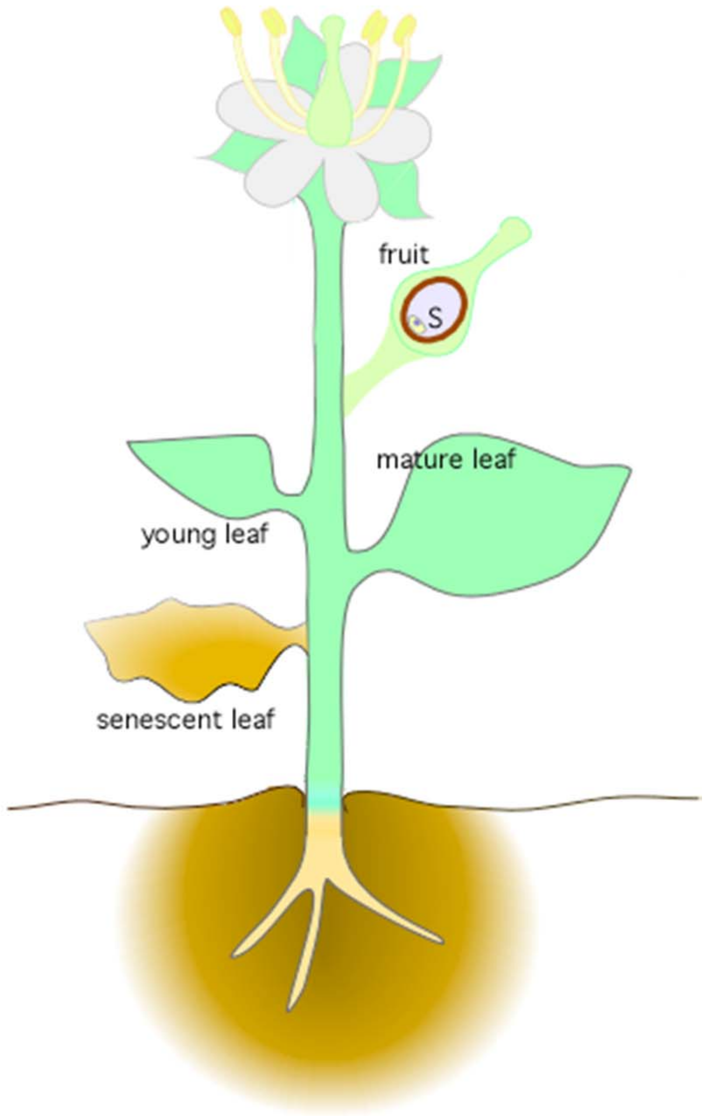
Transport et Signalisation du Fer (TSF)

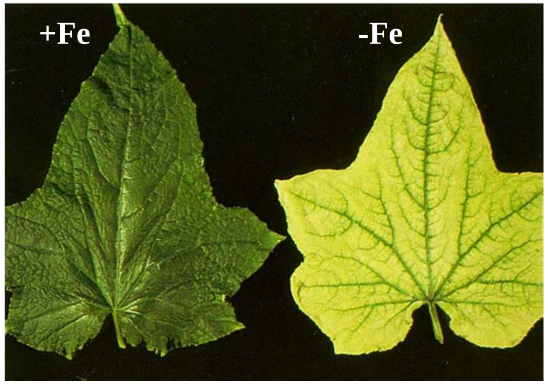


Transport et signalisation du Fer…

Iron in plants

$$Fe^{2+} \underset{e^-}{\overset{e^-}{\rightleftharpoons}} Fe^{3+}$$

Iron is essential

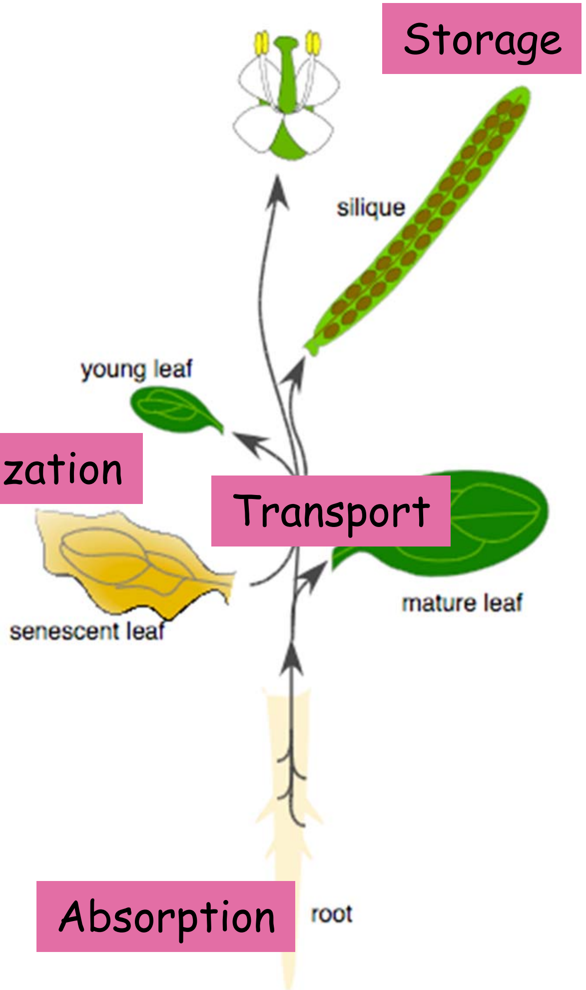


5,63% earth crust
BUT
2/3 arable lands with very low iron availability (alkaline calcareous soils)

$$Fe^{3+} \rightleftharpoons Fe^{2+}$$

pH > 7

$Fe(OH)_3$ insoluble



…but toxic!

Oxidative stress:

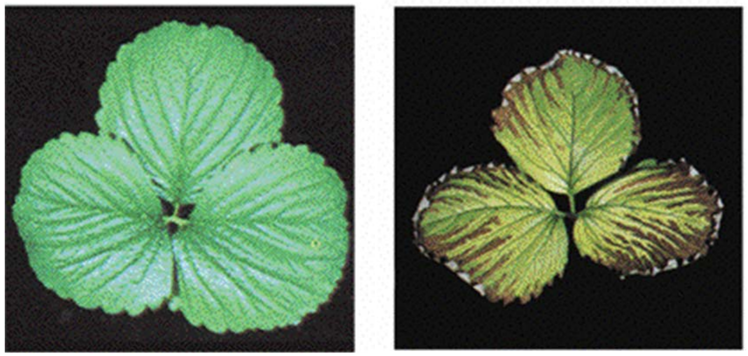
Free radicals production via the Fenton chemistry

Manganese (Mn)

3rd most abundant metal in the Earth’s crust (after Fe and Ti)

Essential for plants and many other living organisms

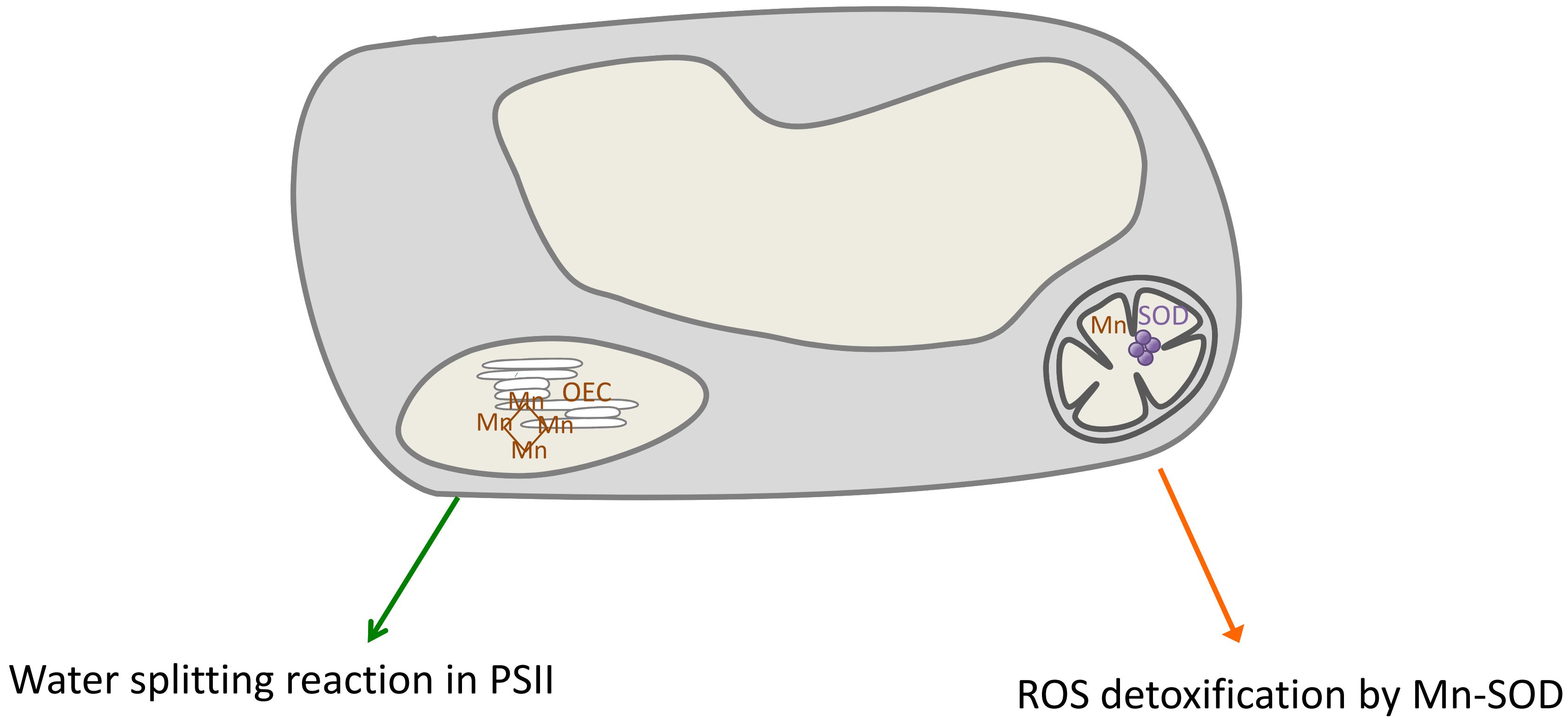
Around 20 identified functions in enzymes and proteins



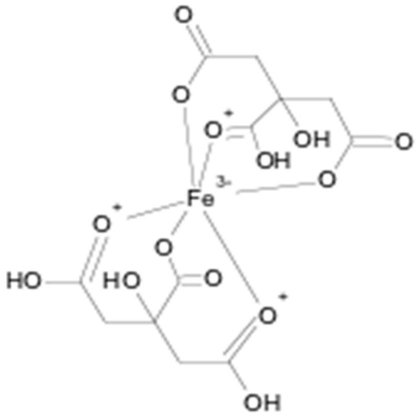
- Mn

++Mn

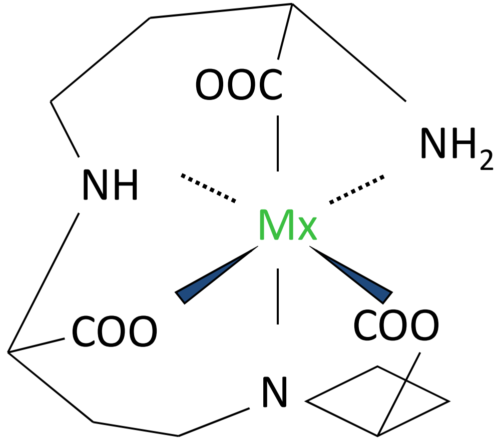
MAIN FUNCTIONS OF MANGANESE IN THE PLANT CELL



Metal ions are very reactive towards organic ligands and readily form metal-ligand complexes (chelates) by coordination bonds.



