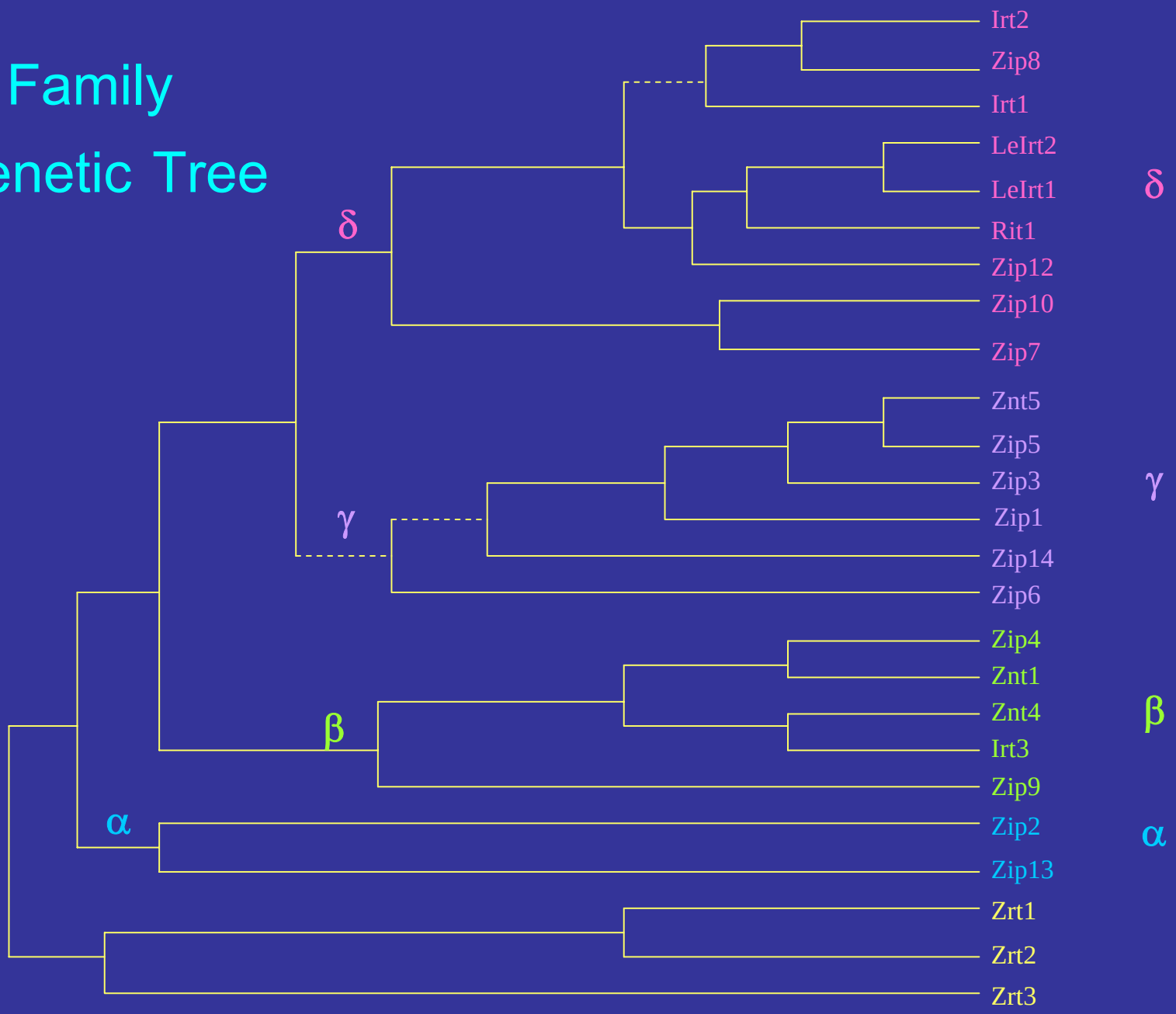
- In “normal” plants there is a low level of *ZIP* expression that is upregulated by Zn deficiency.
- In *T. caerulescens*, *ZNT1* expression is very high under Zn deficient and sufficient conditions.
- Only when *T. caerulescens* is grown on very high levels of Zn is *ZNT1* expression reduced.
- Regulation of Zn transporter expression is altered in *T. caerulescens*, contributing to hyperaccumulation.

Zn Homeostasis in Yeast



From MacDiarmid et al (2000)
EMBO 19:2845

ZIP Family Phylogenetic Tree

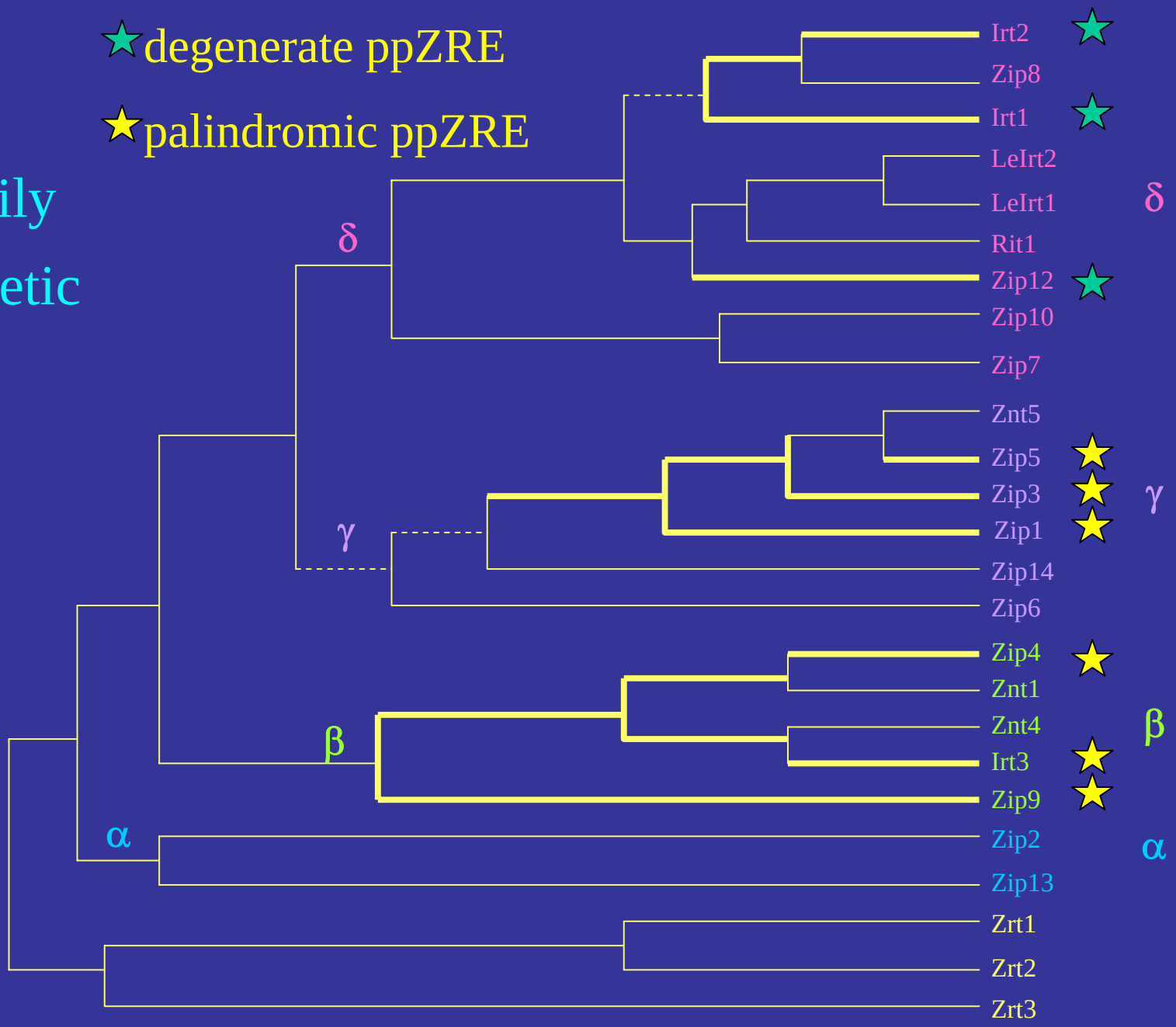


ZIP promoters contain ppZRE(s)

5’-ATG(A/T)CGA(A/C)A(C/T)-3’

ZIP subfamily	One or more ppZREs	No ppZREs
α	.....	***ZIP2, ZIP13***
β	***ZIP4*, ZIP9, IRT3***	.....
γ	***ZIP1, ZIP3*, ZIP5***	***ZIP6, ZIP14***
δ	***IRT1, IRT2, ZIP12***	***ZIP7, ZIP8***

ZIP family Phylogenetic Tree

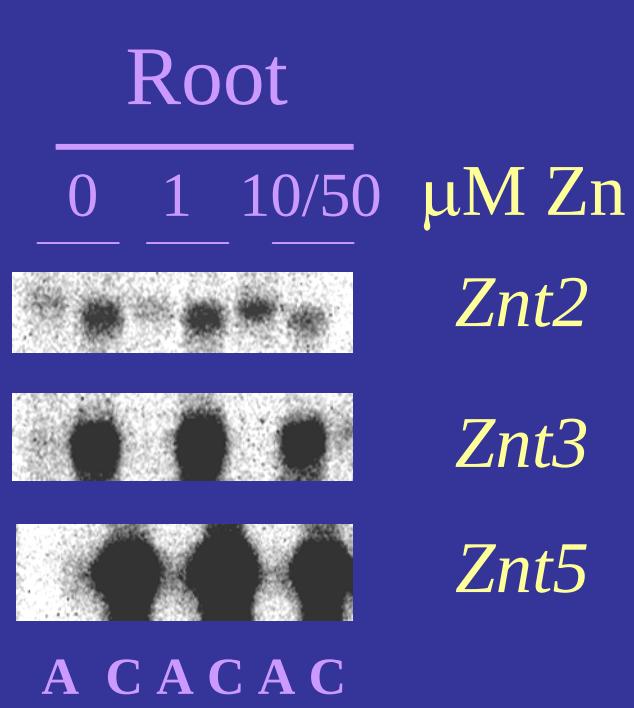


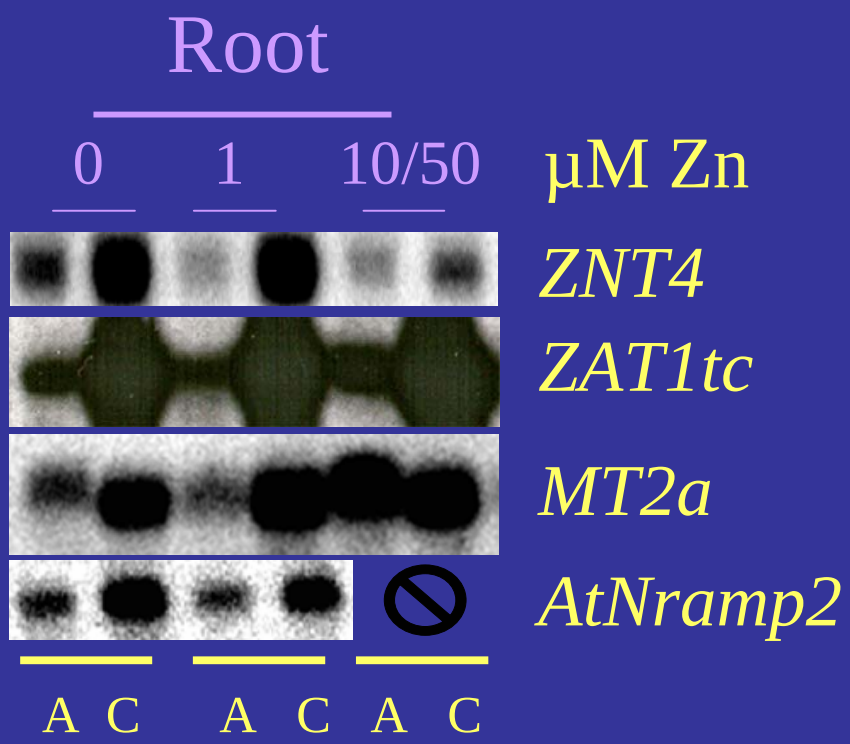
Is Expression of Other Metal Transport Genes Altered in *T. caerulescens?*

- Fourteen *ZIP* and three *IRT* homologues have been identified in *Arabidopsis*
  - We have cloned 5 *ZNT* homologs in *Thlaspi* with more on the way
  - What are their roles in hyperaccumulation and micronutrient homeostasis?
- Other micronutrient/heavy metal transporter families:
  - CAX family of tonoplast divalent cation/H^+ antiporters
  - Nramp family of broad specificity micronutrient/heavy metal transporters
  - ZAT family of tonoplast and PM micronutrient/heavy metal transporters (members of the CDF superfamily)

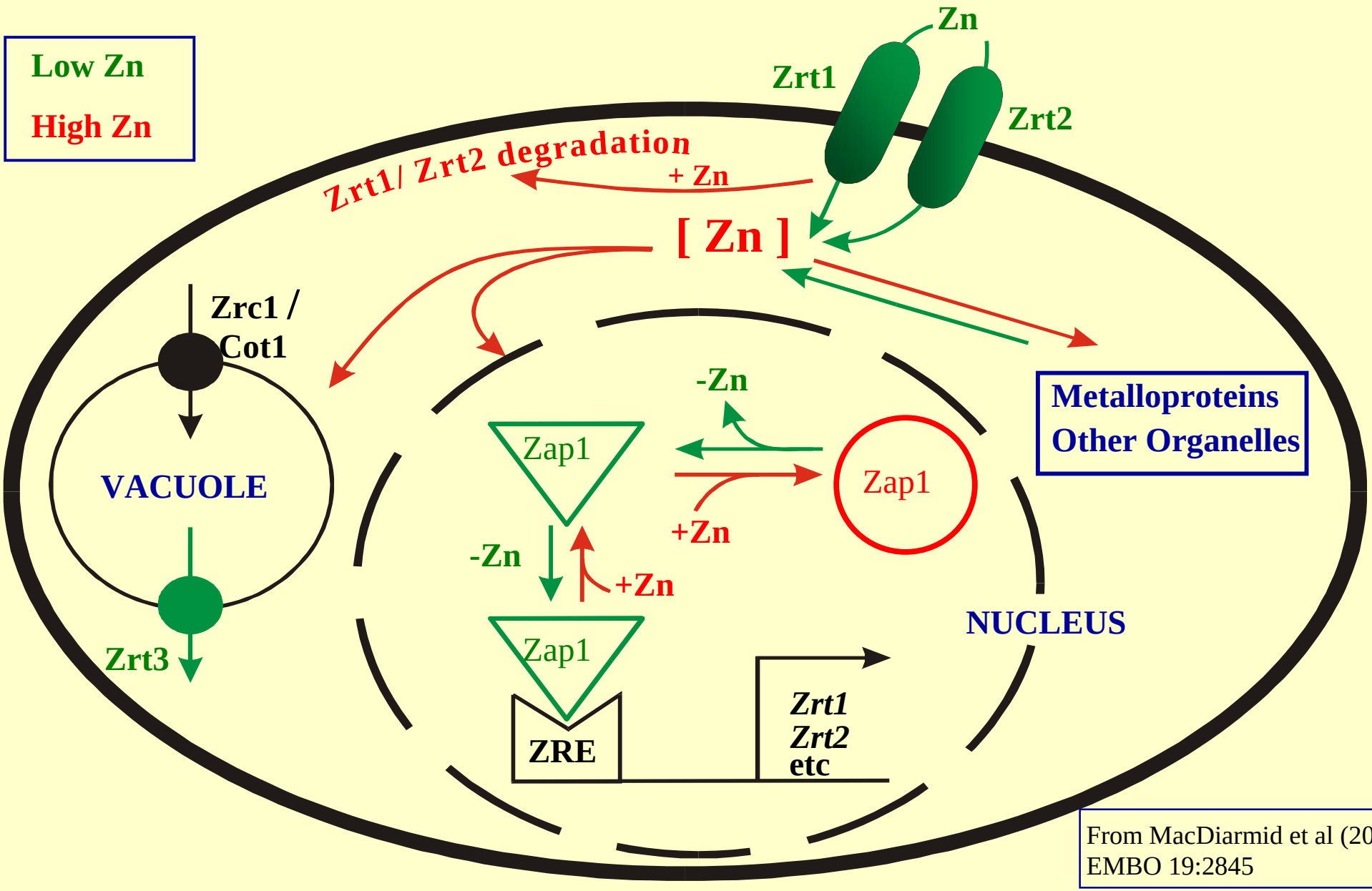
Do any of these play a role in hyperaccumulation or tolerance?