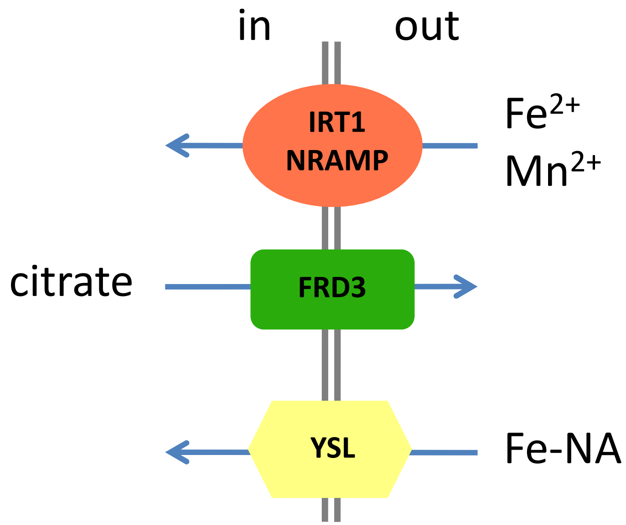
Fe-(citrate)$_2$



Metal-nicotianamine

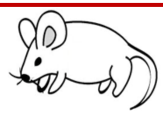
Significance and advantages of chelation:

- increase availability
- prevent formation of insoluble precipitates
- reduce toxicity
- increase mobility
- suppress growth of pathogens



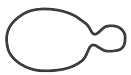
The NRAMP family of metal transporters

Mammals



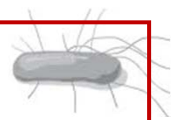
NRAMP1 and NRAMP2: Fe transport
- PM: dietary Fe uptake in intestine)
- Vesicles (resistance against pathogens in macrophages)

Yeast



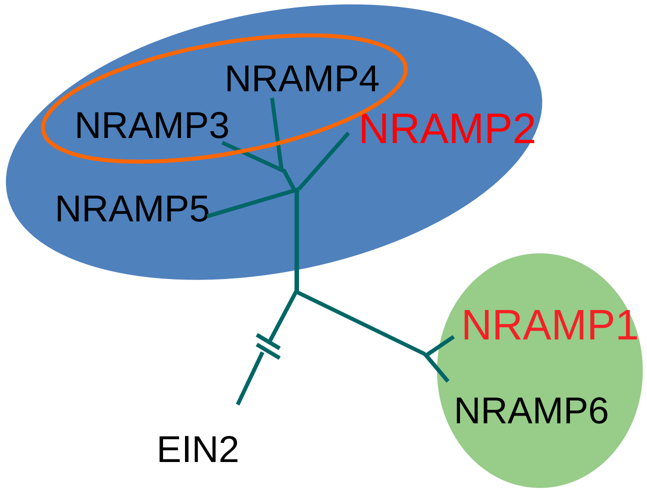
SMF1 and **SMF2: Mn** transport (PM, vesicles)
SMF3: **Fe** transport (vacuole)

Bacteria



MntH: Mn transport (PM)

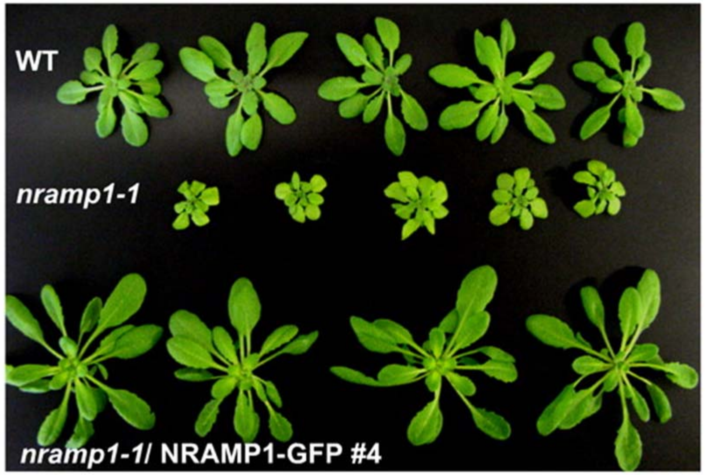
Fe and Mn vacuolar efflux



SUBSTRATES (yeast complementation assay)

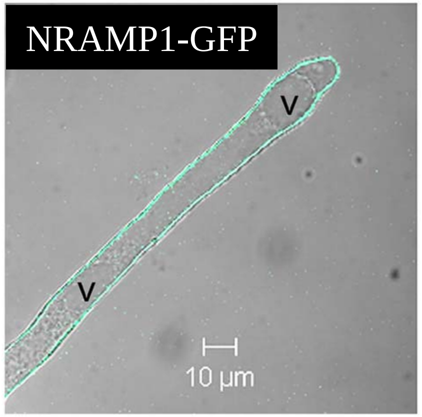
	Fe	Zn	Mn	Cd
NRAMP1	+	-	+	+
NRAMP3	+	+	+	+
NRAMP4	+	+	+	+
NRAMP6	-	-	-	+

The *nramp1-1* knockout Allele of *NRAMP1* Is Hypersensitive to Mn Deficiency.

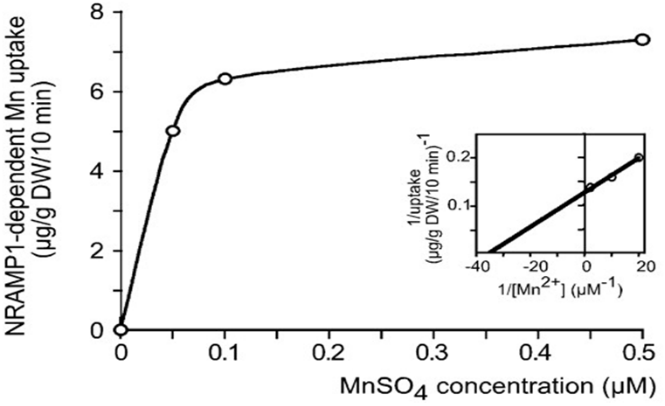


NRAMP is expressed in roots and induced by Mn deficiency.

NRAMP1 is a Plasma Membrane Protein.



NRAMP1-dependent Mn uptake



Km = 30 nM

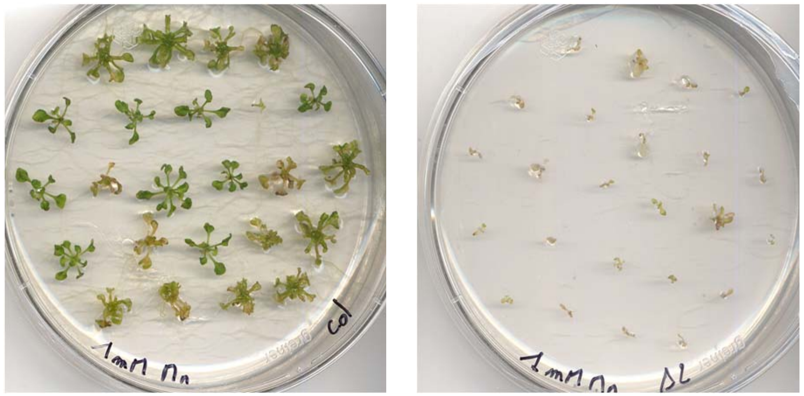
V_{max} = 0.8 ppm/min

Cailliatte et.al. Plant Cell 2010

Mn homeostasis is altered in the *nramp2-1* mutant

nramp2-1 is chlorotic and small

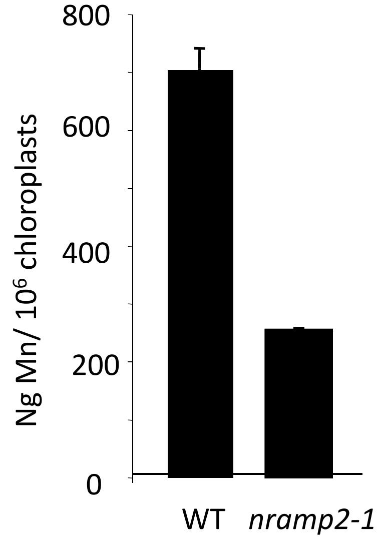
nramp2-1 is sensitive to Mn excess



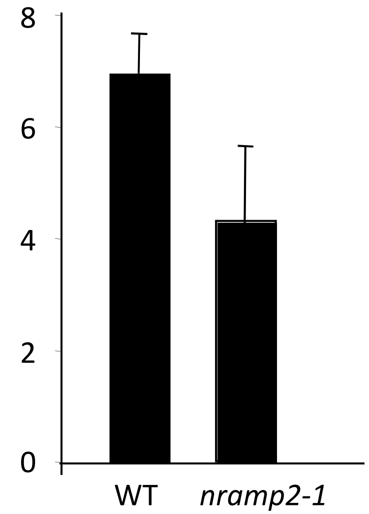
WT nramp2-1

1 mM Mn

Mn in chloroplasts

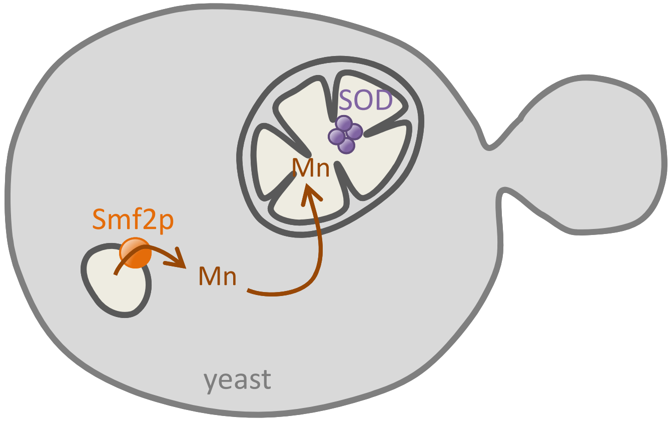


Photosynthetic activity



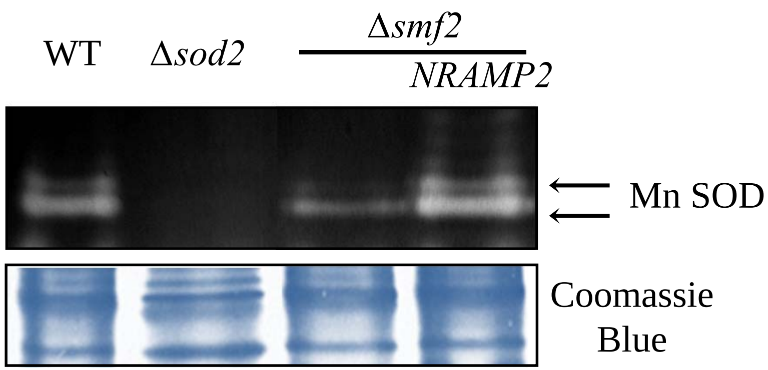
Cailliatte, unpublished

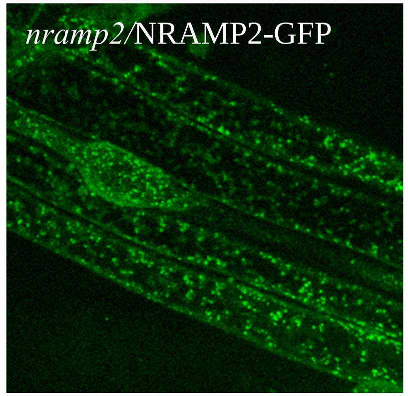
Putative function of NRAMP2 in leaves



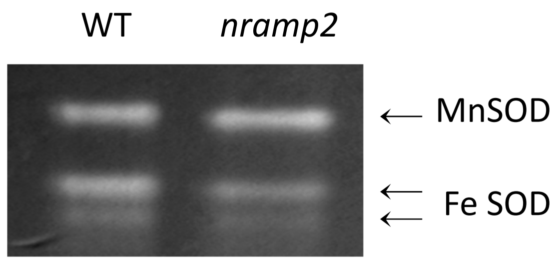
Liu et al., 2001

NRAMP2 rescues growth on –Mn and impaired MnSOD activity of the *smf2* mutant

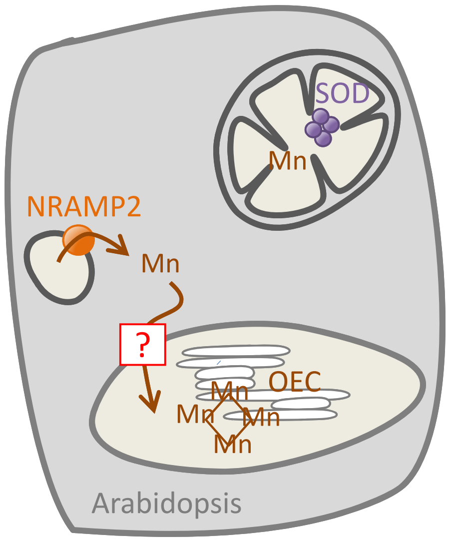


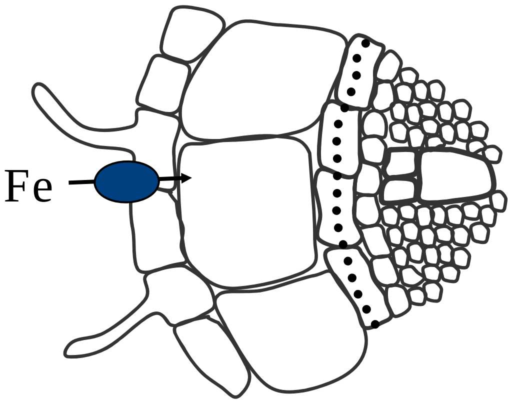


MnSOD activity is not affected in *nramp2-1*

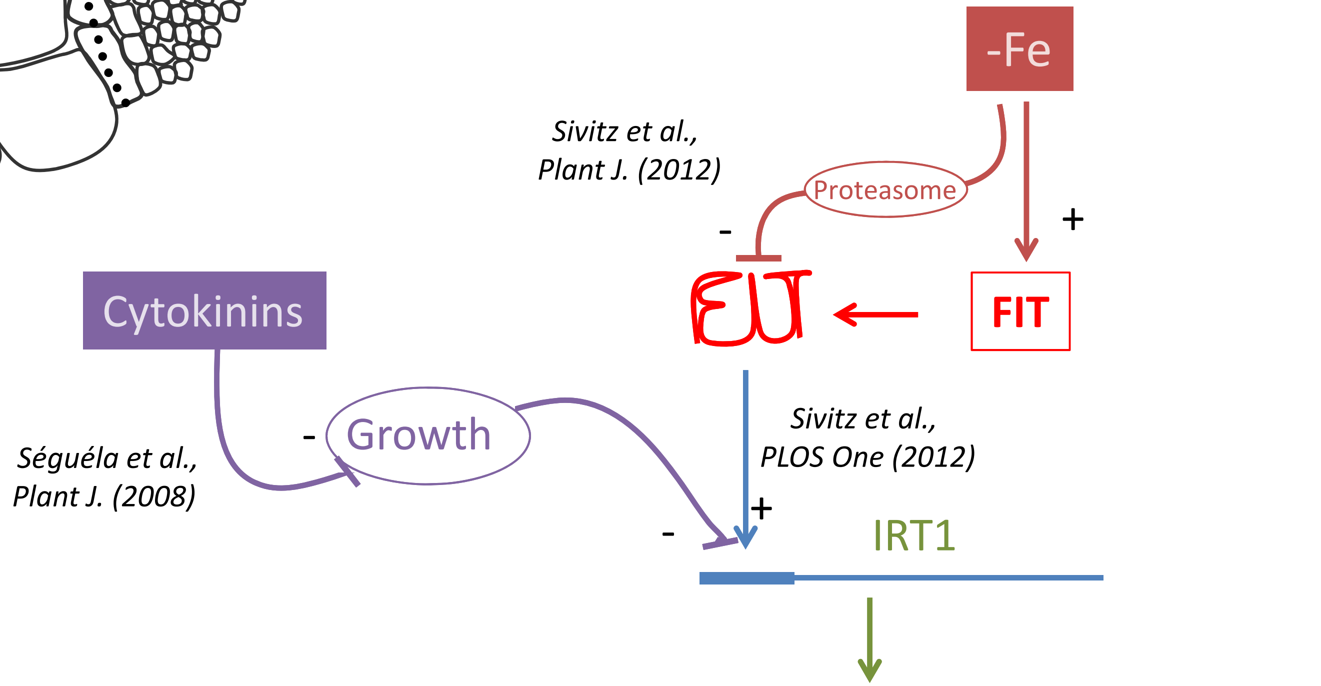


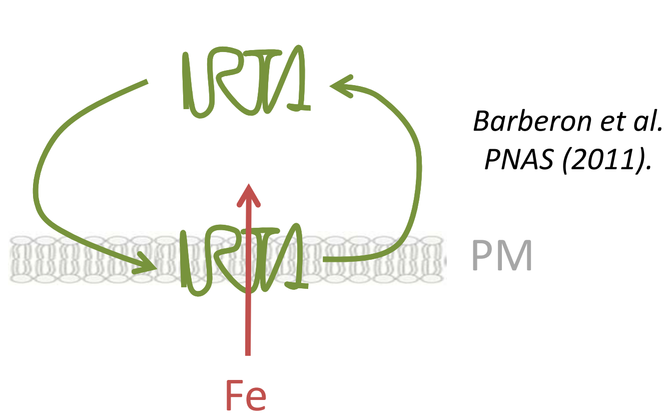
NRAMP2 is located in endomembranes distinct from mitochondria



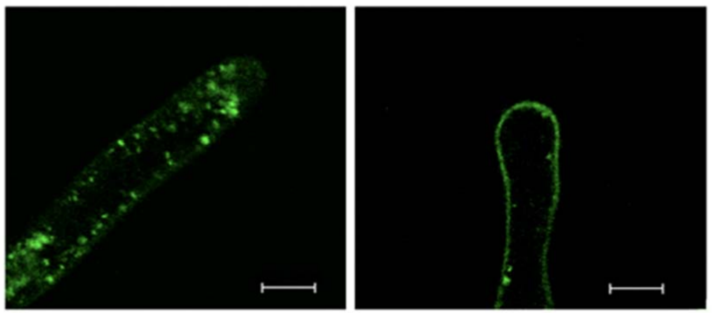


Iron-deficiency signaling pathway



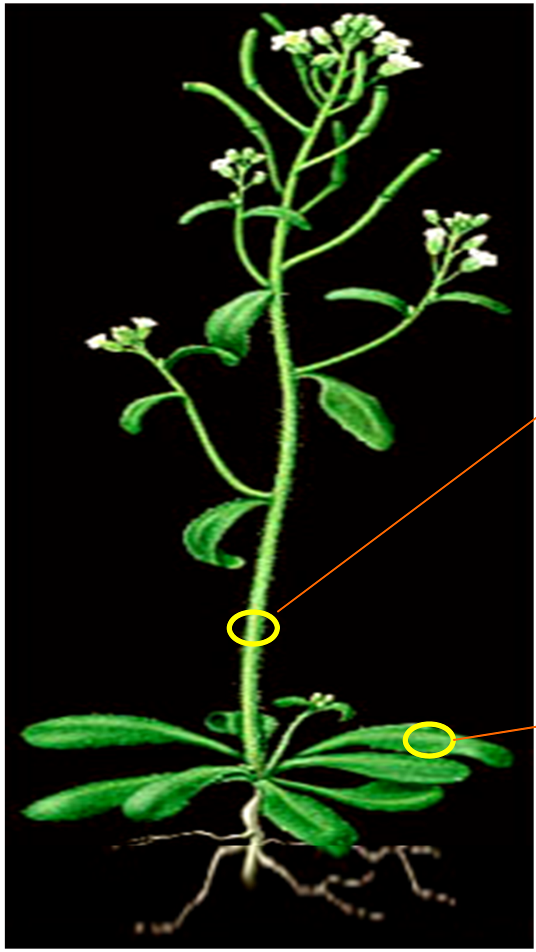


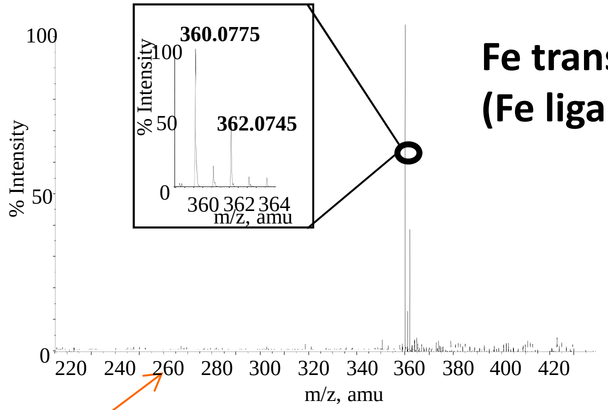
IRT1 est ubiquitinylée puis endocytée



irt1-1/35S::IRT1 *irt1-1*/35S::IRT1$_{K154R,K179R}$

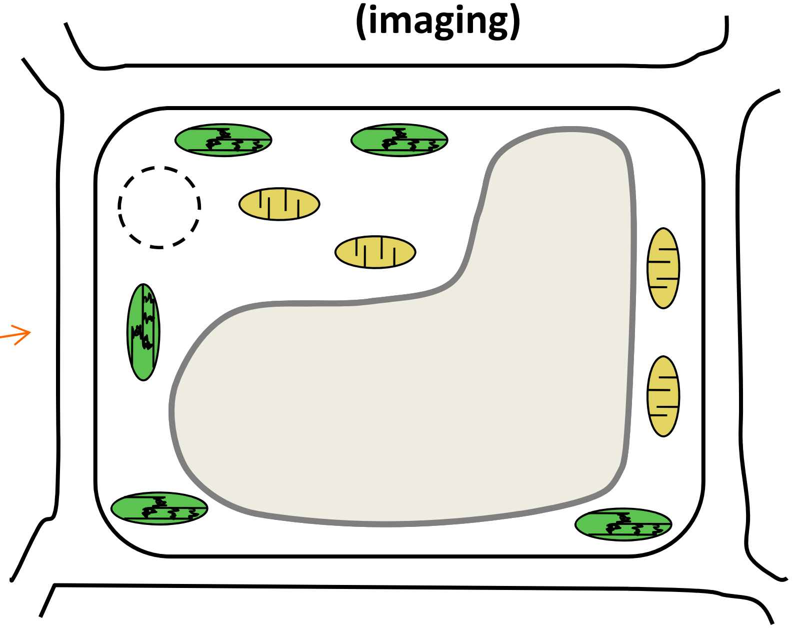
New issues in metal (Fe) homeostasis





Fe transport
(Fe ligands-> speciation)

Fe distribution
(imaging)

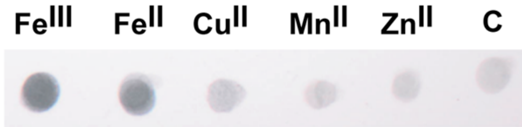


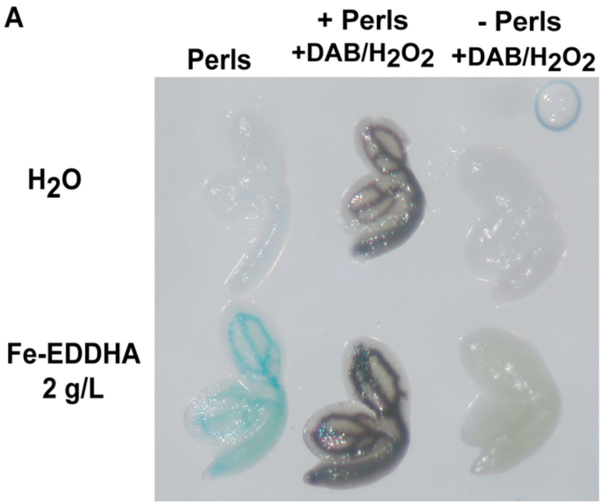
Compartmentalization of Fe: development of a new tool, the Perls/DAB histochemical staining

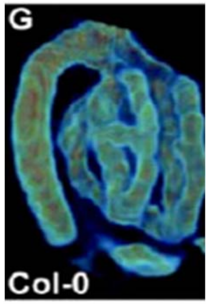
Fe^{III} + ferrocyanide ⟶ [Fe^{III}-ferrocyanide] **Prussian blue**