Heavy Metal-Related Gene Expression in Roots of *T. caerulescens* vs. *T.arvense*



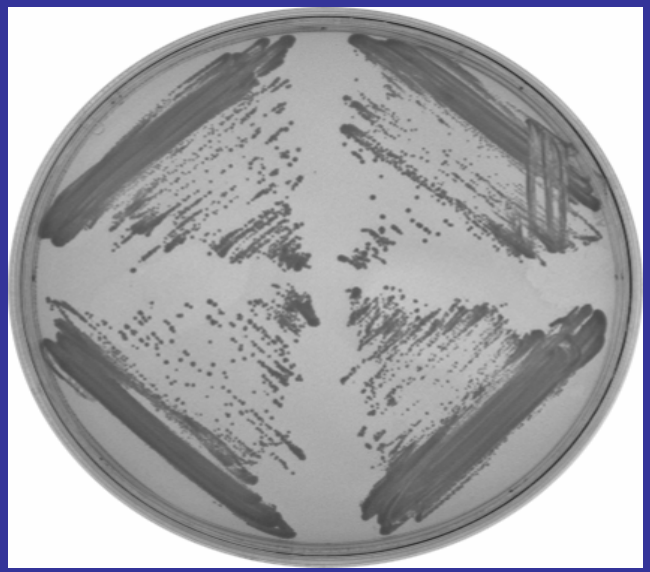


Zn Homeostasis in Yeast

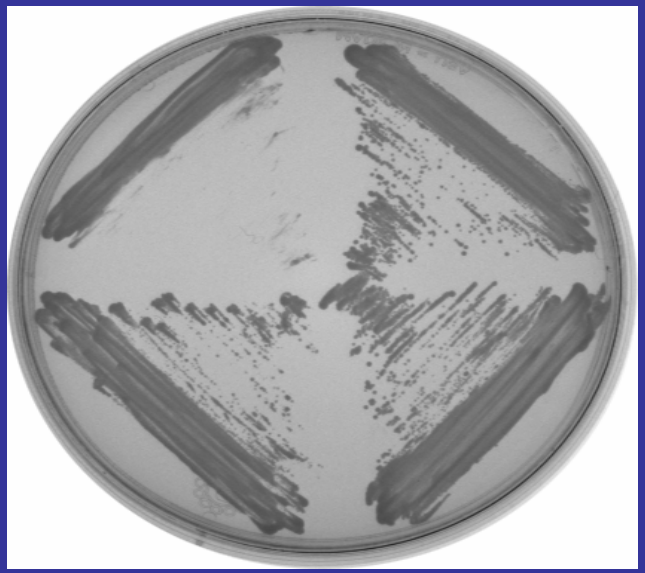


From MacDiarmid et al (2000)
EMBO 19:2845

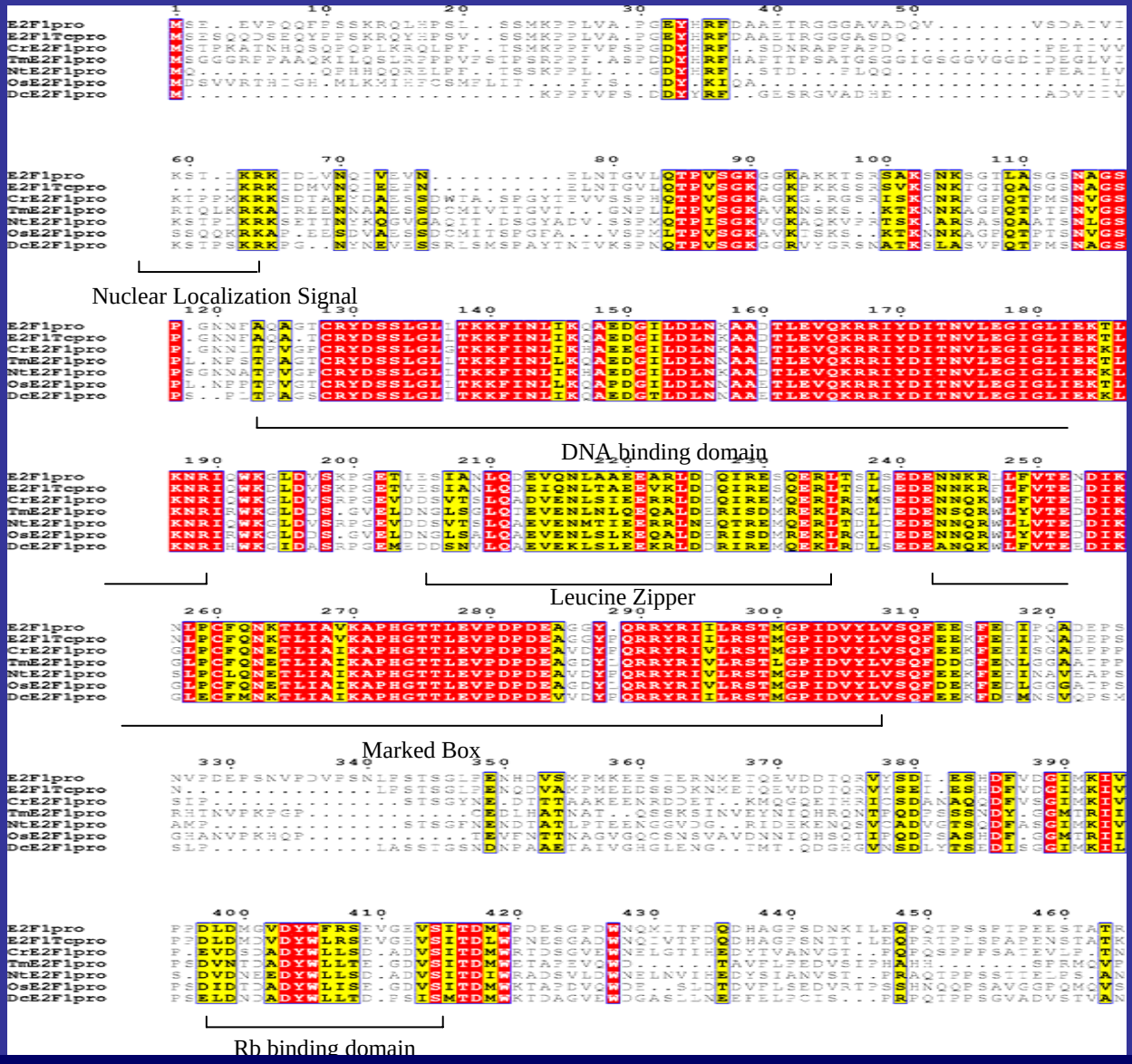
E2F1 and E2F2 Confer Growth on Low Zinc for the ZAP Deletion Mutant, ZHY6



High Zinc

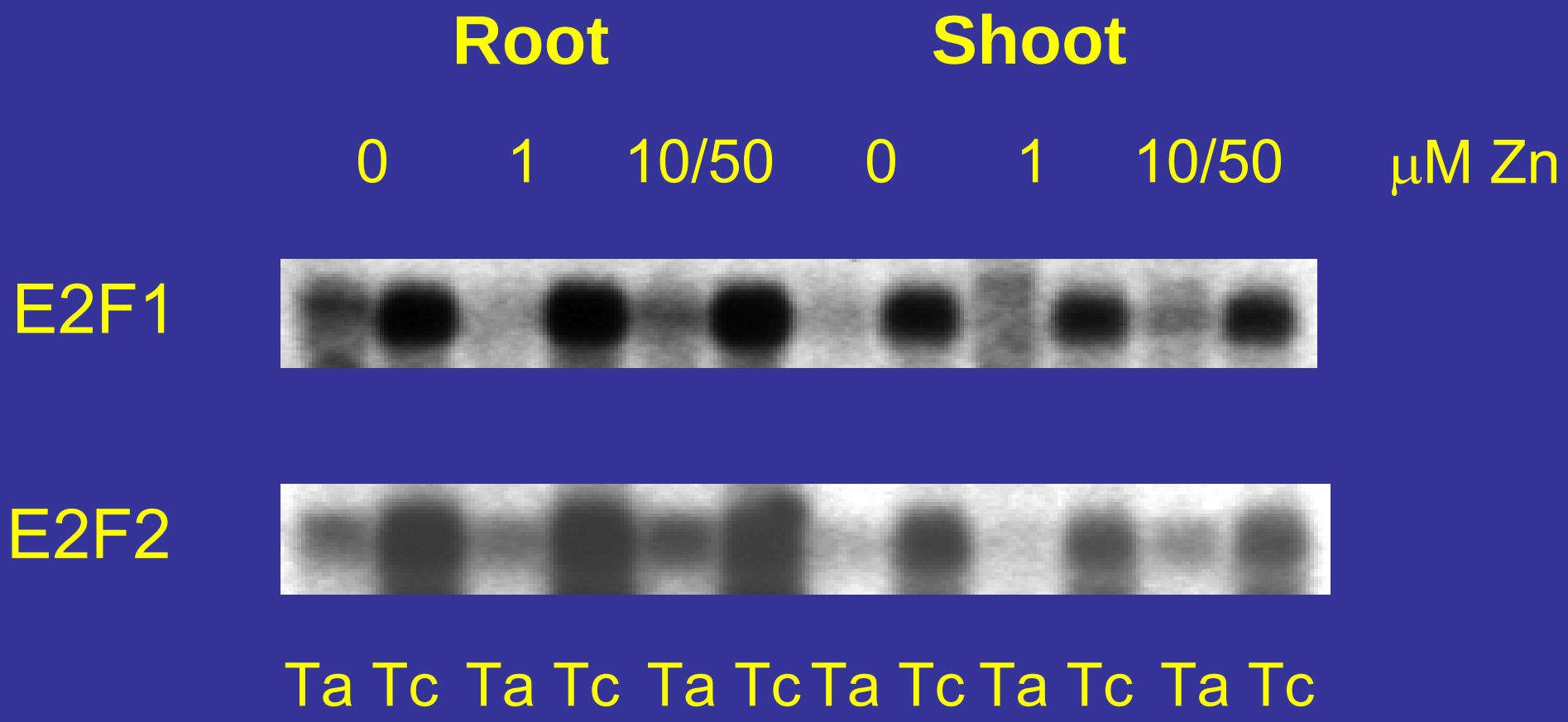


Low Zinc



Significant Sequence Homology among Plant E2F-like proteins

E2F Induced *Zrt1* Expression in Yeast

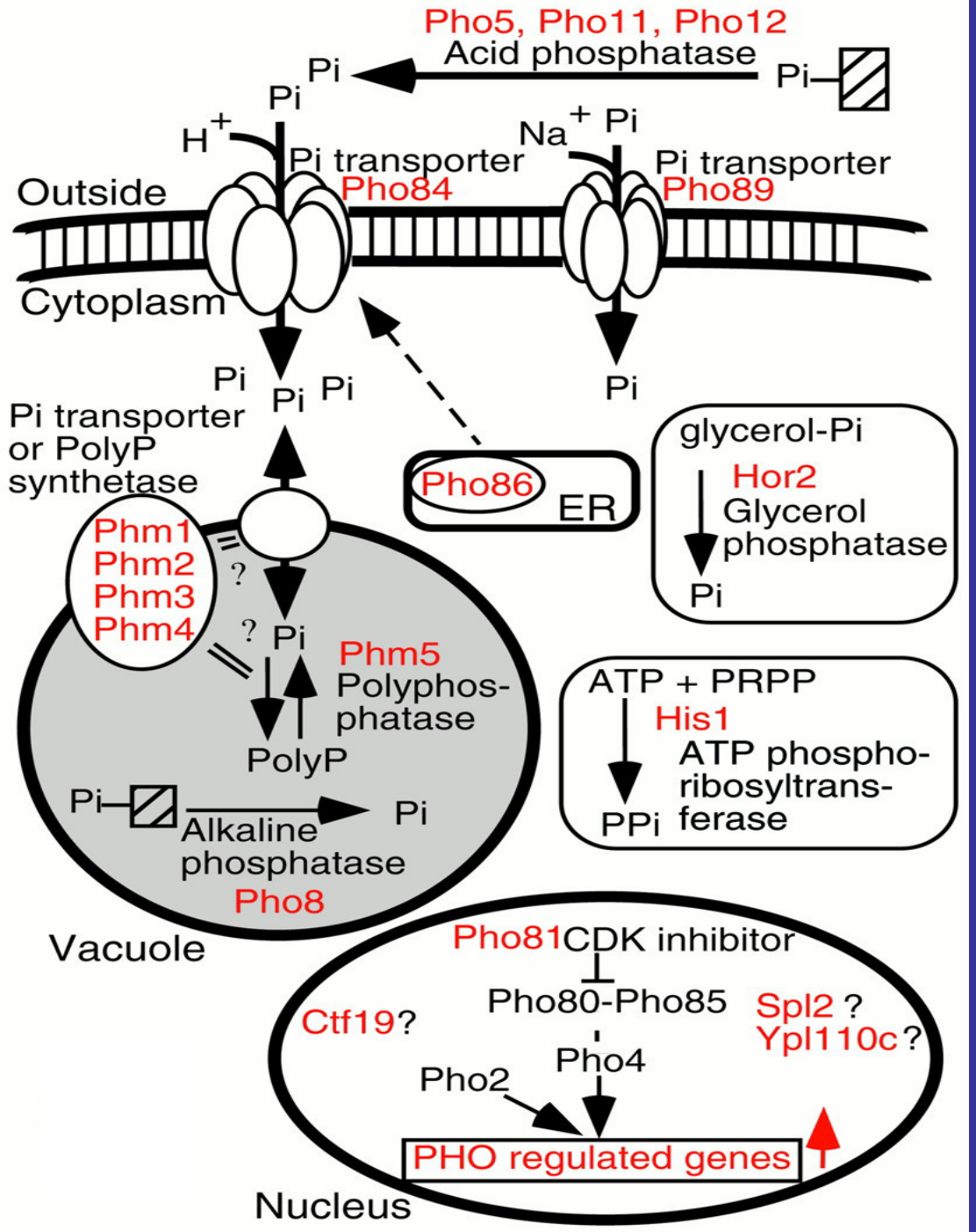


E2F1/E2F2 are members of a family of transcription factors involved in regulation of the cell cycle via a complex involving cyclins and CDKs. How might such a protein be involved in regulation of plant mineral nutrition?