[Fe^{III}-ferrocyanide]+H_2O_2 ⟶ [Fe^{II}-ferrocyanide] + O+ H

DAB **brown polymers**





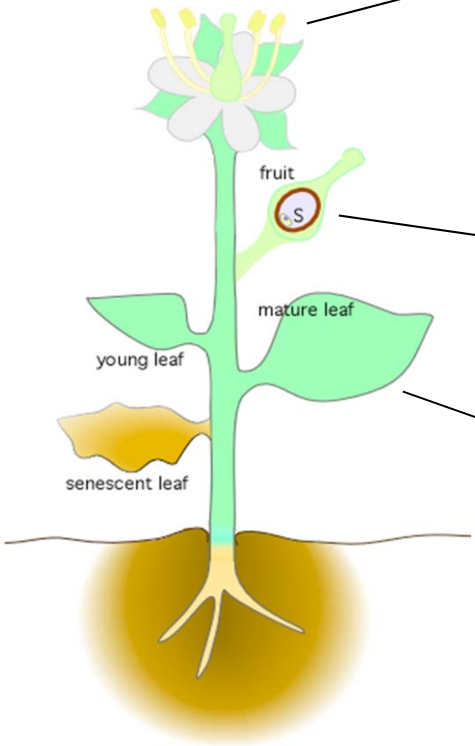


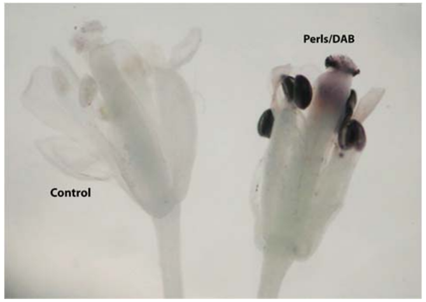
Kim et al, 2006

Roschzttardtz et al., 2009

Compartmentalization of Fe: towards an Atlas of Fe distribution in plants

Identification of Fe pools in the different organs of the plant





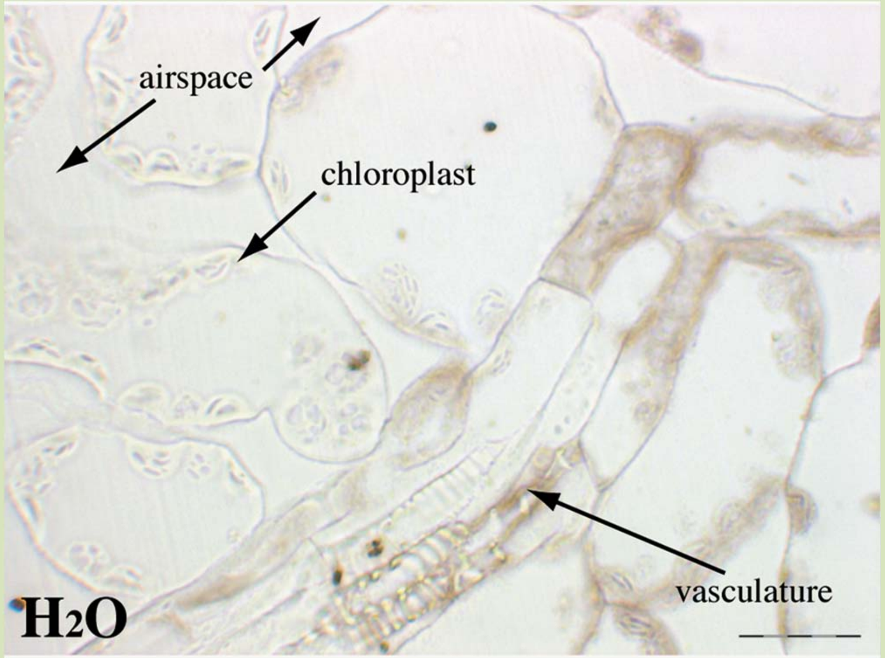
Fe localization in pollen

Fe localization and transport in embryos

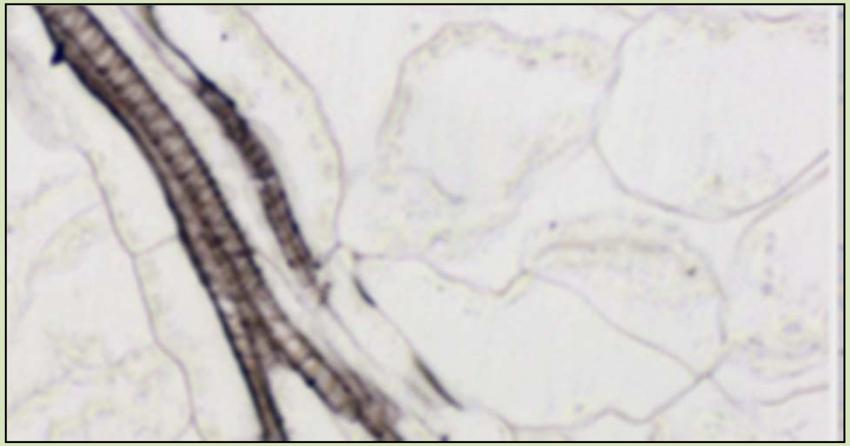
Fe localization and transport in leaf cells

Iron in leaves

Wild-type



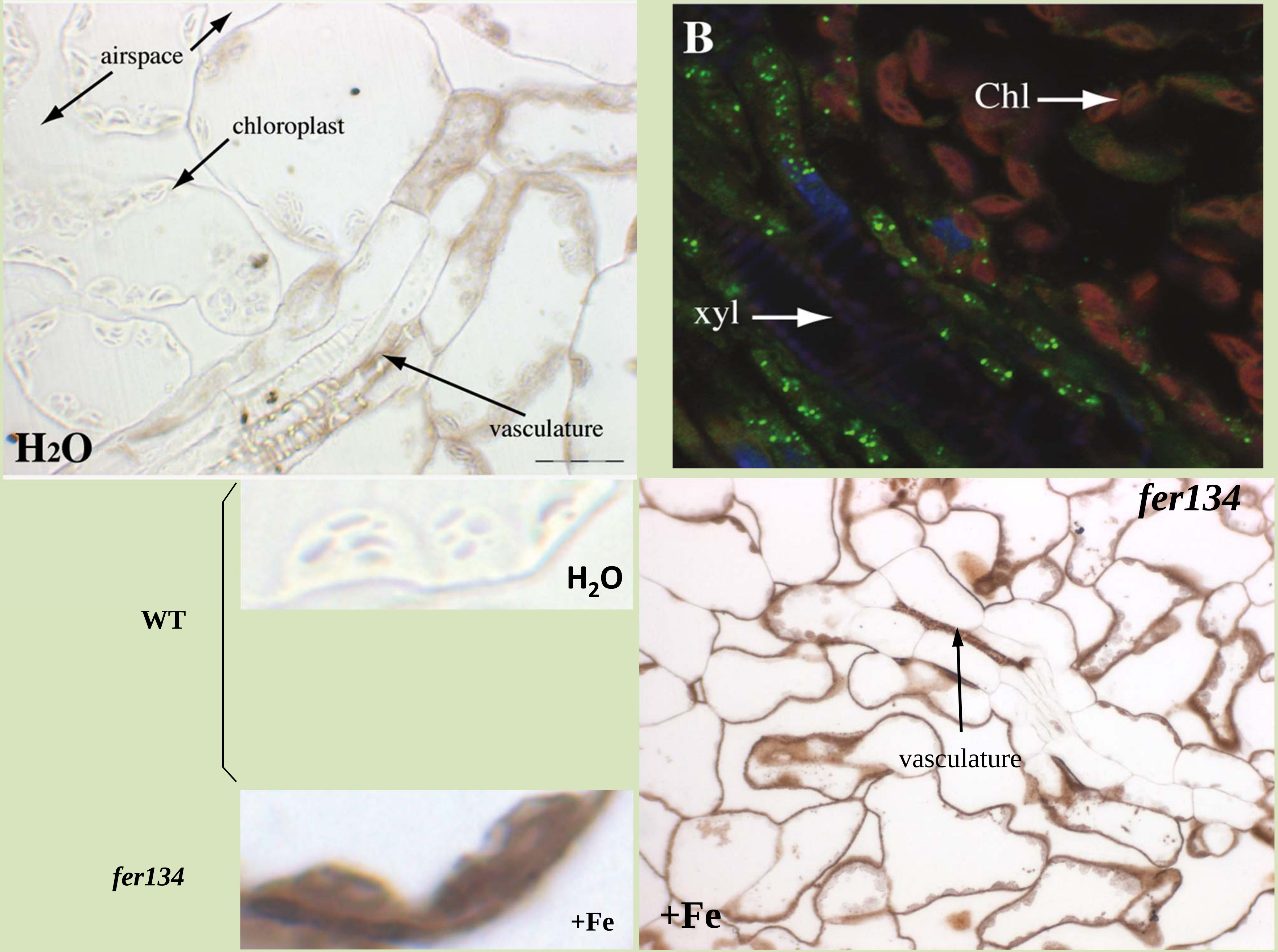
frd3 mutant



Fe blocked in the vascular system
Citrate efflux required for xylem unloading

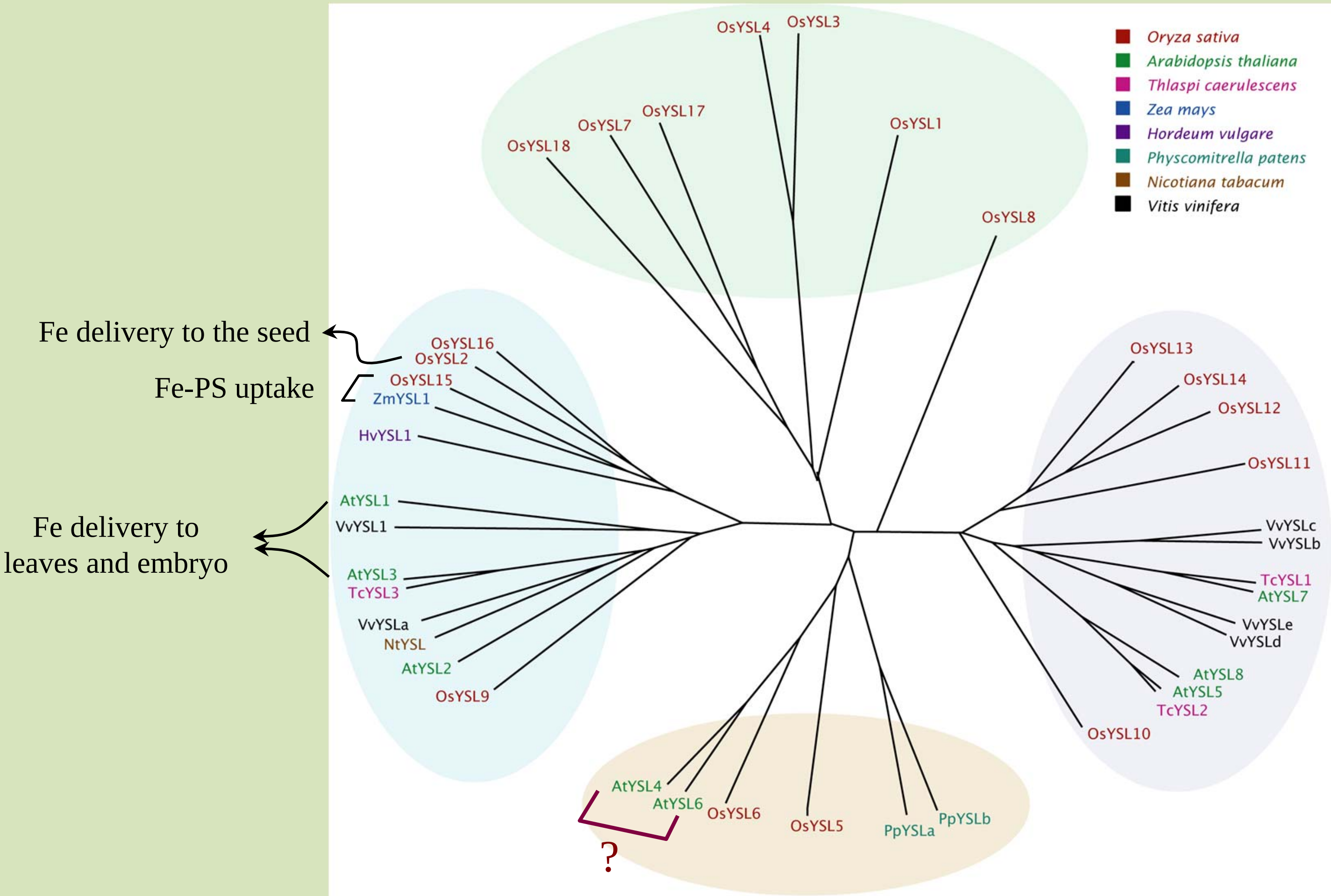
(Roschzttardtz et al., 2011)
(Roschzttardtz et al, in prep)