PHO Regulon in Yeast:

Molecular Regulation of P Nutrition/Acquisition

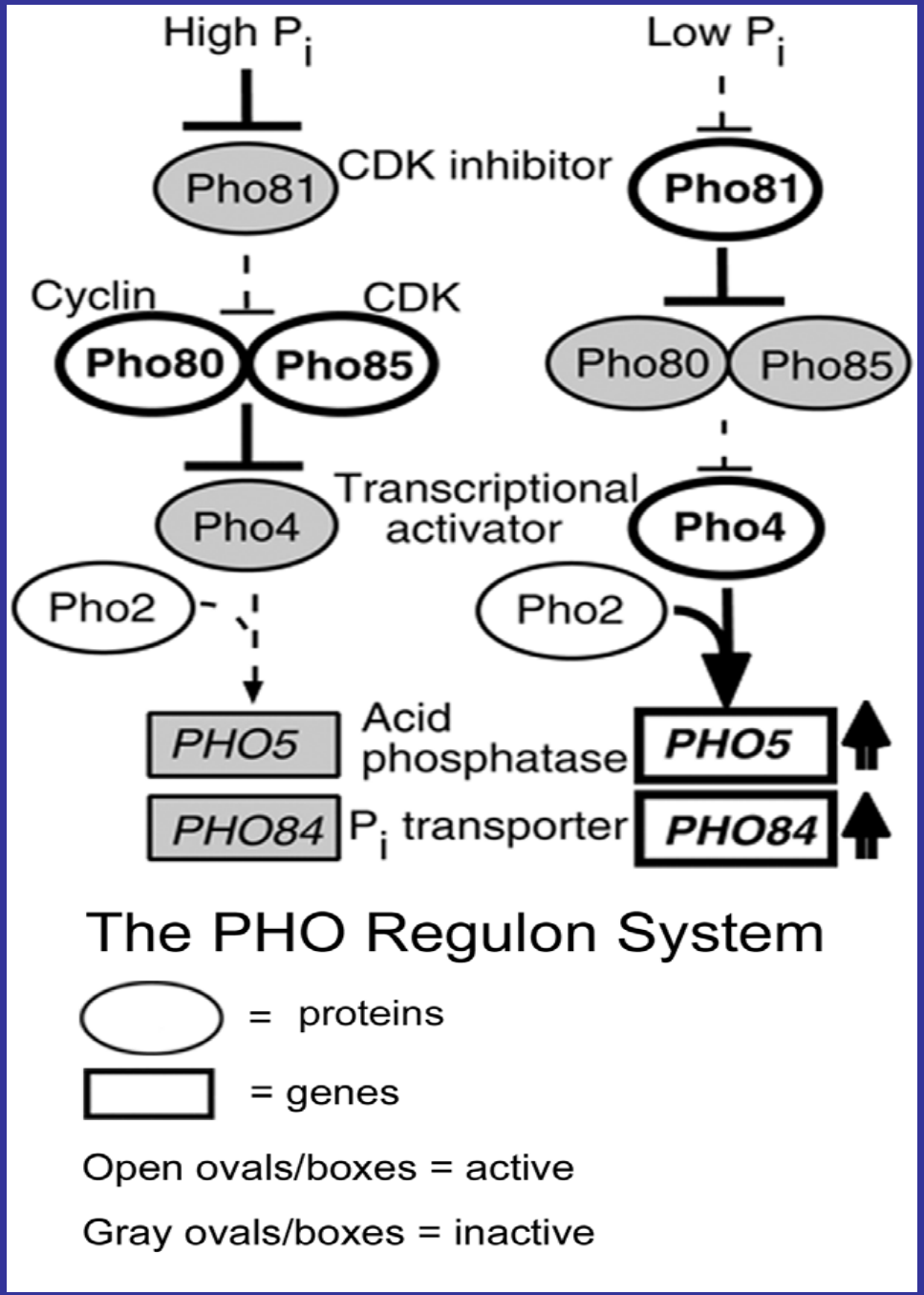
Ogawa, DeRisi and Brown. 2000. Molecular Biology of the Cell 11:4309.



PHO Regulon in Yeast:

Pho regulatory system showing the 5 main regulator proteins (Pho 2, 4, 80, 81, and 85)

Ogawa, DeRisi and Brown. 2000. Molecular Biology of the Cell 11:4309.



Elucidating mechanisms of Zn-dependent gene regulation in *Thlaspi caerulescens*

- Promoter Analysis
  - We have cloned *Thlaspi ZNT1* promoter via genome walking and *Arabidopsis ZIP4* promoter via PCR (*ZNT1* promoter contains the ppZRE found in *ZIP4*)
  - Expression analysis using promoter-reporter gene constructs and protoplast transient expression assay – reciprocal assays
  - Promoter deletion analysis to identify functional ZREs

34% identity between ZIP4 and ZNT1 promoters

```
1    AACTCCCCATCTTA-CAAAGTT-ACCGTCCTTTTAGCTTAAGCTGCCTACTTCTCATCCTT-TTTCAGCTTAAGCTACTCC---------------TAATC--ATCCTT--TTAAACCTA pAtZp4promoter
1    GACTGAAGATGGCAGCAATGTGGAGAGGGAAGAGAGGAAGAGCTTTCTGGAAATATTAATTATGTTGGTTGGGAATATACCAAGGGAGAGGTGAAACAATCCAATCCTTAACCCAAGCTA pZnprom_typeI_F

99   CGGCTT--TAAGTTTTTTTTTAACTC---ATATAATCTTCTGCAGTAGACTTGACTTAATCGGATTTTCTGTTTCATGAACTTGTTGGTAGTGTG-GAACAAATGGGAAAATGAATATTT pAtZp4promoter
121  CCTCCTAATAATCCTTTTTTTTAGCTTCCGGTTTAGTGTGTTGAAGTTGTTAAACTCCTTTTATAACCGTTGTTATGTAATCGTTTTTGTATTTCATGAACAAATGGATGAA-GAATATTT pZnprom_typeI_F

213  T---TGGAACAAATTGATTTTCTGTTTCATAT-TAAGTTAAATCATTCTGTTTCCACTGAAATAAATTGTTTTCCAAAAATCACTCCGTTTATTATGTCTTTGTTTTTAAGAAATAAAAG pAtZp4promoter
240  TAGTTGGAACCA-TCATTACAATATTTTACTTGTCAACTAAAA-ATGTAAATGCAATTAGCACTCTCCATCTACAATCTGTCAACCT-TGTGCAATCAATAAAAATGTAGATAATAATAC pZnprom_typeI_F

329  TGAGAAAACAGAATAACGCGAAAATGTCGACATATTTGGCTAAGTATAGACAAGATTGGGAAGCTCTGTTTAGTTAT--GCGT---CAGTC--TCTCATCAGTGTTCAACTGCCACGGAG pAtZp4promoter
357  TAGCATCTTTTAACTTTAATATTATTTTCCAAATTTTGTGTGGAAAAAAATGAAAAGAGAGAATAACGTGAAAATGTCGACATAATTAGCCAAATATACAAGTGTACAAGTGCCACGGAG pZnprom_typeI_F

442  CGAACCGATTCCTAATTGCAACGTCCCGAGTCCATAGAATGTCGACACTCTTTCACTCTTTCTCCAAGTTGCCTCCTTTGAGTCCTTTCTCATATTTTATAGACTCACTTTCTGTTTCTT pAtZp4promoter
477  CGAGCGAGCGGTT--CCGCAAC-TCCAACTTGCTGAGAATGTCGACACTCTTTCGCTCGTC-----GATT-CCTCCTTT---TTTCTAATC-TGCTTT-TCGGTTAGCTTTCTGCTCCTT pZnprom_typeI_F

562  GATCCCGAGGAAGAAGAAGAATAAACTCTT-GTTCCCATGATCTTCGTCGATGTTAGT---------ACCTTGAGATTCGATT--CGTCTCTCCGATTCTTTATAATCG-GAATTTATTG pAtZp4promoter
583  GATCCCGAGAAA-AAGAAGAAGACACTCTTTGTTCCCATGATCATCGCCGATGTTAGTCATATATCCACCTTGGGATTCGTTTTCCGTCTCTCCACTTTTCTATATTCTTGAATTTACTG pZnprom_typeI_F

669  GTTT--------GTGGAATCTGATTTT-GGGTTCTCTTCTATTGCTCTTATAGGTTCTTTGGAAATTGTTTCCTCTATACTCGTTTGGATCAGGAAGAGACTCTCTCTCAGGTATTTCAT pAtZp4promoter
702  TTTTCTCTTTTTGTGGAATCTGATTTTCGAATTCTCTTCTCTTGGTCTTA--GCTTCTTTGGAAATCGTTTCCTCTATACTTGTTCAGATCAGGAAGAGATTCTCTCTCAGGTATTTCGT pZnprom_typeI_F

780  CGAACACTTACCCTACAATTTAGTGAAATTTCTGTGTGATGTAGAATTCTCTAGATTCTTCTAATCGAGTTTTAGCTGAGAAAATTAGTGAAATGAAATGAGATTTTCCATTGATTGCT- pAtZp4promoter
820  CCAACACTTGTTTCAT--TTCAACC---TCACAATTCAGTG-AGA---CTCTG--TTTCACCGCTCAA---TTATCTGTCTTCTTCCATCT--TCCAATCGAATTTCCCCTTGATTGCTC pZnprom_typeI_F

899  -AGATTCCAAAACTTTAGCTTGTTGTTCTGAATTGCACTTATTTGTTGTGTTGTATGGACCATGGAAACATCCAAAAGAGTCCATTTTGCAGA--------TCATTCCC---GA------ pAtZp4promoter
924  TAGATTCCGAAACTGTAA---ATTGTTCTGAATTGCCCTC-TCTGTTGC--TCTATGGACCATGAAAGC-CGAAAAAGAGTCCATTTTGCAGACAATGGCTTCATCTCCCACGAAAATCC pZnprom_typeI_F

1001 -----GACA                                                                                                                  pAtZp4promoter
1037 TCTGTGATG                                                                                                                  pZnprom_typeI_F
```

ZIP4 promoter ZRE

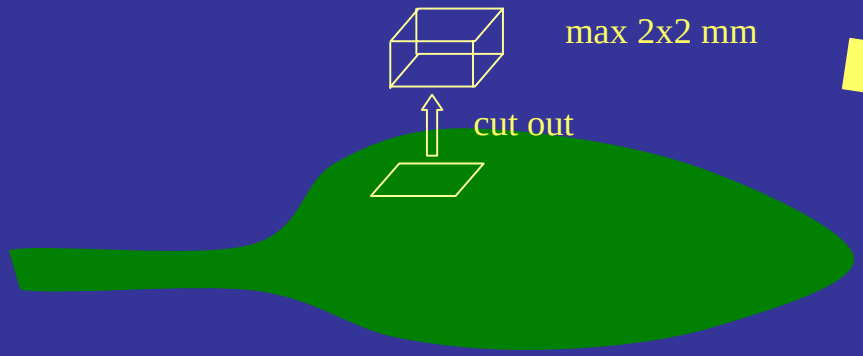
ZNT1 promoter ZRE

Elucidating mechanisms of Zn-dependent gene regulation in *Thlaspi caerulescens*

- Transcription factor analysis – E2F1/2:
  - Test E2F1/2 DNA binding to *ZNT1* promoter via DNAse I footprinting and electromobility shift assays
  - Characterize T-DNA knock-out line for *AtE2F2*
  - Search for other trans-factors using yeast 1-hybrid analysis

A Novel Quantitative *in situ* mRNA Hybridization Technique for Rapidly Determining Cell Specific Heavy Metal Transporter Gene Expression

Overview of *in situ* mRNA hybridization method



vacuum infiltrate with alkaline fixation solution

dehydrate

extract hydrophobic compounds

rehydrate

digest proteins

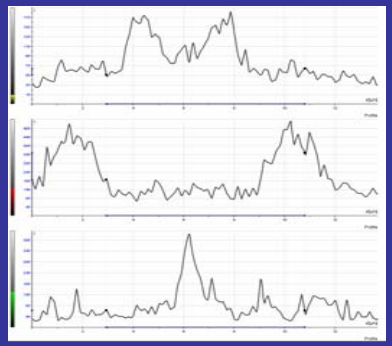
postfixate

(denature)

hybridize with fluorescently labeled oligonucleotides

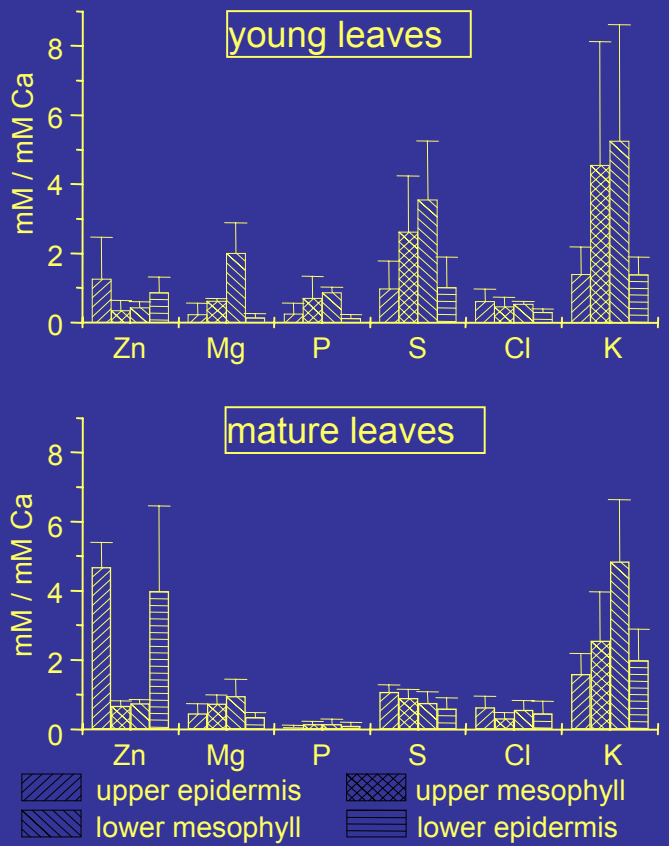
record images in CLSM

quantify

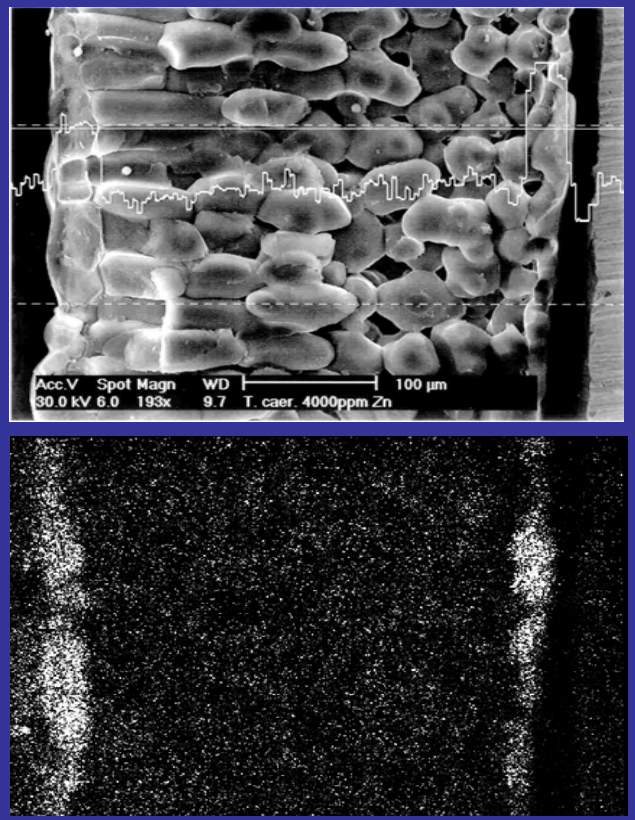


Compartmentation of metals in leaves of *Thlaspi* and *Alyssum*
Zn/Cd/Ni accumulation in epidermal vacuoles

Compartmentation in leaves of *Thlaspi caerulescens*



Concentrations of elements in leaf tissues

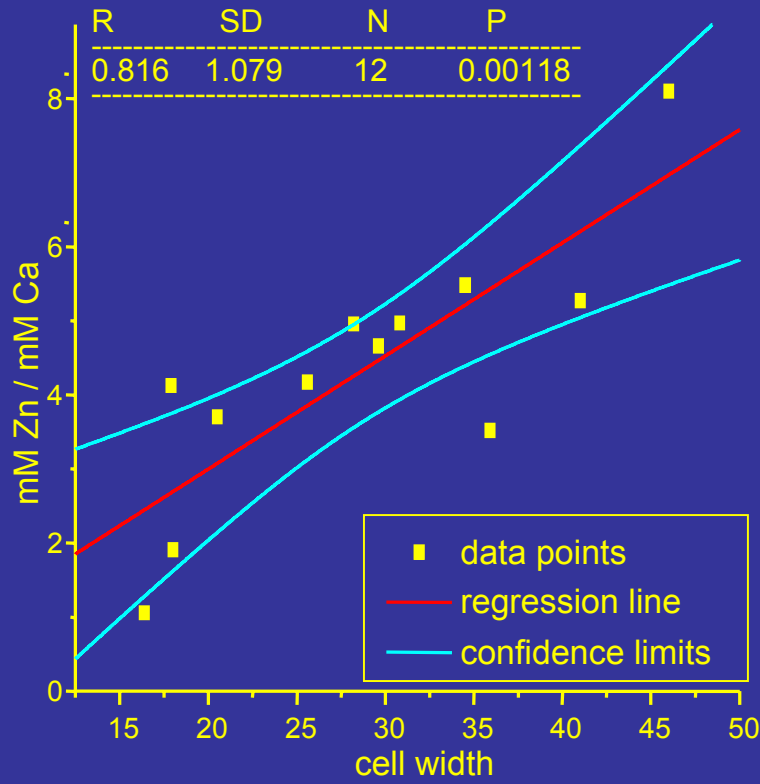


Zn K α line scan and dot map of a leaf

Linear Regression: Y = A + B * X

Parameter	Value	Error
A	-0.057	1.027
B	0.153	0.034

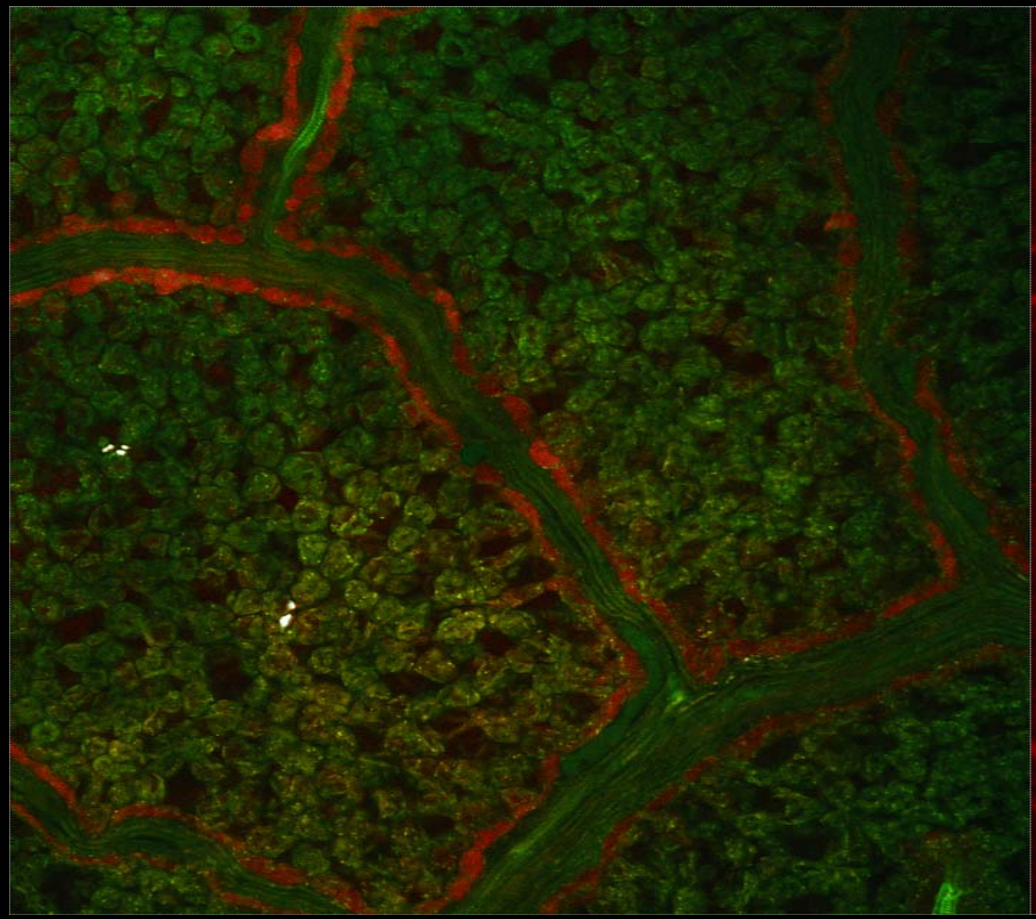
R	SD	N	P
0.816	1.079	12	0.00118



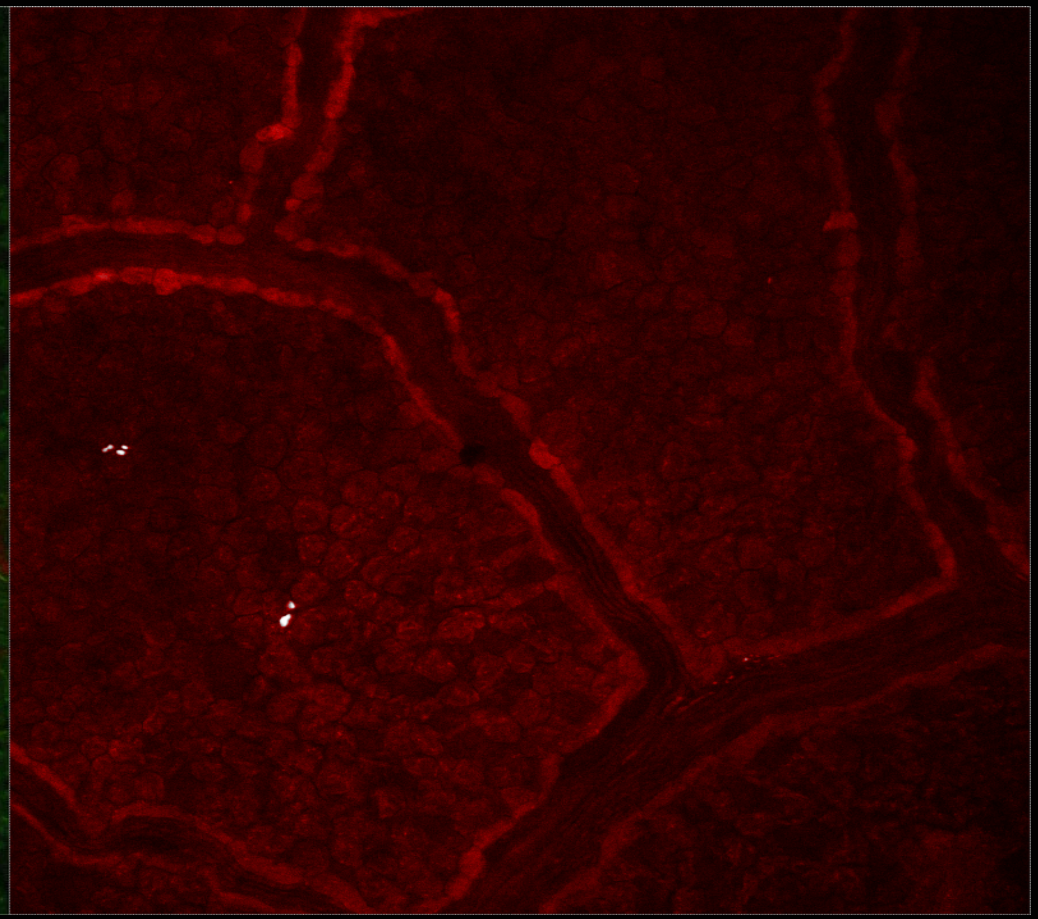
Correlation between cell size and zinc concentration

Küpper H, Zhao F, McGrath SP (1999) Plant Physiology 119, 305-11

ZNT1 mRNA localization in young leaves of *Thlaspi caerulescens*:
Mesophyll, antisense probe

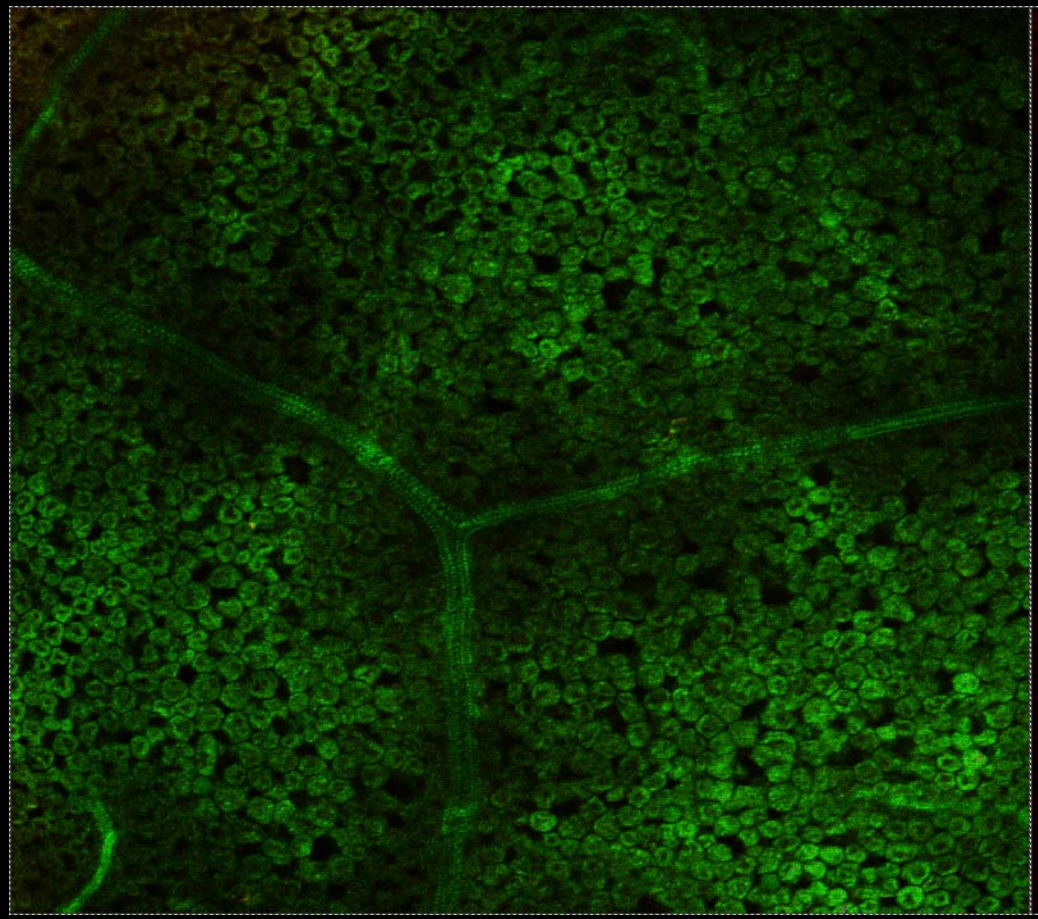


Overlay of green autofluorescence and red oligonucleotide fluorescence

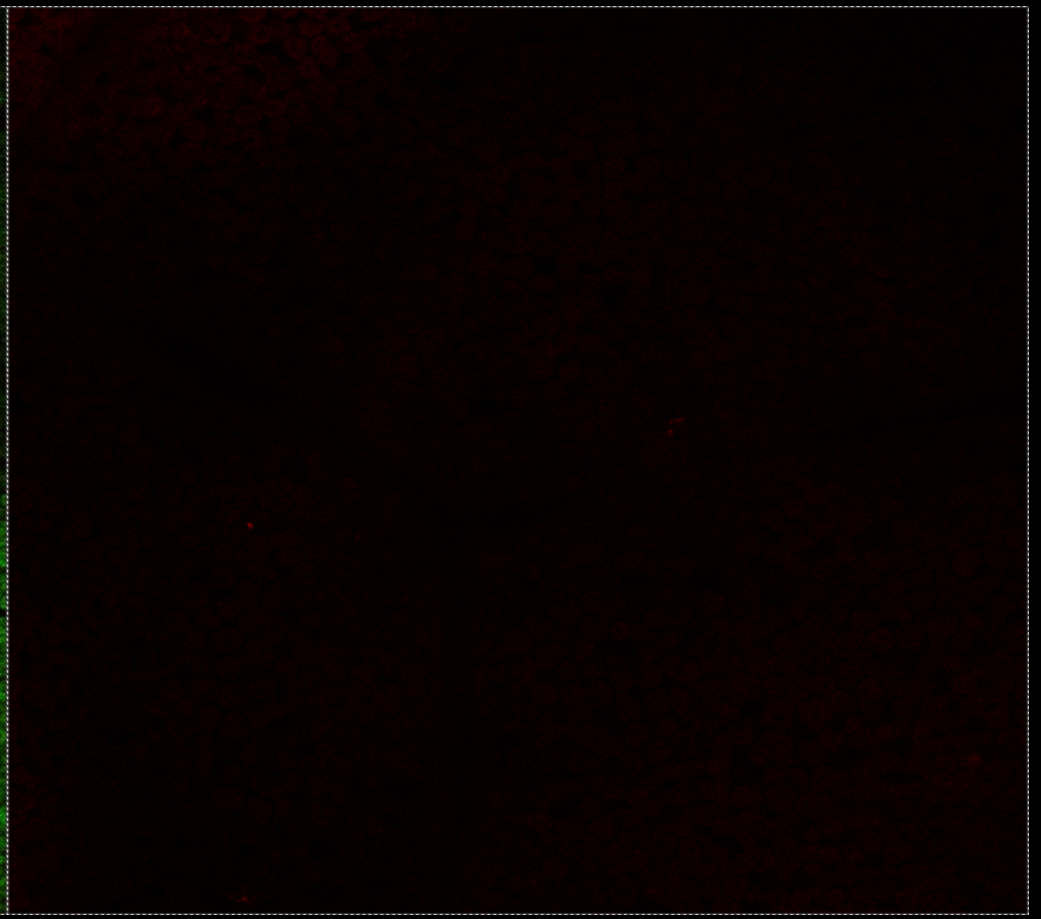


Red oligonucleotide fluorescence

ZNT1 mRNA localization in young leaves of *Thlaspi caerulescens*:
Mesophyll, control with sense strand probe