Iron in leaves



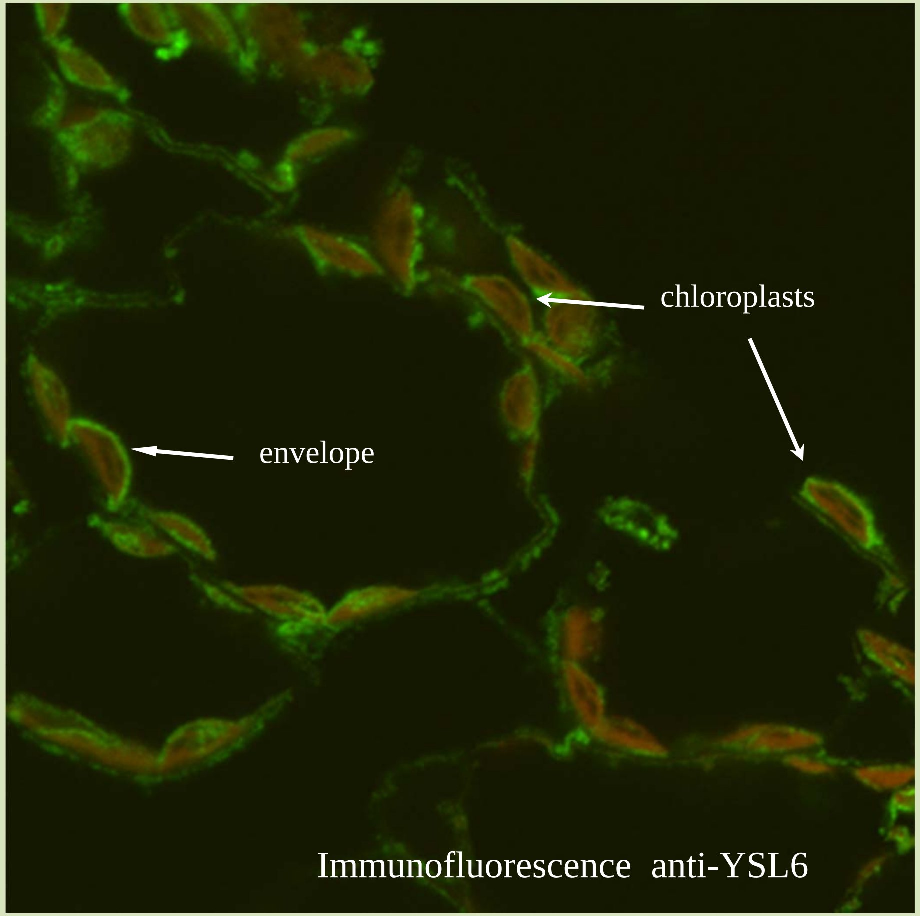
Iron in leaves

Characterization of YSL4 and YSL6, two Fe-nicotianamine transporters

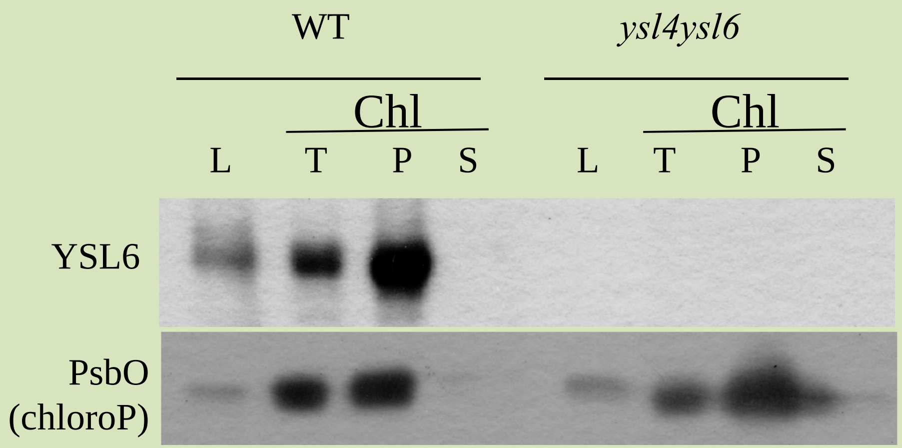


Iron in leaves

YSL6 is a protein of the chloroplast envelope



Leaf of a 3 week-old soil-grown plant



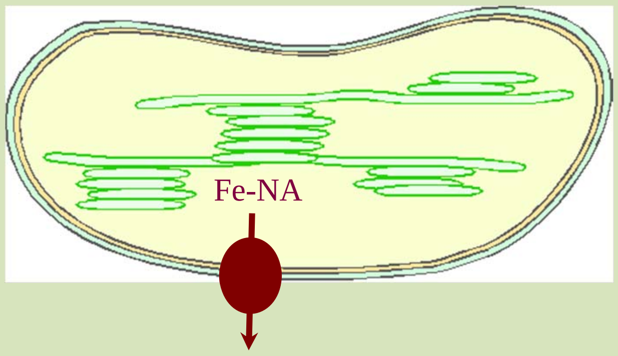
T: total proteins
P: membrane pellet
S: soluble fraction

(Divol et al., submitted)

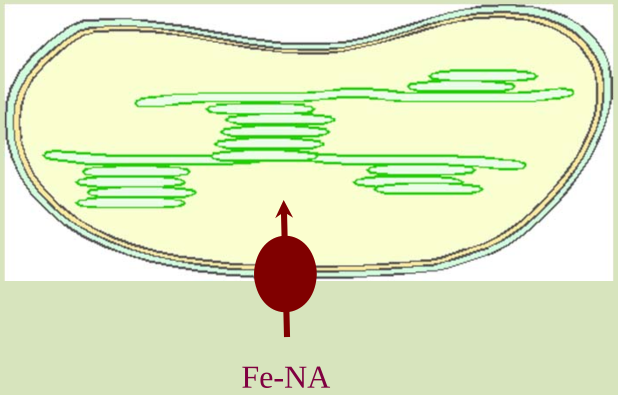
Iron in leaves

Two hypotheses for YSL6 activity

Effux from the chloroplast

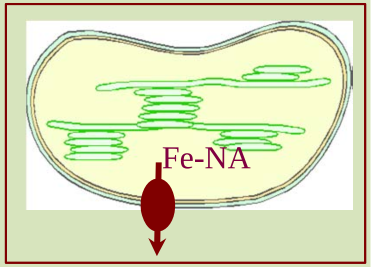


Influx into the chloroplast

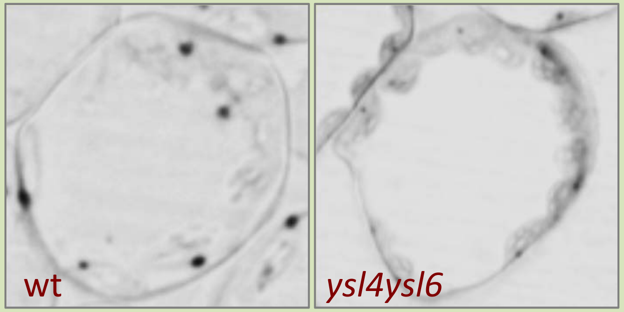


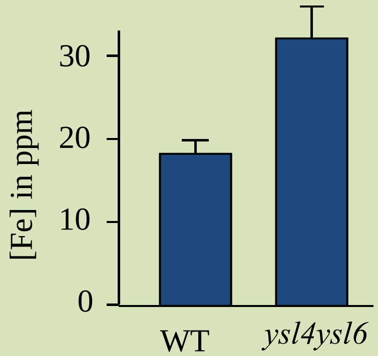
Iron in leaves

Arguments for a function of YSL4 and YSL6 in Fe release from the chloroplasts



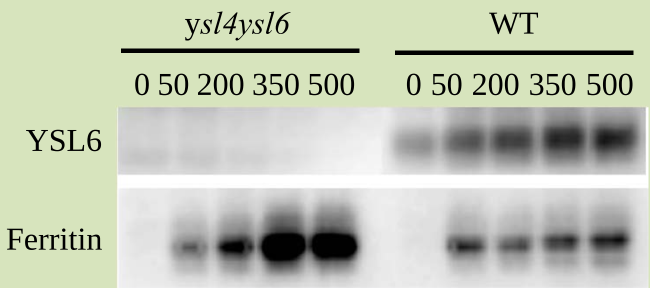
ysl4 ysl6 chloroplasts contain more Fe





ysl4 ysl6 is hypersensitive to Fe excess

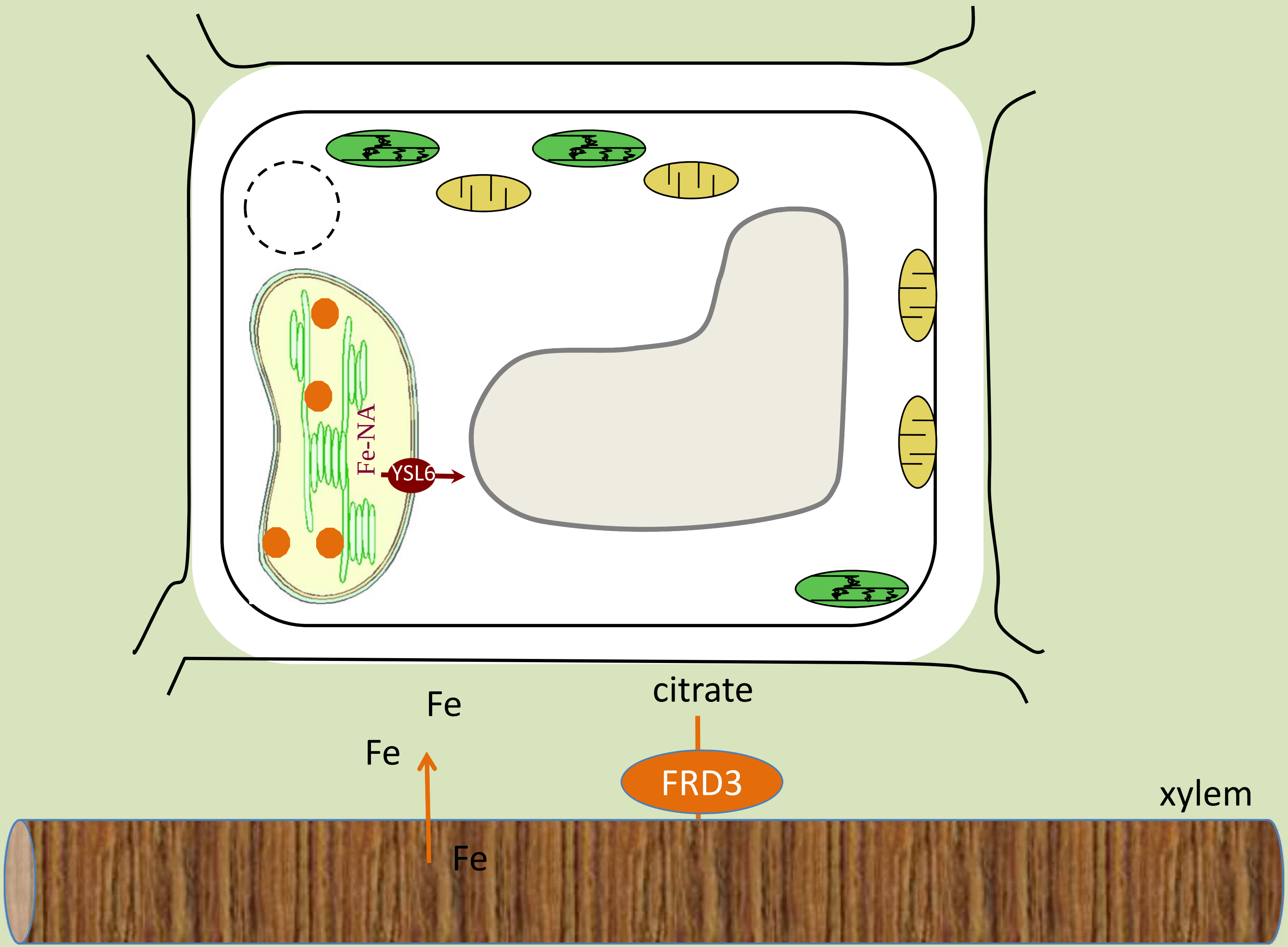
The abundance of ferritins is elevated in the *ysl4 ysl6* mutant



(Divol et al., submitted)

Iron in leaves

Mechanism of Fe distribution in leaf cells

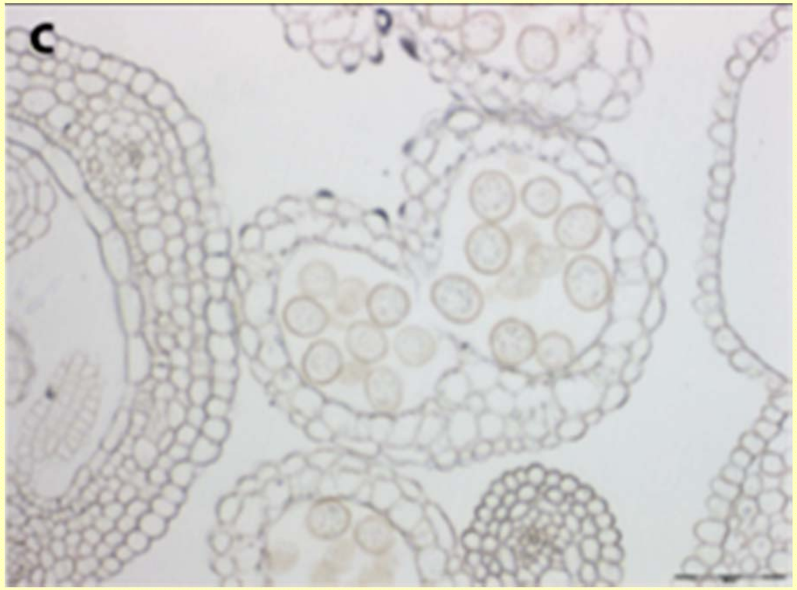


Iron in pollen

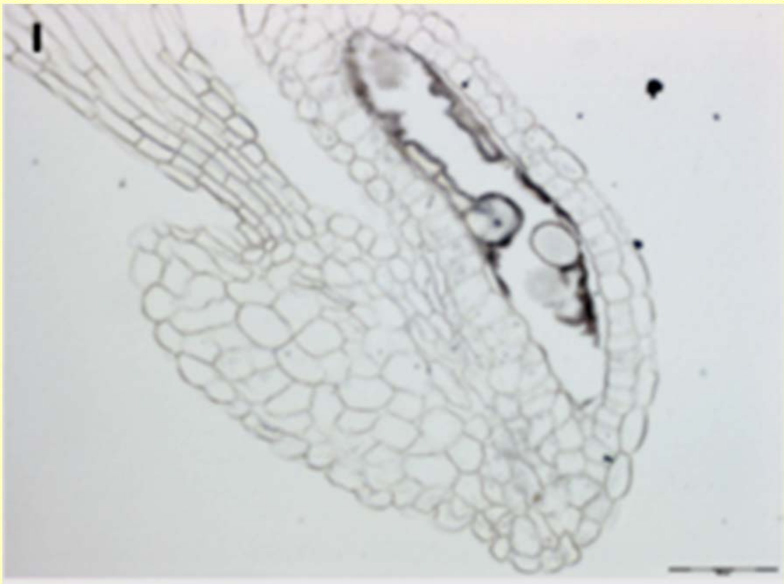
Iron fails to enter the pollen grains in the *frd3* mutant

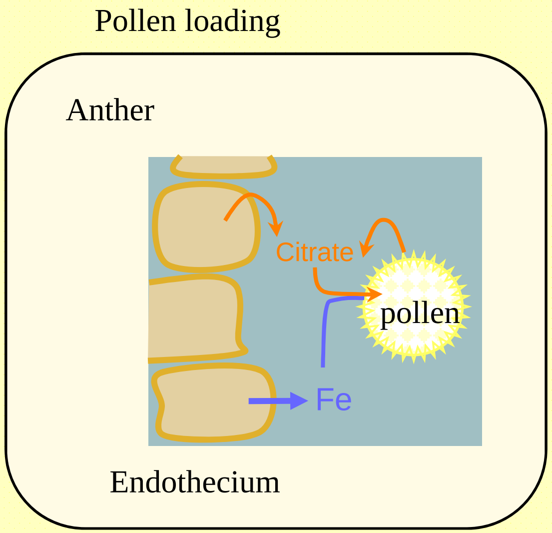
Perls/DAB staining

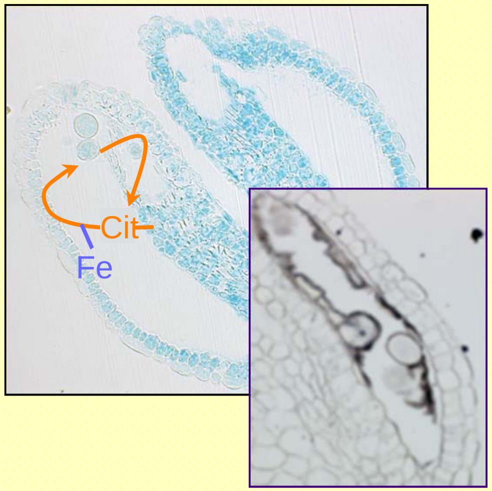
WT



frd3

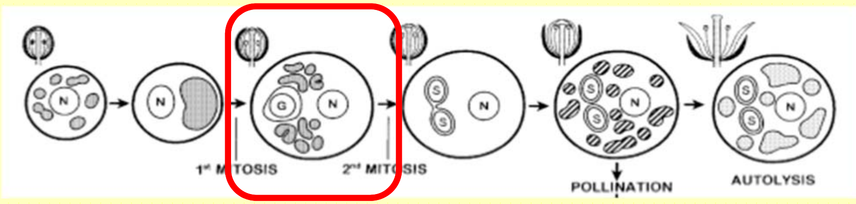






(Roschzttardtz et al., Plant Cell 2011)

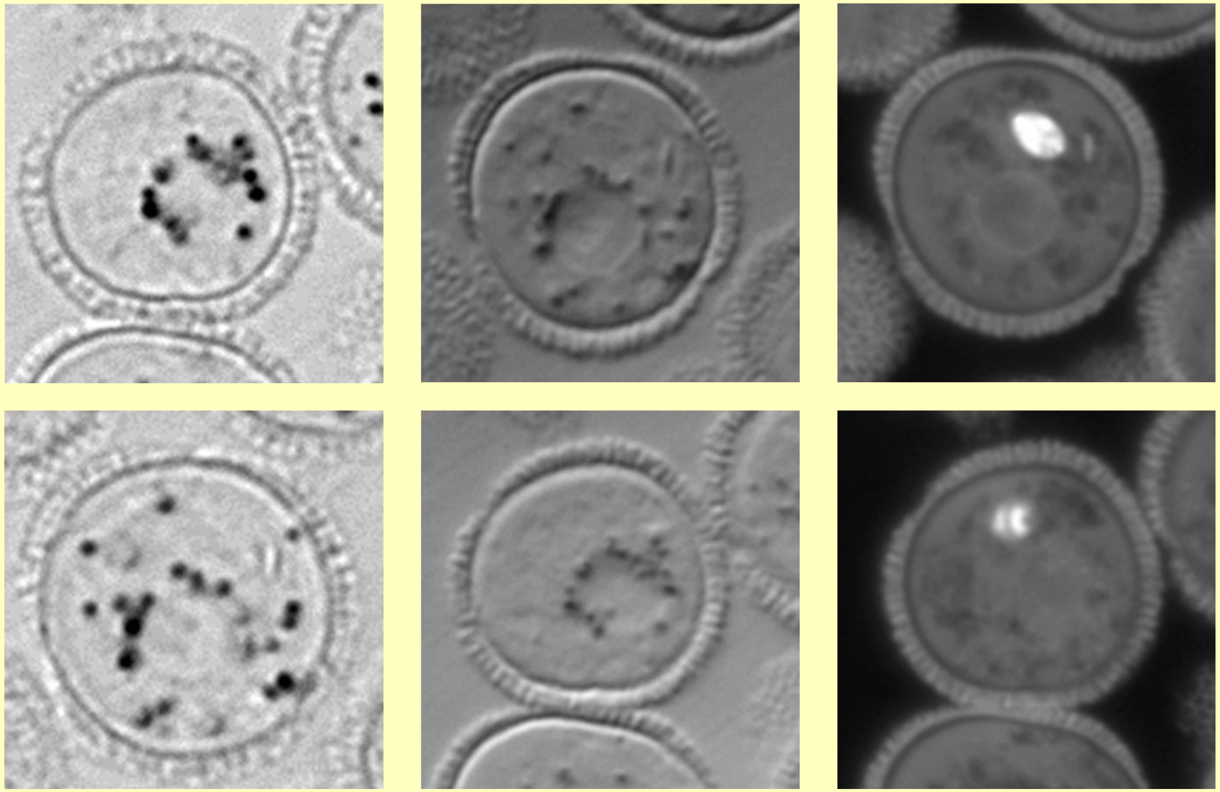
Iron in pollen



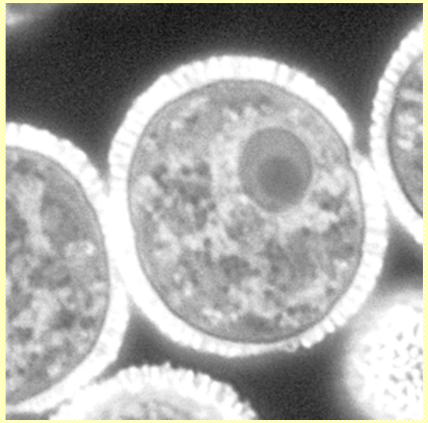
Perls/DAB

Perls/DAB+DIC

DAPI (generative nuclei)

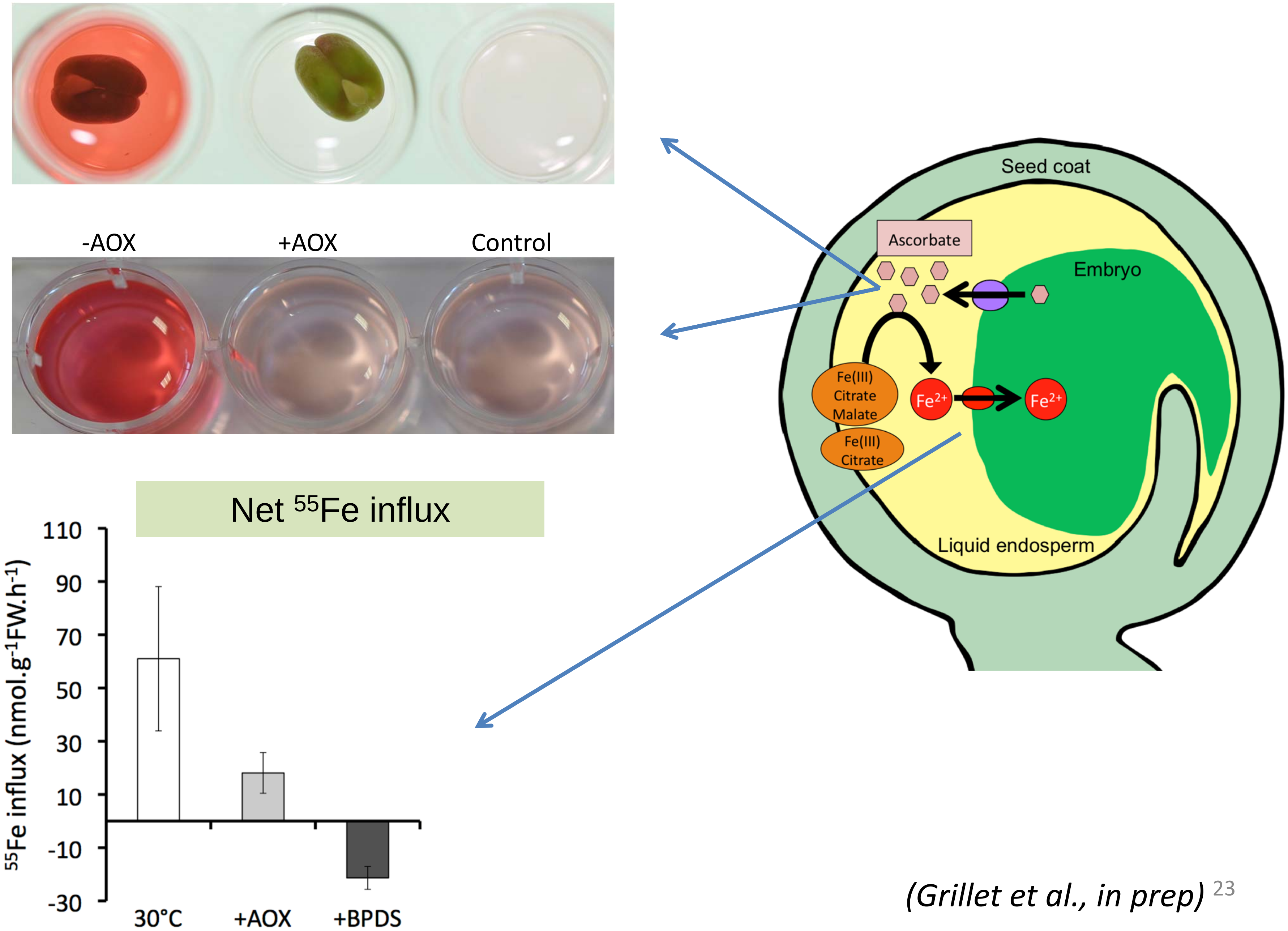


DiOC6 (mitochondria)



Is it Fe in mitochondria?