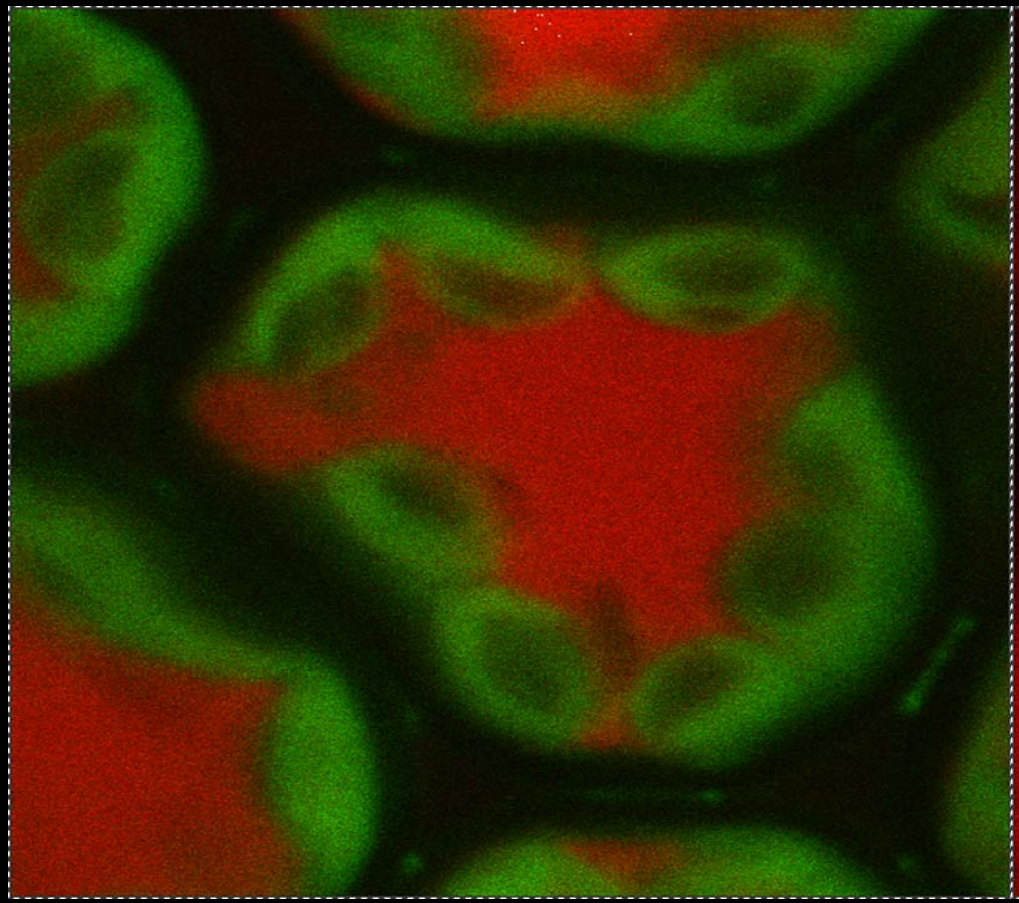


Overlay of green autofluorescence and red oligonucleotide fluorescence

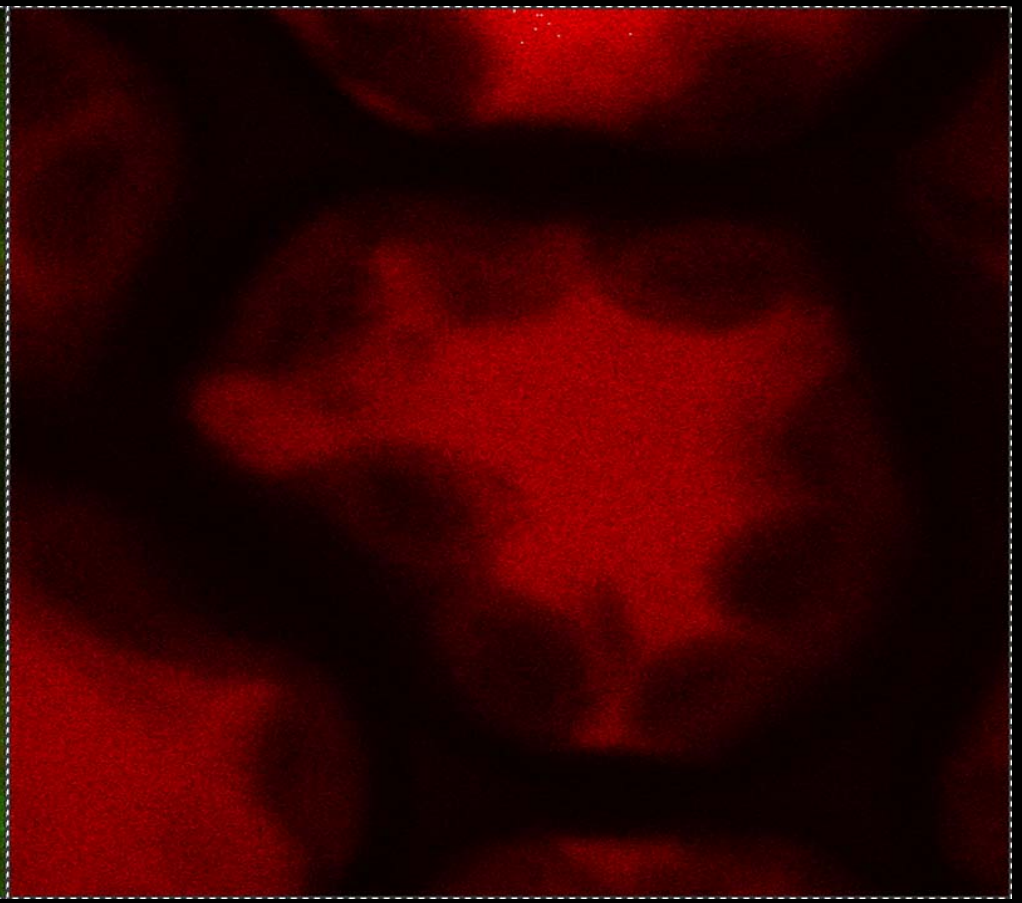


Red oligonucleotide fluorescence

ZNT1 in young leaves of *Thlaspi caerulescens* Prayon:
mesophyll, antisense probe

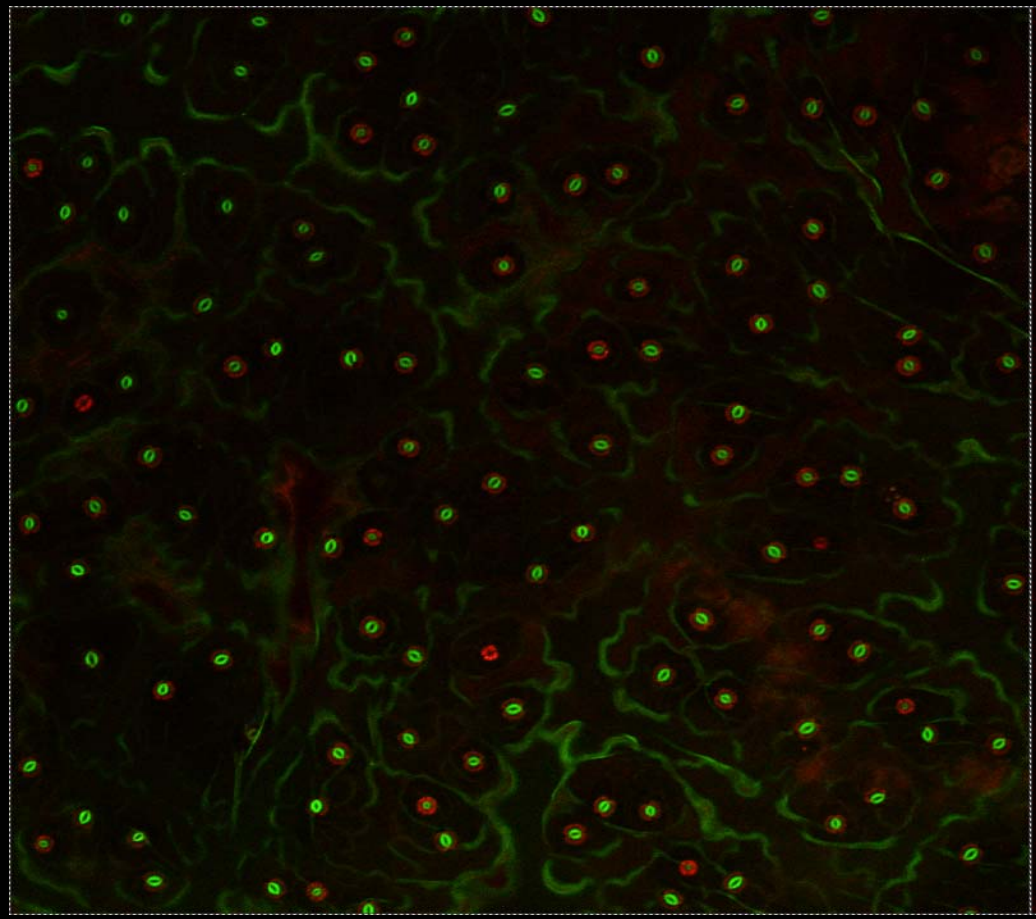


overlay of green autofluorescence and
red oligonucleotide fluorescence

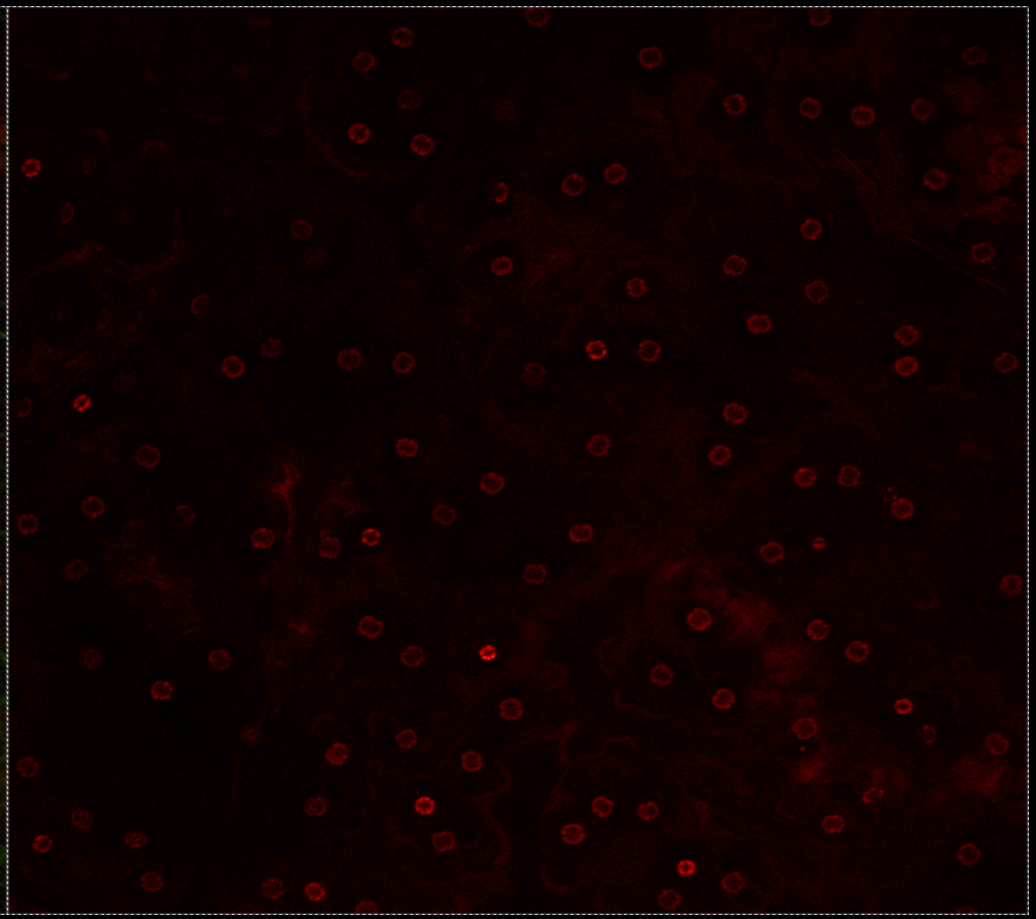


red oligonucleotide fluorescence

ZNT1 mRNA localization in young leaves of *Thlaspi caerulescens*:
Epidermis, antisense probe

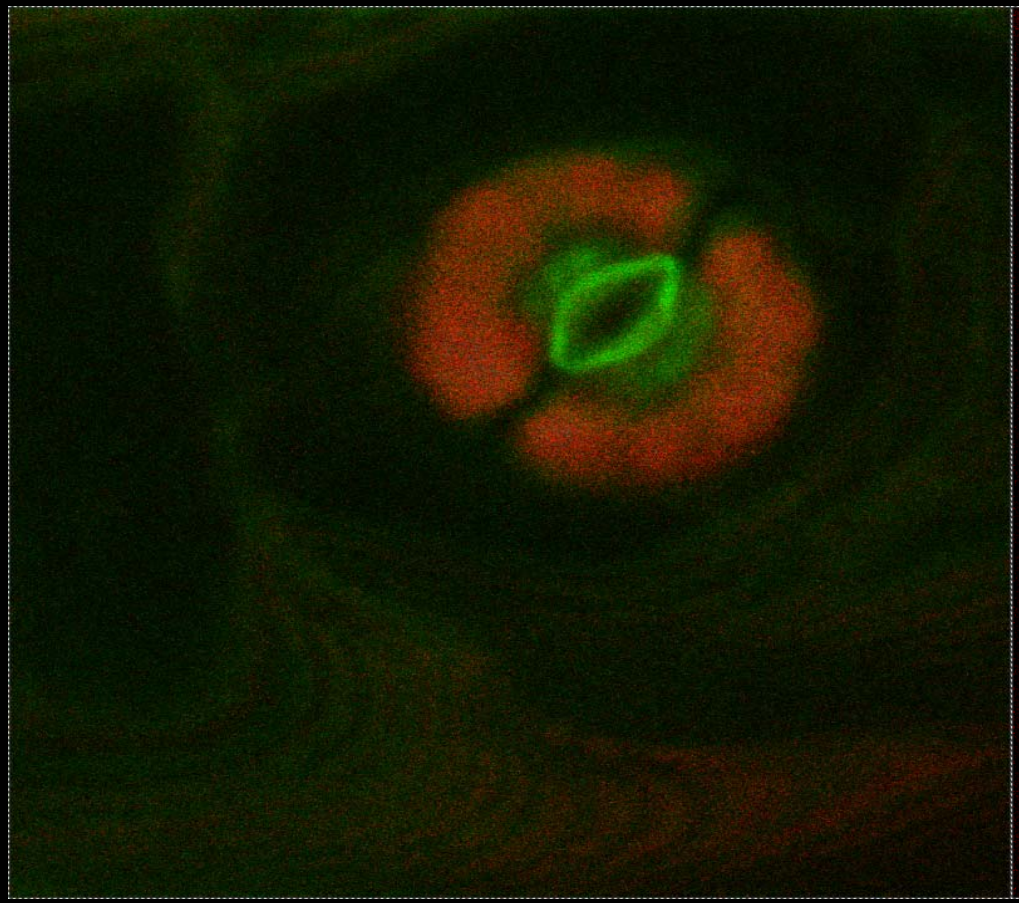


Overlay of green autofluorescence and red oligonucleotide fluorescence

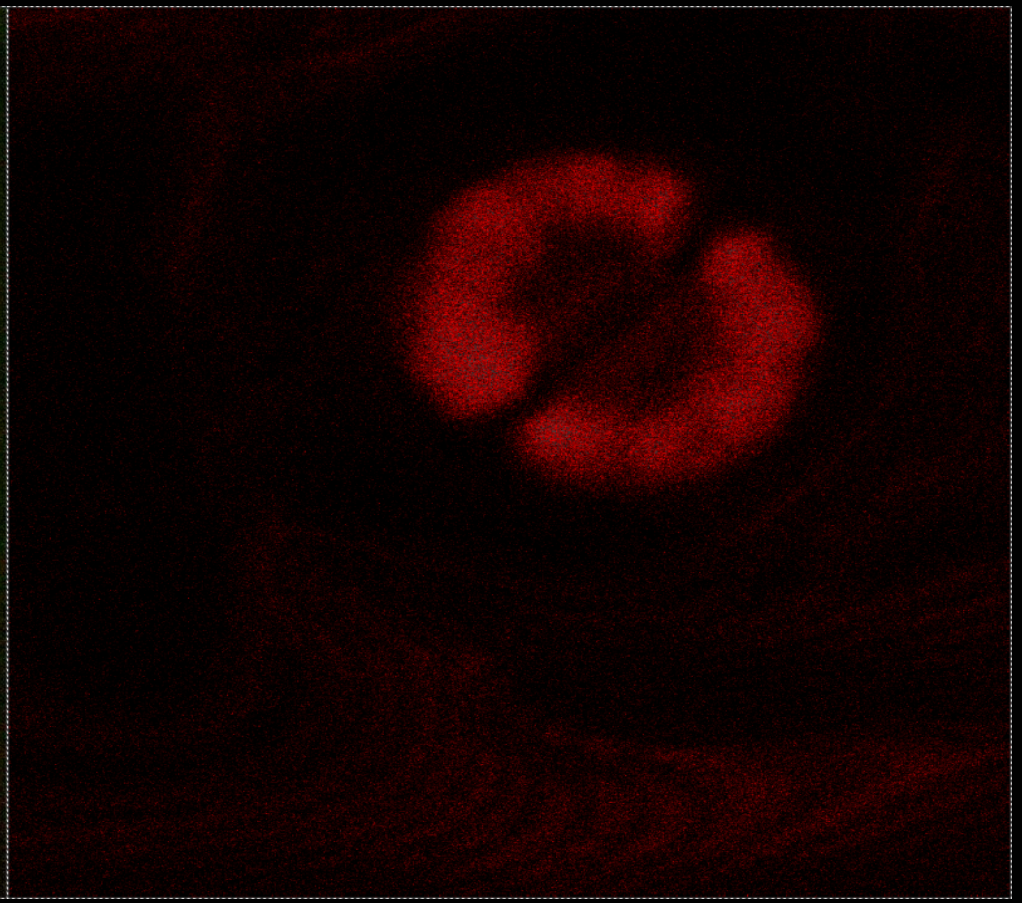


Red oligonucleotide fluorescence

ZNT1 mRNA localization in young leaves of *Thlaspi caerulescens*:
Stomata, antisense probe



Overlay of green autofluorescence and red oligonucleotide fluorescence



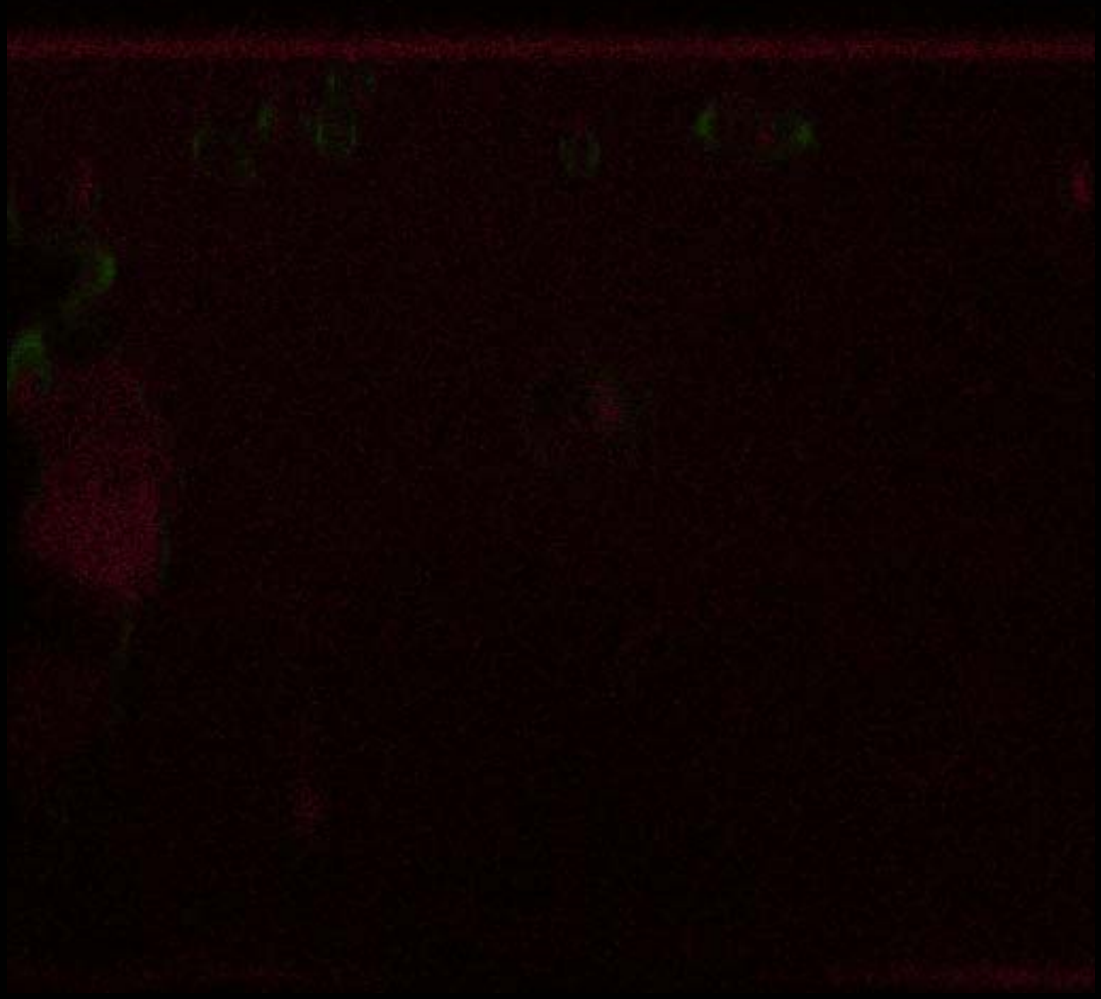
Red oligonucleotide fluorescence

Summary of *ZNT1* Leaf Localization Studies

1) *ZNT1* expressed primarily in photosynthetic cells - guard cells in epidermis and mesophyll cells in leaf interior (high stringency & high affinity conditions)
2) *ZNT1* appears to be involved in "normal" leaf Zn nutrition and not hyperaccumulation
3) Related members of Thlaspi ZIP family expressed in specialized large epidermal cells (metal accumulating cells?) and around veins (transfer from xylem to leaf?)

ZNT1 mRNA localization in roots of *Thlaspi caerulescens*:

Starting at tip of mature root, antisense probe



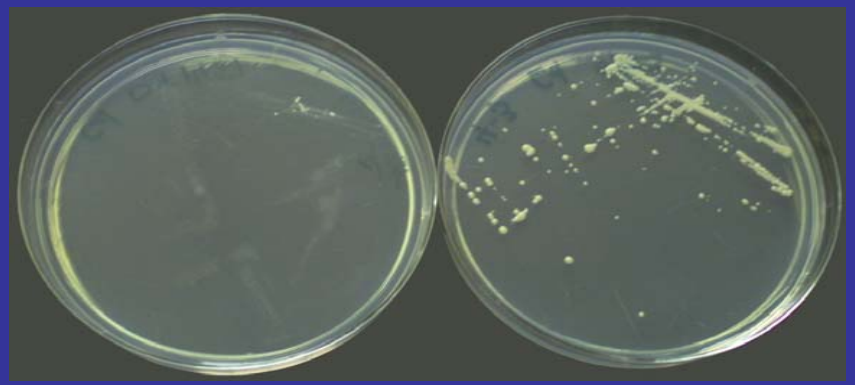
Series of optical cross sections moving back from root tip -
overlays of green autofluorescence and red oligonucleotide fluorescence

Searching for Thlaspi Cadmium Tolerance Genes

Zn Tolerance Genes – Melinda Klein

Cd Tolerance Genes – Ashot Papoyan

- Yeast transformed with *T. caerulescens* cDNA library are grown on high Zn or Cd plates.
- Tolerance phenotype is confirmed by replating; insert is isolated and identified.



Susceptible and Tolerant yeast growing on metal enriched SD plates.

Thlaspi Genes Conferring Cd Tolerance in Yeast

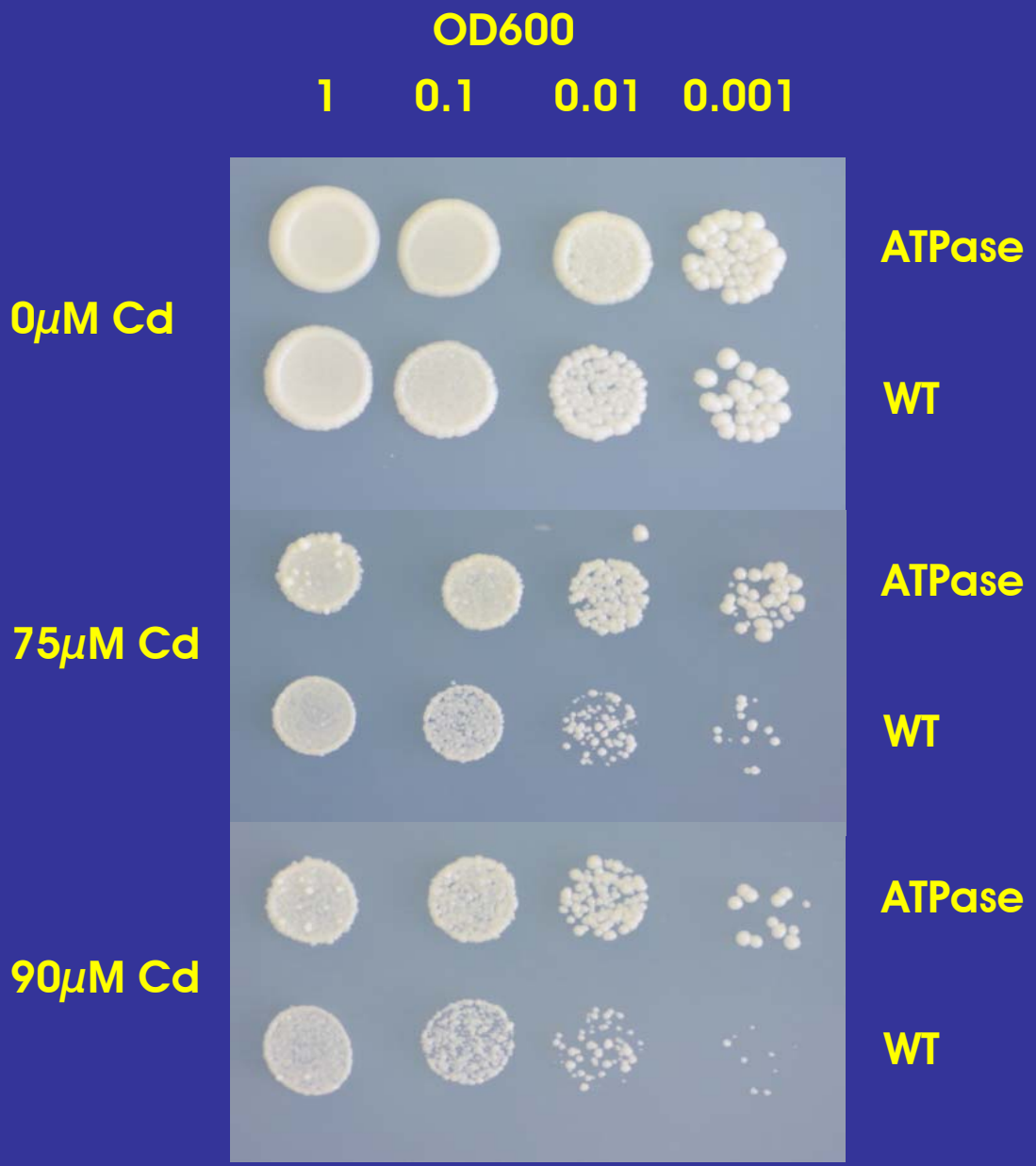
Clone	Closest Match and Function	Accession #	GenBank Hit	Reference
1	*Brassica juncea* metallothionein-	AY486002	Y10849	Schafer et al. (1998)
	like protein			
2	*Arabidopsis thaliana*	AY486003	L15389	Zhou et al. (1994)
	metallothionen			
3	*Arabidopsis thaliana*	AY486004	U15130	Yeh et al. (1994)
	metallothionen			
4	*Arabidopsis thaliana*	AY486005	NM114760	Town et al.(2003)
	60S ribosomal protein L13			
5	*Arabidopsis thaliana* putative	AY486006	NM_118925	Town et al (2003)
	protein			
6	*Sinapis alba* subunit of	AY486007	Y07498	Wenng et al. (1989)
	of photosystem II			
7	*Arabidopsis thalina* light	AY486008	Z12120	Park et al. (1992)
	regulated kinase			
8	*Arabidopsis thalina* putative	AY486009	CAB87795	Rieger et al. (2000)
	protein			
9	*Arabidopsis thalina* putative	AY486001	AF412407	Richaud et al.(2001)
	heavy metal P-type ATPase			