Ascorbate efflux as a ne strategy for Fe reduction and uptake



(Grillet et al., in prep)

THE PAST

C. Curie (DR2)
P. Czernic (MCF)
S. Mari (CR1)
G. Vert (CR1)

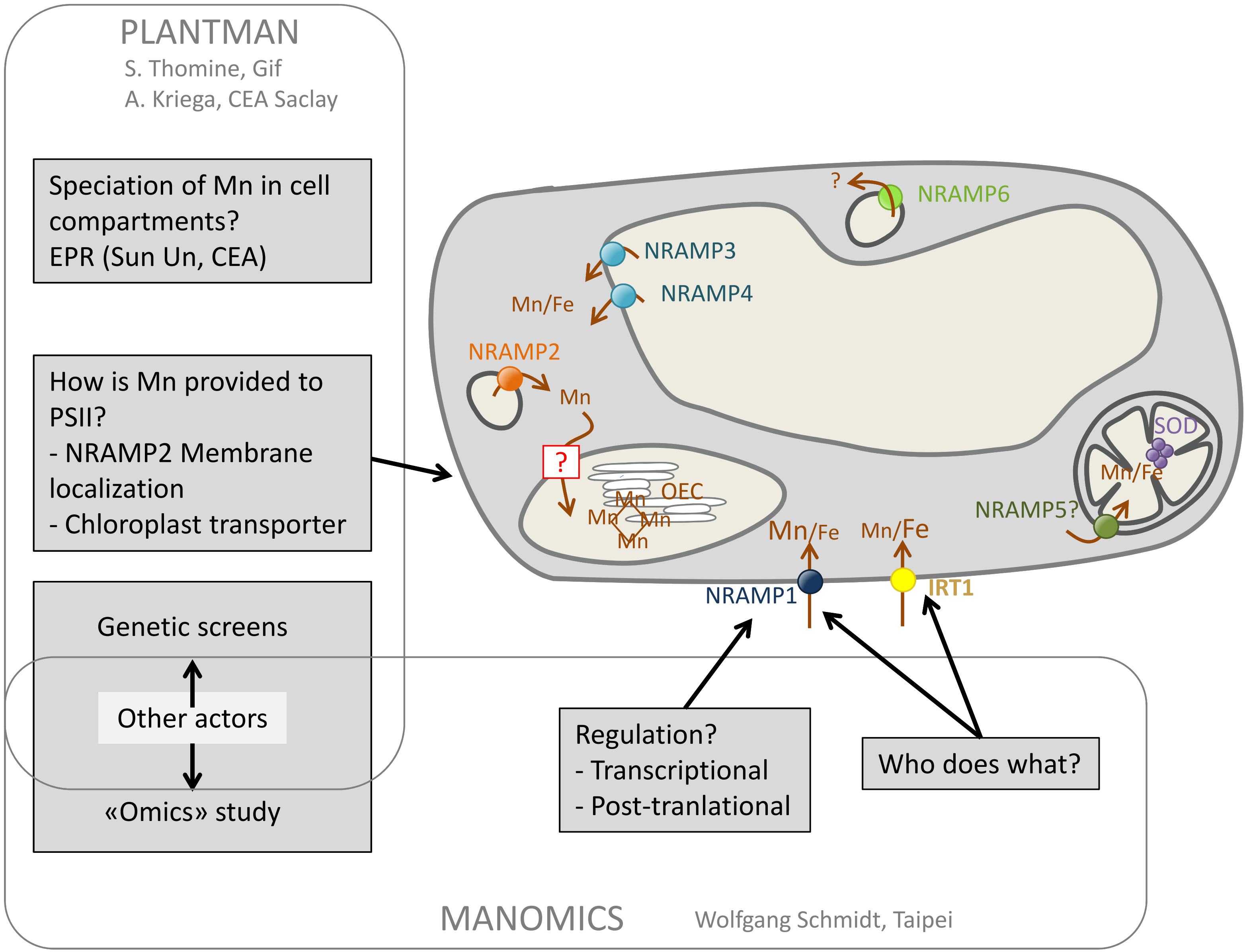
M. Seguela
M. Barberon
A. Sivitz
E. Zelazny
R. Blanvillain
D. Couch
F. Divol
H Roschzttardtz
R. Cailliatte
G. Cassin
L. Grillet

THE PRESENT

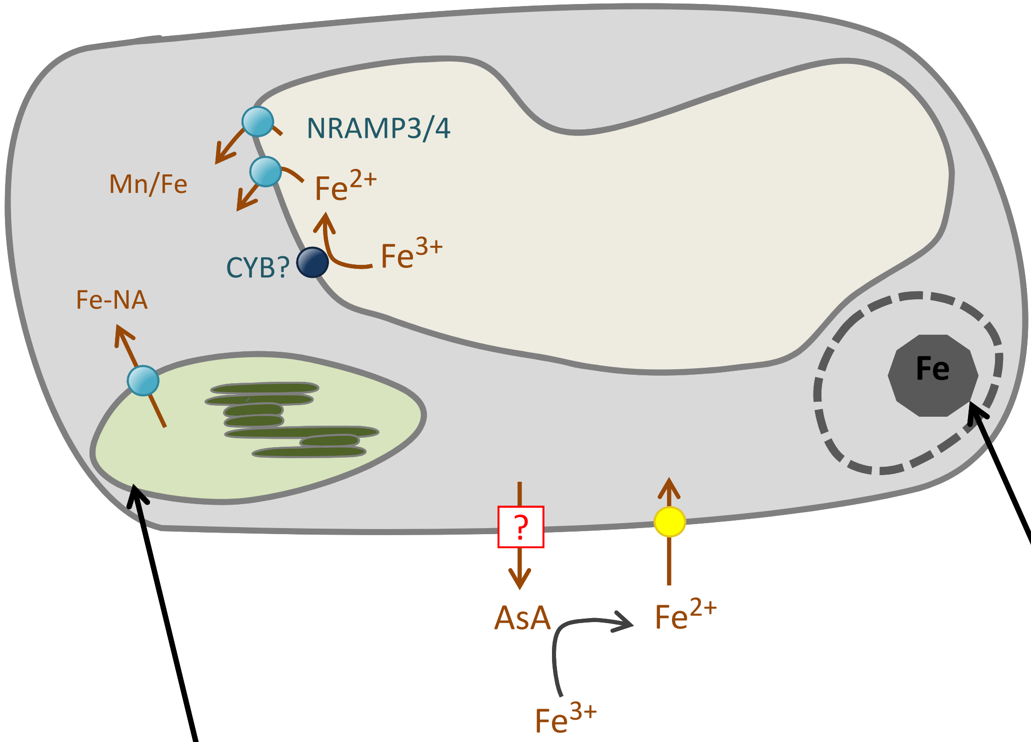
C. Curie (DR2)
S. Mari (CR1)
O. Richard (CR1)
C. Alcon (IE)
S. Loubet (TR)
S. Chay (TR)

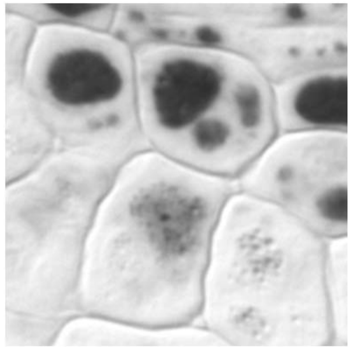
M. Hoang (PhD)
L Castaings (Postdoc)
S. Gutierrez (Postdoc)
L. Wei (Postdoc)

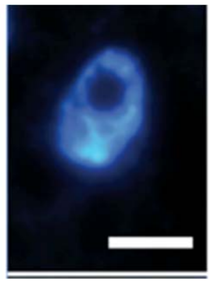
DETERMINANTS OF MANGANESE HOMEOSTASIS



NEW STRATEGIES OF IRON TRANSPORT AND CONTROL OF SUBCELLULAR DISTRIBUTION







Regulation of Fe transport and availability (transporters ligands…) in the chloroplast

SUBCELIF

Julio Saez, Perpignan
Laurent Ouerdane, Pau
Richard Ortega, Bordeaux

What is the function of nucleolar Fe

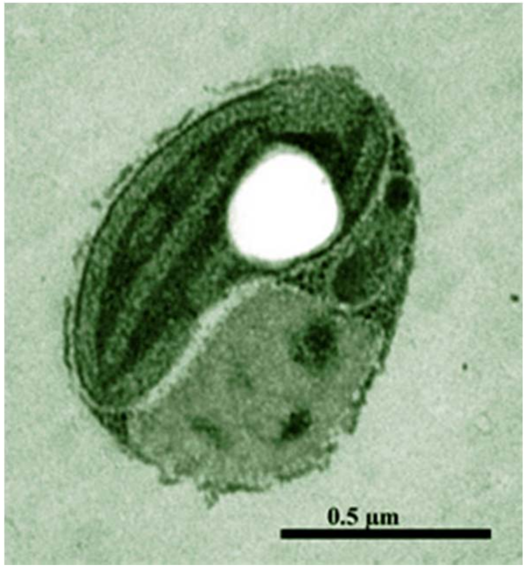
A genetic screen for disturbed iron distribution (*did*)

homozygous T-DNA library (Salk)

Perls-DAB assay

did mutants

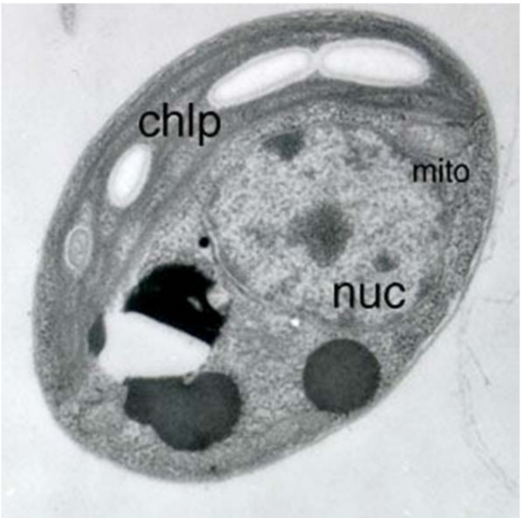
Towards the establishment of microalgea as unicellular photosynthetic models to study metal homeostasis



Ostreococcus tauri

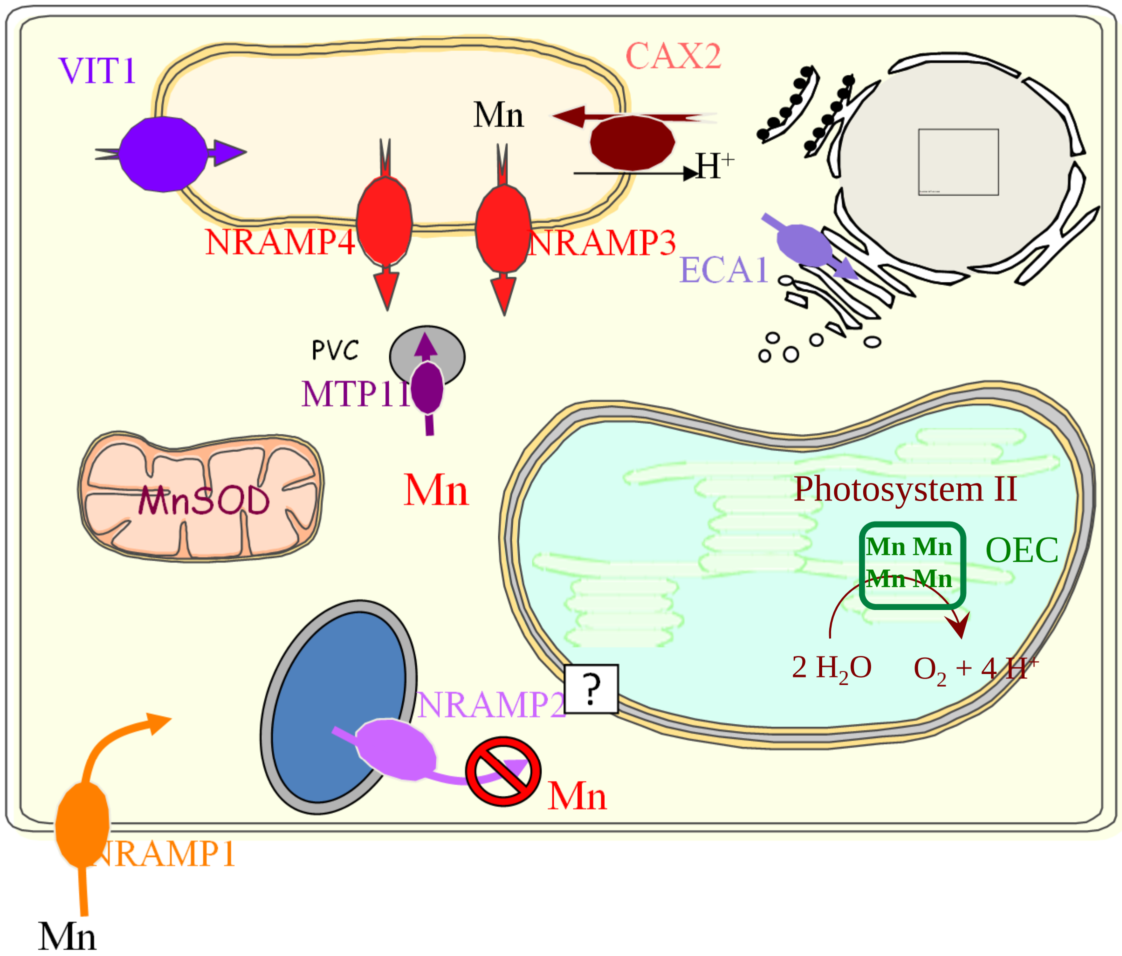
-discovered in 1994 by Claude Courties, in the Thau bassin
-largely widespread in the oceans
- « coccoïd » cell without wall
-1 chloroplast, 1 mitochondrion
-smallest photosynthetic eucaryotic cell (≈ 1µm)