Alignment of 3’ ends of *Arabidopsis* and *Thlaspi* HMA4’s

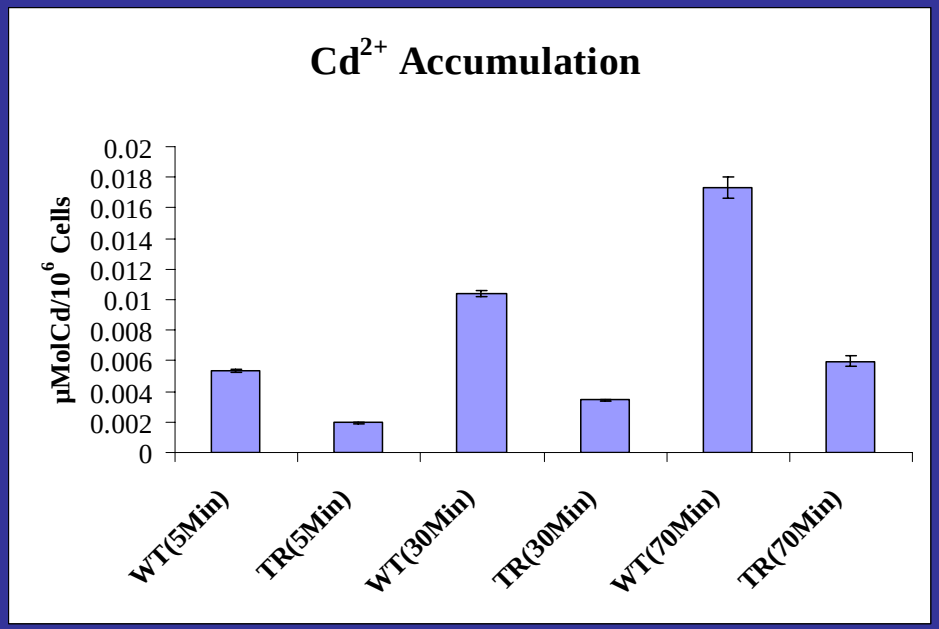
```
Athma4 ASTSKLNGRKLEGDDDYVVDLEAGLLTKSGNGQCKSSCCGDKKNQENVVMMKPSSKTSSD 776
Tchma4 --------KKLEGVDDQGLDLEAGLLSKS---QCNSGCCGDQKSQEKVMLMRPASKTSSD 768
       :**** **  :*******:**   **:*.****:*.**:*::*:*:******
Athma4 HSHPGCCGDKKEEKVKPLVKDGCCSEKTRKSEGDMVSLSSCKKSSHVKHDLKMKGGSGCC 836
Tchma4 HLHSGCCGEKKQESVK-LVKDSCCGEKSRKPEGDMASLSSCKKS---NNDLKMKGGSSCC 824
       * *.****:**:*.** ****.**.**:**.****.********   ::********.**
Athma4 ASKNEKGKE-VVAKSCCEKPKQQVESVGDCKSGHCEKKKQAEDIVVPVQIIGHALTHVEI 895
Tchma4 ASKNEKLKEAVVAKSCCED-KEKTEGNVEMQILNLERGSQKK----------------- 865
       ****** ** ********. *::.*.  : :  : *: .* :
Athma4 ELQTKETCKTSCCDSKEKVKETGLLLSSENTPYLEKGVLIKDEGNCKSGSENMGTVKQSC 955
Tchma4 ---VGETCKSSCCGDKEKAKETRLLLASEDPSYLEK-------EERQTTEANIVTVKQSC 915
       . ****:***..***.*** ***:**:.****        : :: . *: ******
Athma4 HEKGCSDEKQ--TGEITLASE---EETDDQDCSSGCCVN-EGTVKQSFDEKKHSVLVEKE 1009
Tchma4 HEKASLDIETGVTCDLKLVCCGNIEVGEQSDLEKGMKLKGEGQCKSDCCGDEIPLASEED 975
       ***.. * :   * ::.*..    *  ::.* ..*  :: **  *..   .: .:  *::
Athma4 GLDMETGFC-------------------CDAKLVCCGNTEGEVKEQCR----LEIKKEE 1045
Tchma4 SVDCSSGCCGNKEELTQICHEKTCLDIVSCDSKLVCCGETEVEVREQCDLKKGLQIKNEG 1035
       .:* .:* *                    **:******:** **:***     *:**:*
Athma4 HCKSGCCGEEIQTGEITLVSE--EETESTNCSTGCC---------------VDKEEVTQ 1087
Tchma4 QCKSVRCGDEKKTEEITEETDNLKSESGDDCKSPCCGTGLKQEGSSSLVNVVVESGESGS 1095
       :***  **:* :* ***  ::  :. .. :*.: **               *:. *  .
Athma4 TCHEKPASLVVSG---LEVKKDEHCESSHRAVKVETCCKVKIP--EACASKCRDRAKR-H 1141
Tchma4 SCCSKEGEIVKVSSQSCCASPSDVVLSDLEVKKLEICCKAKKTPEEVRGSKCKETEKRHH 1155
       :* .* ..:*  .     .. .:   *. .. *:* ***.* .  *. .***::  ** *
Athma4 SGKSCCRSYAKELCSHRHHHHHHHHHHHVSA 1172
Tchma4 VGKSCCRSYAKEYCSHRHHHHHHHHHVGAA- 1185
        ********** *************  .:
```

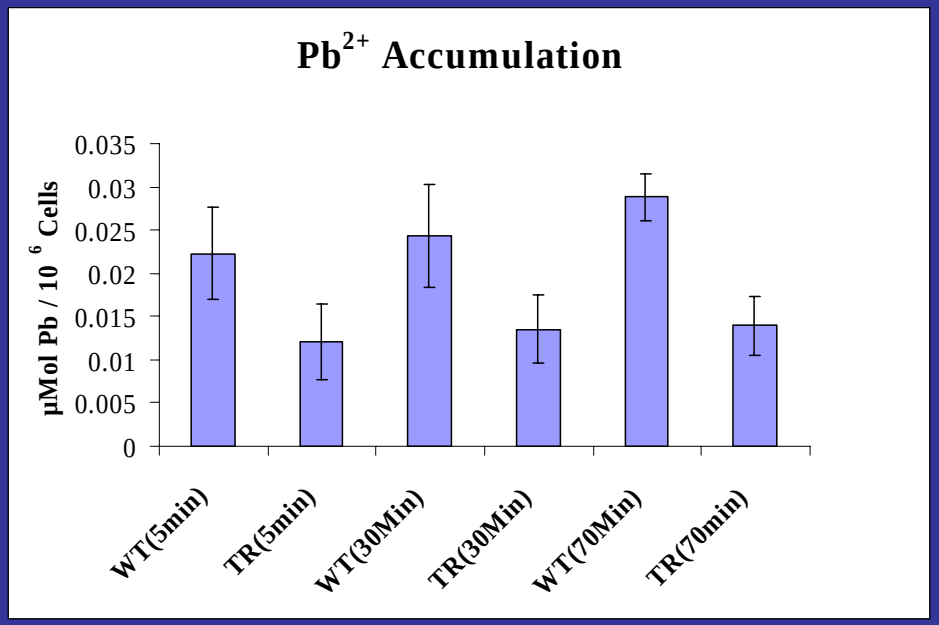
* HMA Domain (**GMTCxxC**) is missing
* *Arabidopsis* and *Thlaspi* HMA4s are 71% Identical

Cd Tolerance for WT and *TcHMA4* Transformed Yeast

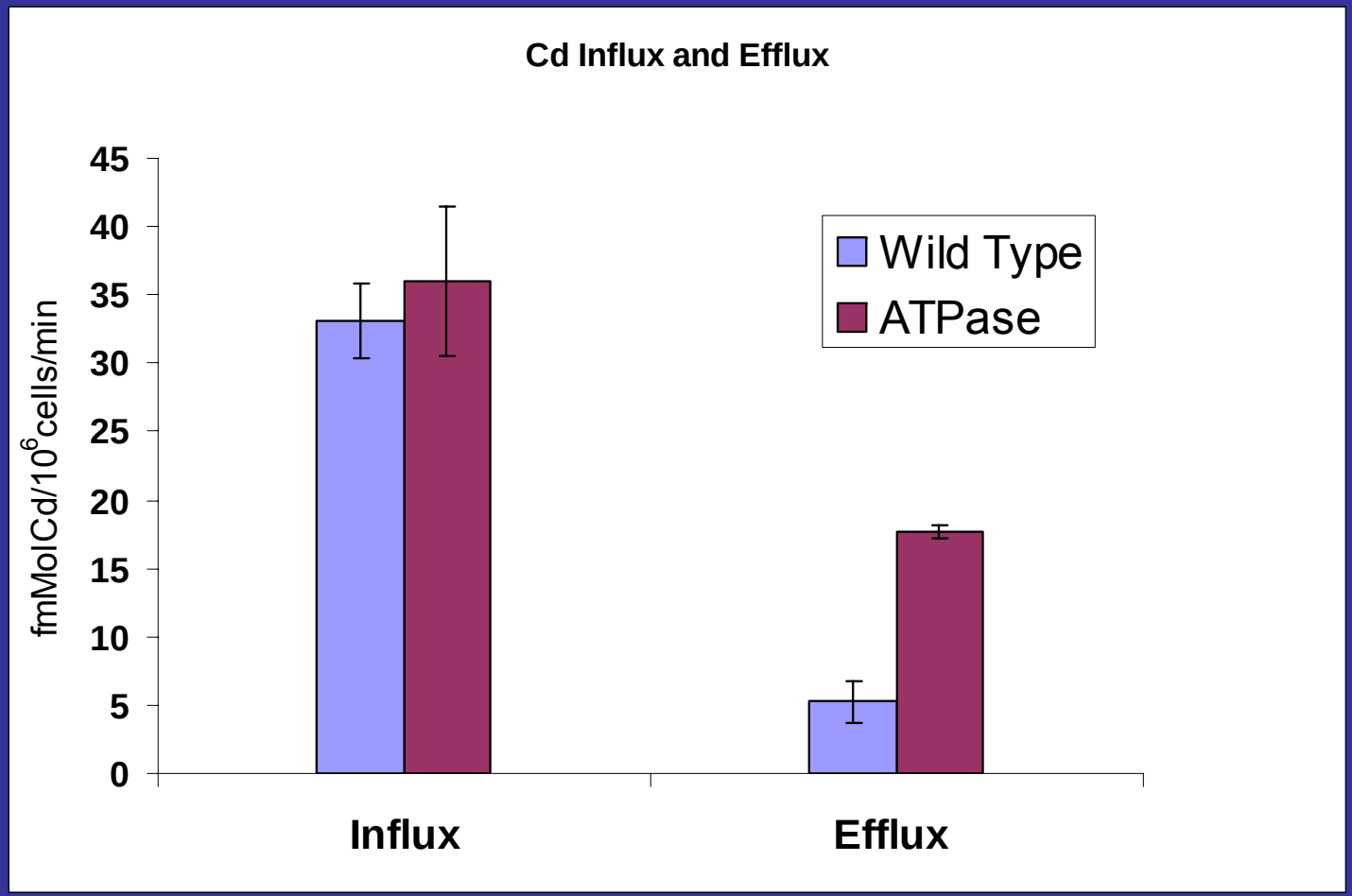


Cd^{2+} and Pb^{2+} Accumulation for WT and *TcHMA4* Transformed Yeast

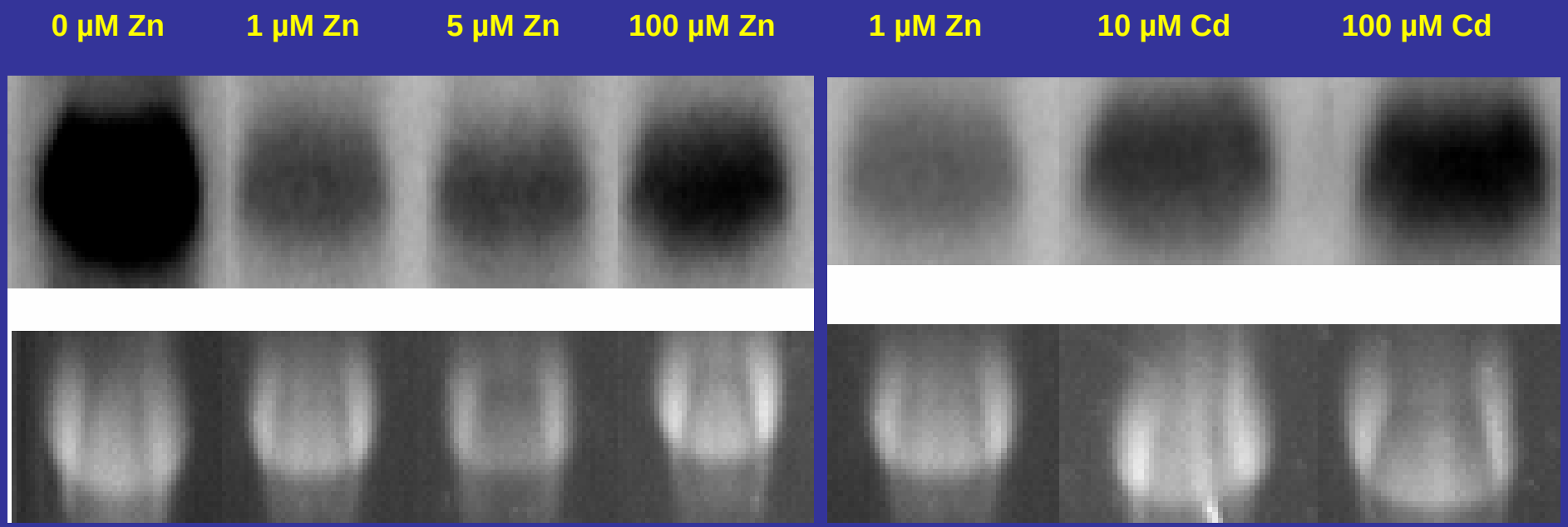




Quantification of Unidirectional ^{109}Cd Influx and Efflux in WT and *TcHMA4* Transformed Yeast



Root *TcHMA4* Gene Expression in Response to Changes in Plant Zn and Cd Status



	0 µM Zn	1 µM Zn	5 µM Zn	100 µM Zn	1 µM Zn	10 µM Cd	100 µM Cd
Root	8.9	273	409	239	273	580	4641
Shoot	29	960	3055	5805	0.2	1228	5680

µg Zn / g dwt

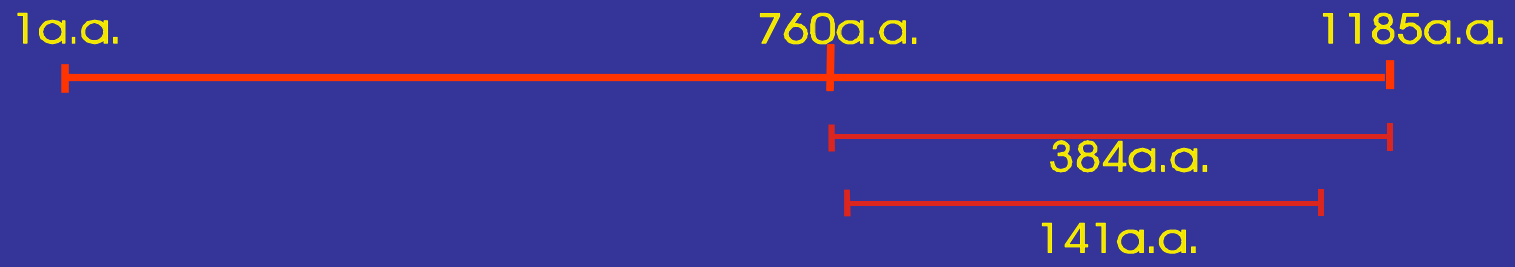
µg Cd / g dwt

TcHMA4 gene expression strongly induced both by Zn deficiency and high Cd and Zn

Does TcHMA4 Play a Role in Both Zn Nutrition and Heavy Metal Hyperaccumulation?

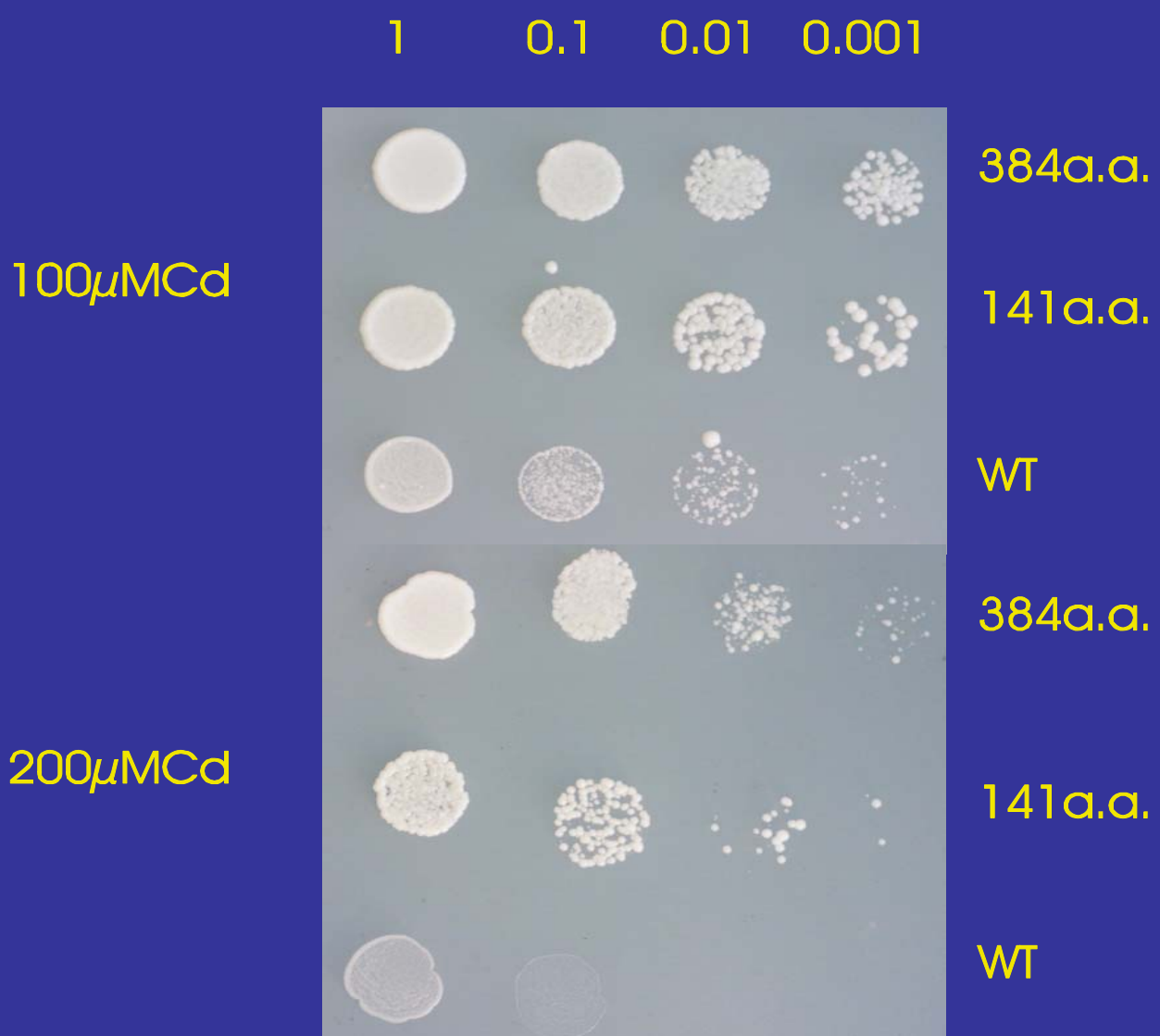
- Expressed primarily in the root
- Under Zn deficiency does it function to provide n to the shoot?
- In high Zn/Cd soils, does it function to translocate metals to the shoot?
- Preliminary results suggest it is expressed near root xylem
- Is it an efflux transporter involved in xylem loading and root-to-shoot transport?

Alignment Of Partial Clones of *TcHMA4*

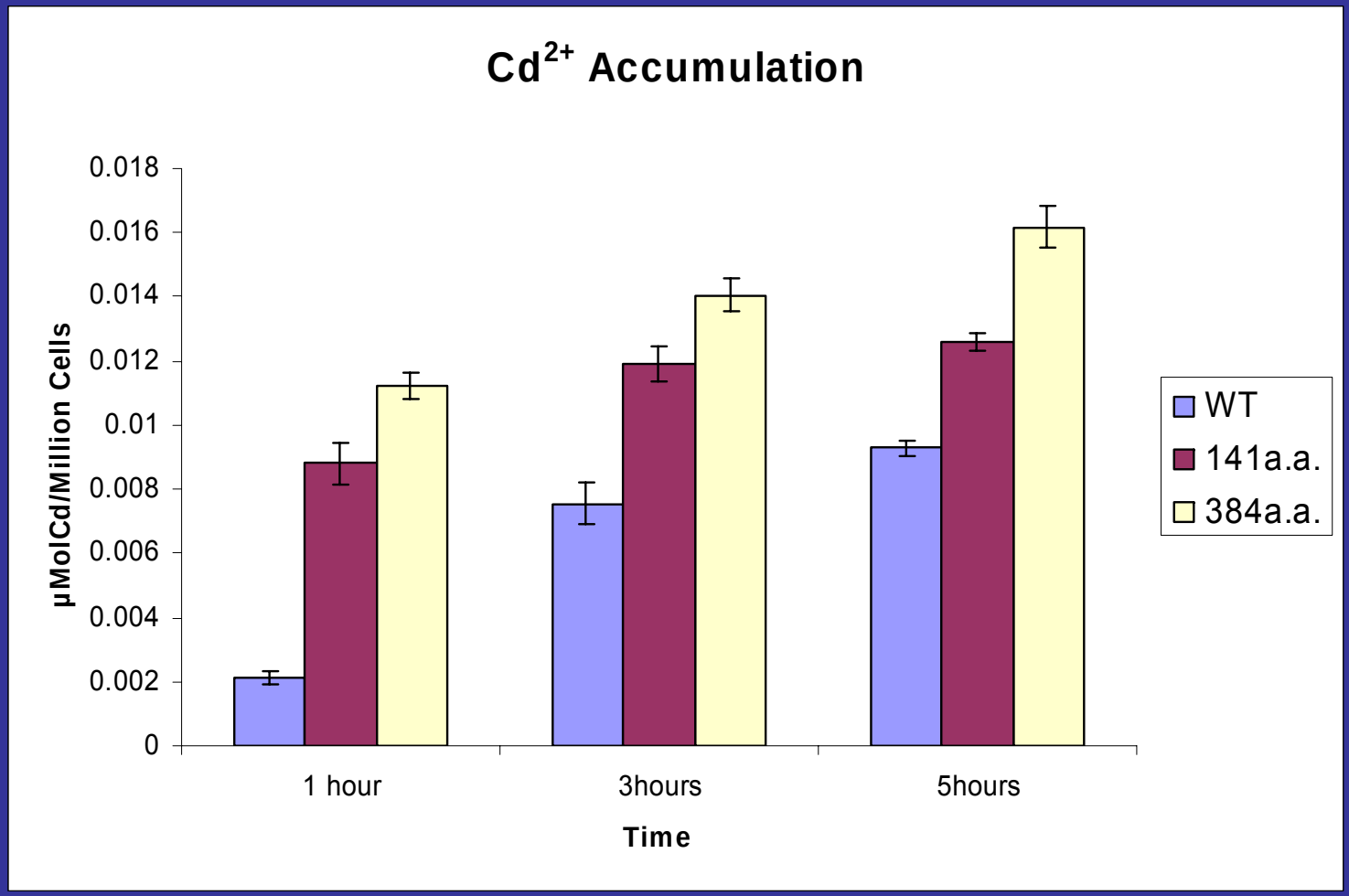



```
384aa           ----------------------------------------------MASLSSCKKSNNDLK 15
141aa           MLMRPASKTSSDHLHSGCCGEKKQESVKLVKDSCCGEKSRKPEGDMASLSSCKKSNNDLK 60
                                                              ***************

384aa           MKGGSSCCASKNDKLKEVVVAKSCCEDKEKAEGNVEMQILNLEKGSQKKVGETCKSSCCG 75
141aa           MKGGSSCCASKNDKLKEVVVAKSCCEDKEKAEGNVEMQILNLEKGSQKKVGETCKSSCCG 120
                ************************************************************

384aa           DKEKAKETRLLLASEDPSYLEKEERQTTEANIVTVKQSCHEKASLDIETGVTCDLKLVCC 135
141aa           DKEKAKETRLLLASED---------------------------------------------- 141
                ****************

384aa           GNIEVGEQSDLEKGMKLKGEGQCKSDCCGDEIPLASEEDSVDCSSGCCGNKEELTQICHE 195
141aa           ------------------------------------------------------------

384aa           KTCLDIVSCDSKLVCCGETEVEVREQCDLKKGLQIKNEGQCKSVRCGDEKKTEEITEETD 255

384aa           NLKSESGDDCKSLCCGTGLKQEGSSSLVNVVVESGESGSSCCSKEGEIVKVSSQSCCASP 315

384aa           SDVVLSDLEVKKLEICCKAKKTPEEVRGSKCKETEKRHHVGKSCCRSYAKEYCSHRHHHH 375

384aa           HHHHHVGAA 384
```

Cd Tolerance for WT Yeast and Yeast Transformed with Partial *TcHMA4* Clones

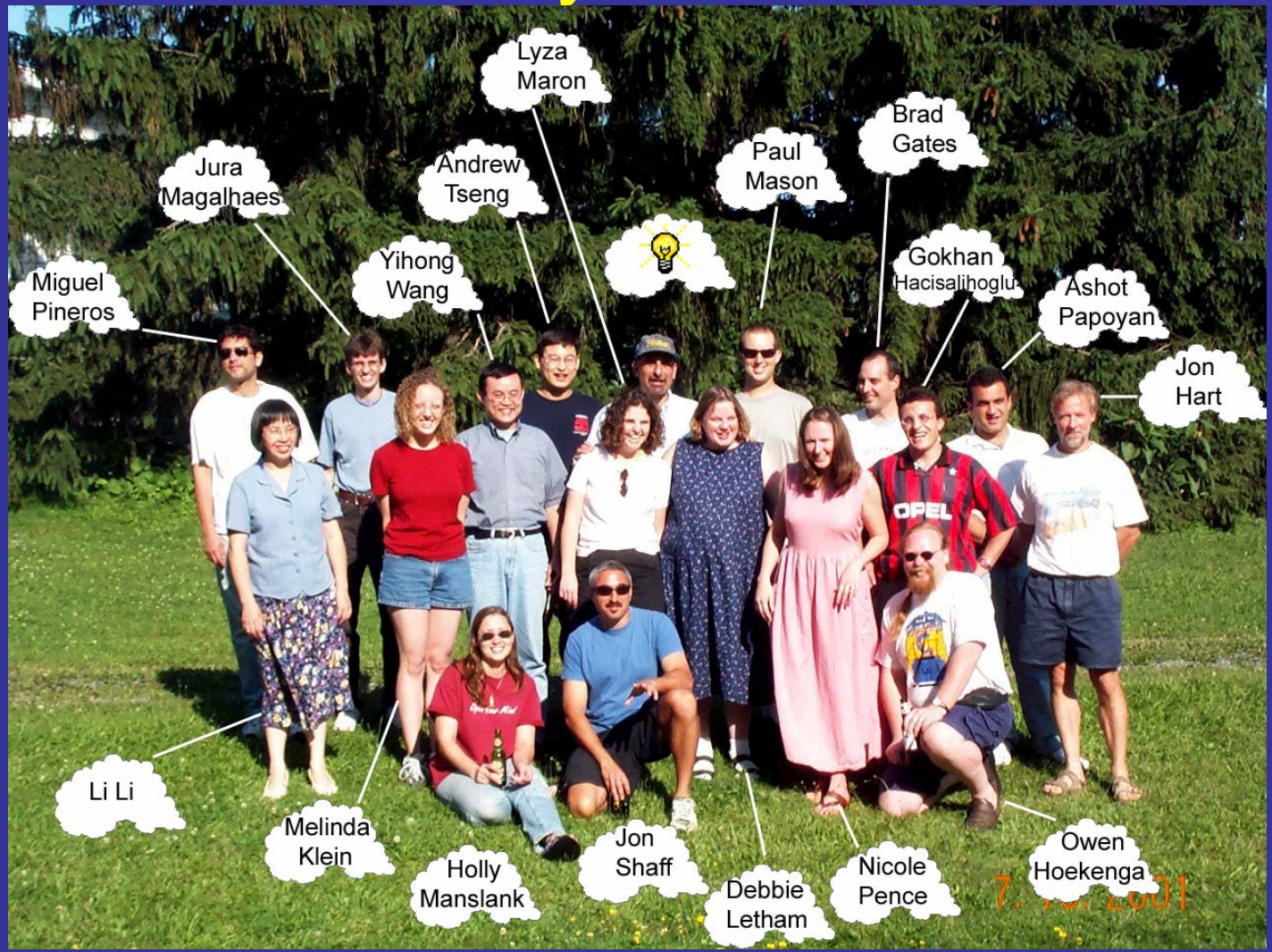


Cd^{2+} Accumulation for WT Yeast and Yeast Expressing Partial *TcHMA4* Clones



When expressed in plants, will they confer enhanced Cd tolerance and accumulation for phytoremediation purposes?

Who Actually Did the Work?



Not Shown: Hendrik Kupper and Laura Seib (*in situ* gene expression techniques), Randy Clark (sorghum Al tolerance genes)