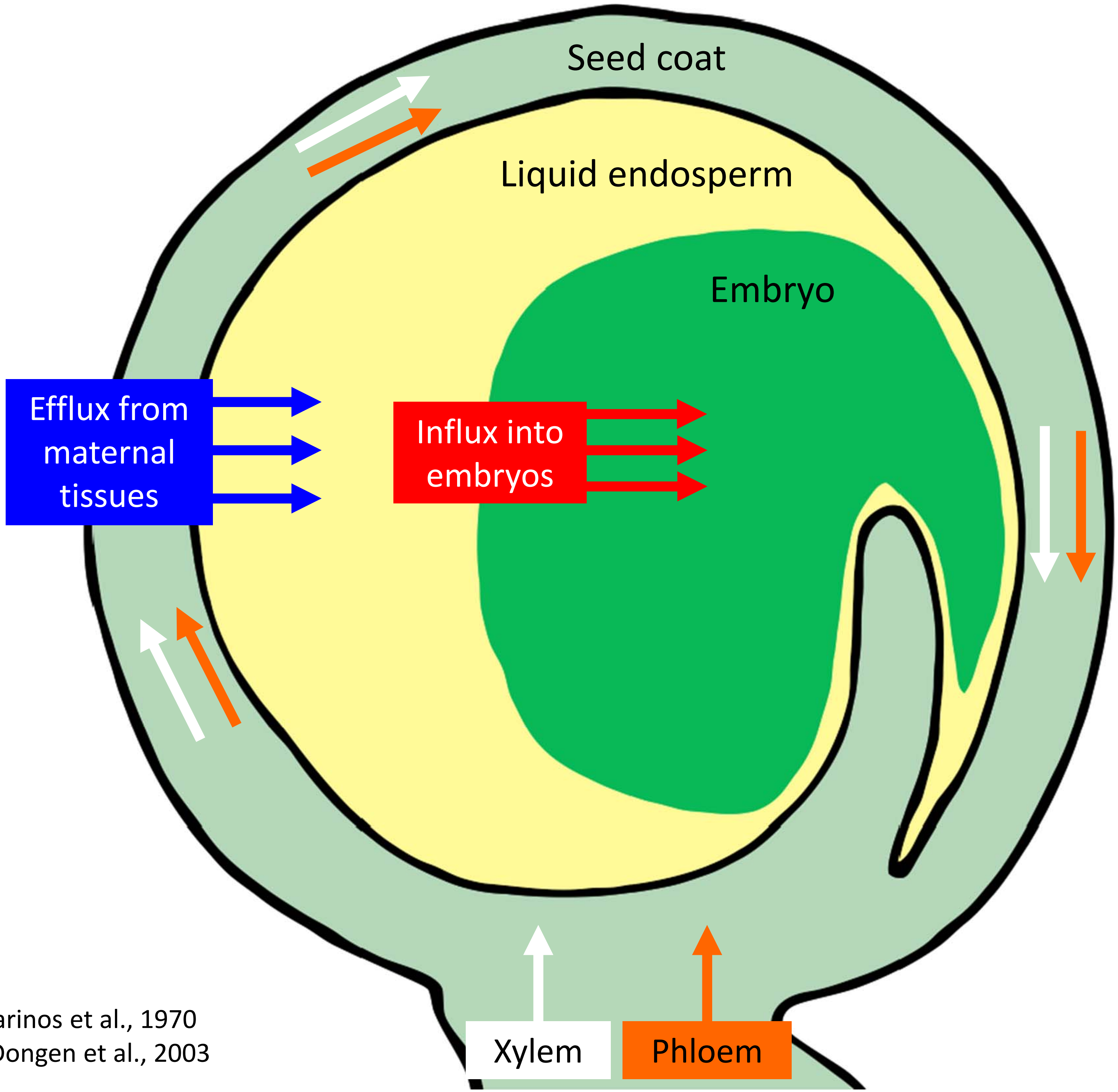
Mn uptake, Mn/Fe subcellular distribution, NRAMP function



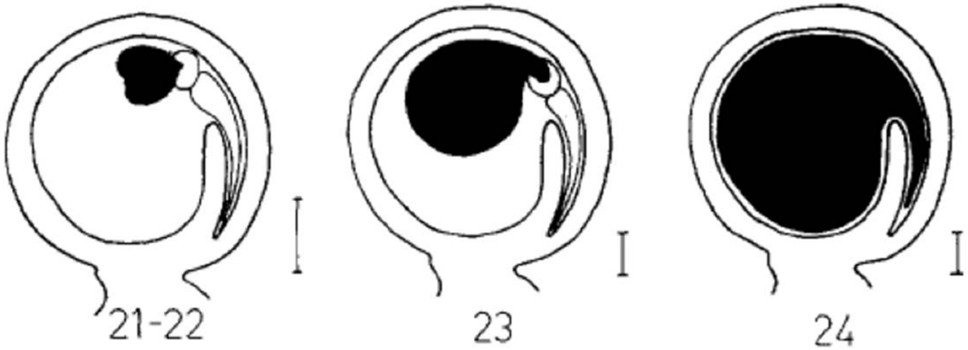
Chlorella variabilis NC64A
-discovered in 1890 by Martinus Willem Beijerinck
- « coccoïd » cell with cell wall (≈2-10 µm)
-non motile with 1 chloroplast
-photosynthetic endosymbiont of *Paramecium bursaria*
-facultative symbiosis
-no reproduction cycle (no genetics)

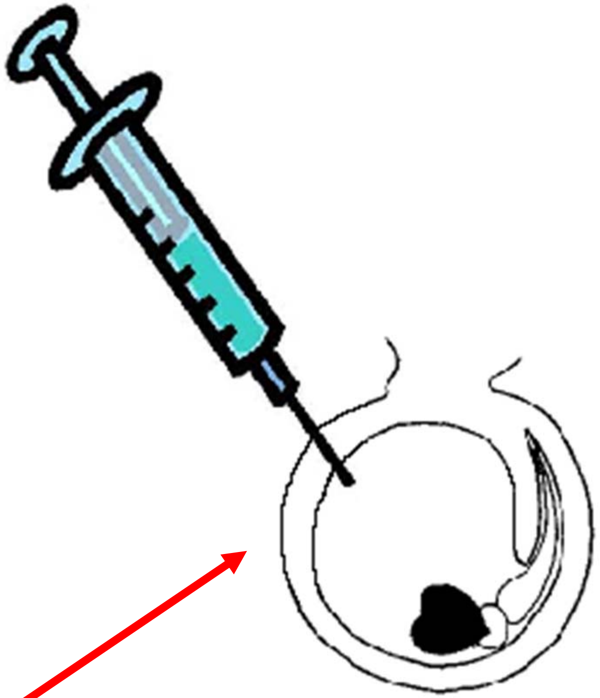
NRAMP1 and NRAMP2 are physiological Mn transporters



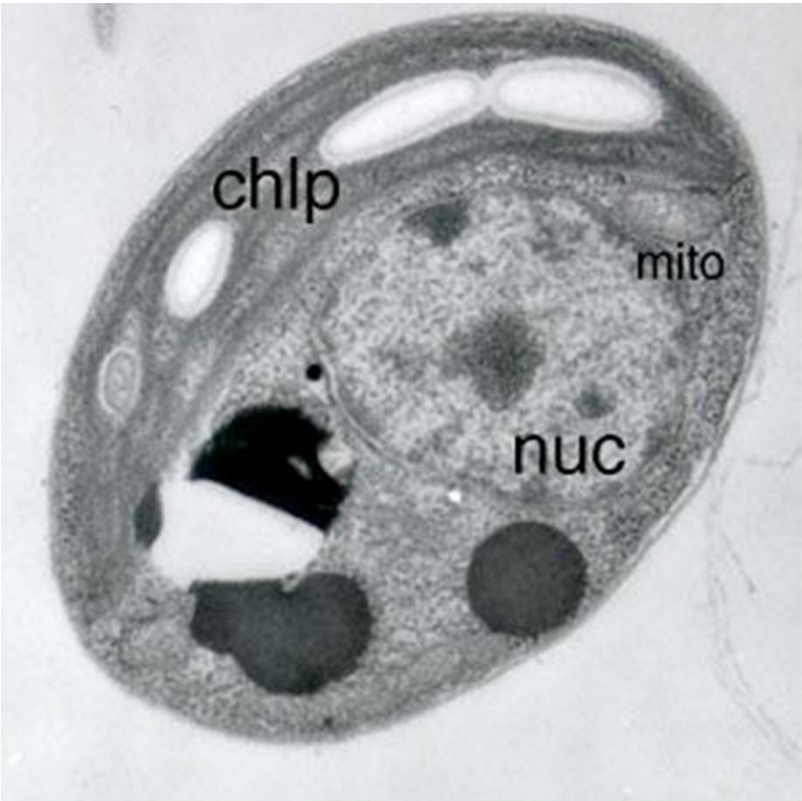


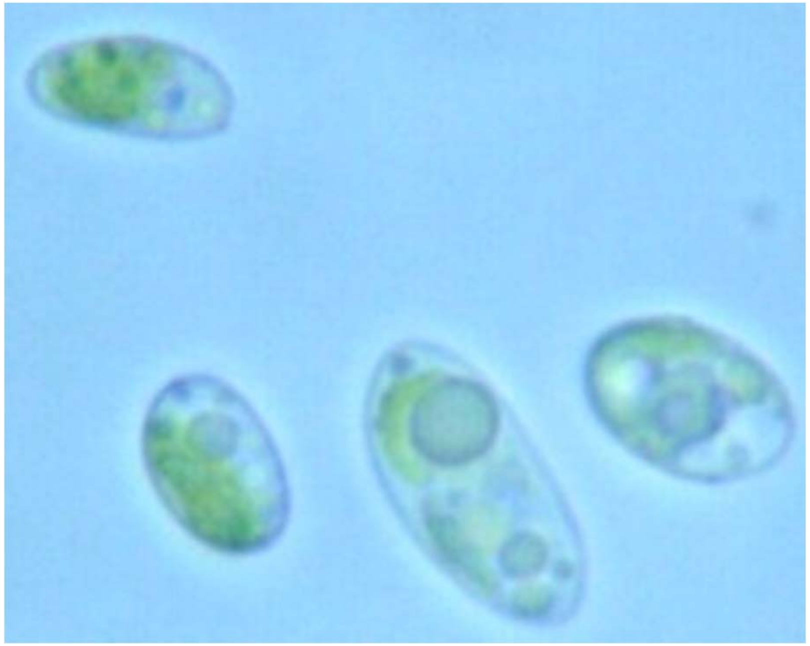
From Marinos et al., 1970
and Van Dongen et al., 2003





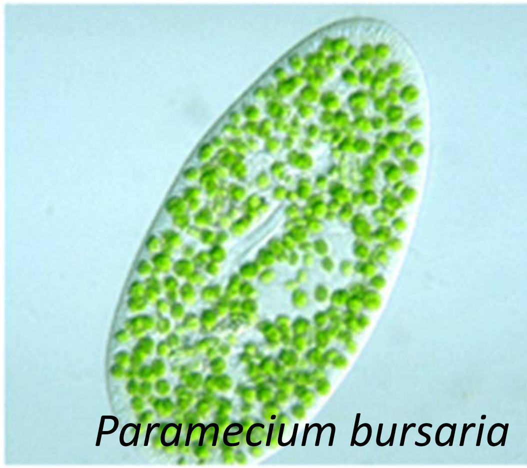
Embryo sac liquid
pH = 5.5





Chlorella variabilis NC64A

-découvert en 1890 par un microbiologiste hollandais Martinus Willem Beijerinck
-cellule « coccoid » avec paroi (≈2-10 µm)
-non motile avec 1 chloroplaste
-endosymbionte photosynthétique du protozoaire unicellulaire *Paramecium bursaria*
-symbiose facultative
-pas de cycle de reproduction (pas de génétique)



Paramecium bursaria

PROJET : HOMEOSTASIE DU FER ET DU MANGANESE CHEZ OSTREOCOCCUS ET CHLORELLA

Prélèvement du manganèse
Mouvements Mn et Fe intracellulaires
Fonction NRAMP

1/Etat des lieux/ Descriptions des modèles

A/description biologie du fer et du manganèse chez ces 2 espèces

=>croissance sous carence ou excès métaux et mesure de la biomasse, photosynthèse, contenu chlorophyllien, contenu métallique, d'activité ferric reductase….uptake…

+ mesure expression de gènes candidats (test)

⇒Purification chloroplastes

B/identification des gènes de l'homéostasie Fe/Mn : séquences génomiques

= inventaire du nombre minimal de transporteurs nécessaire à l'homéostasie des métaux d'une seule cellule (NRAMP; ZIP, CDF, CAX,HMA, ABC)

et/ou

=analyse de quelques gènes plus ciblés de l'équipe

C/identification des gènes de l'homéostasie Fe/Mn : transcriptome/protéome?

= carence ou excès Fe/Mn

= total ou fraction chloroplastique

PROJET : HOMEOSTASIE DU FER ET DU MANGANESE CHEZ OSTREOCOCCUS ET CHLORELLA

2/Evolution de fonction

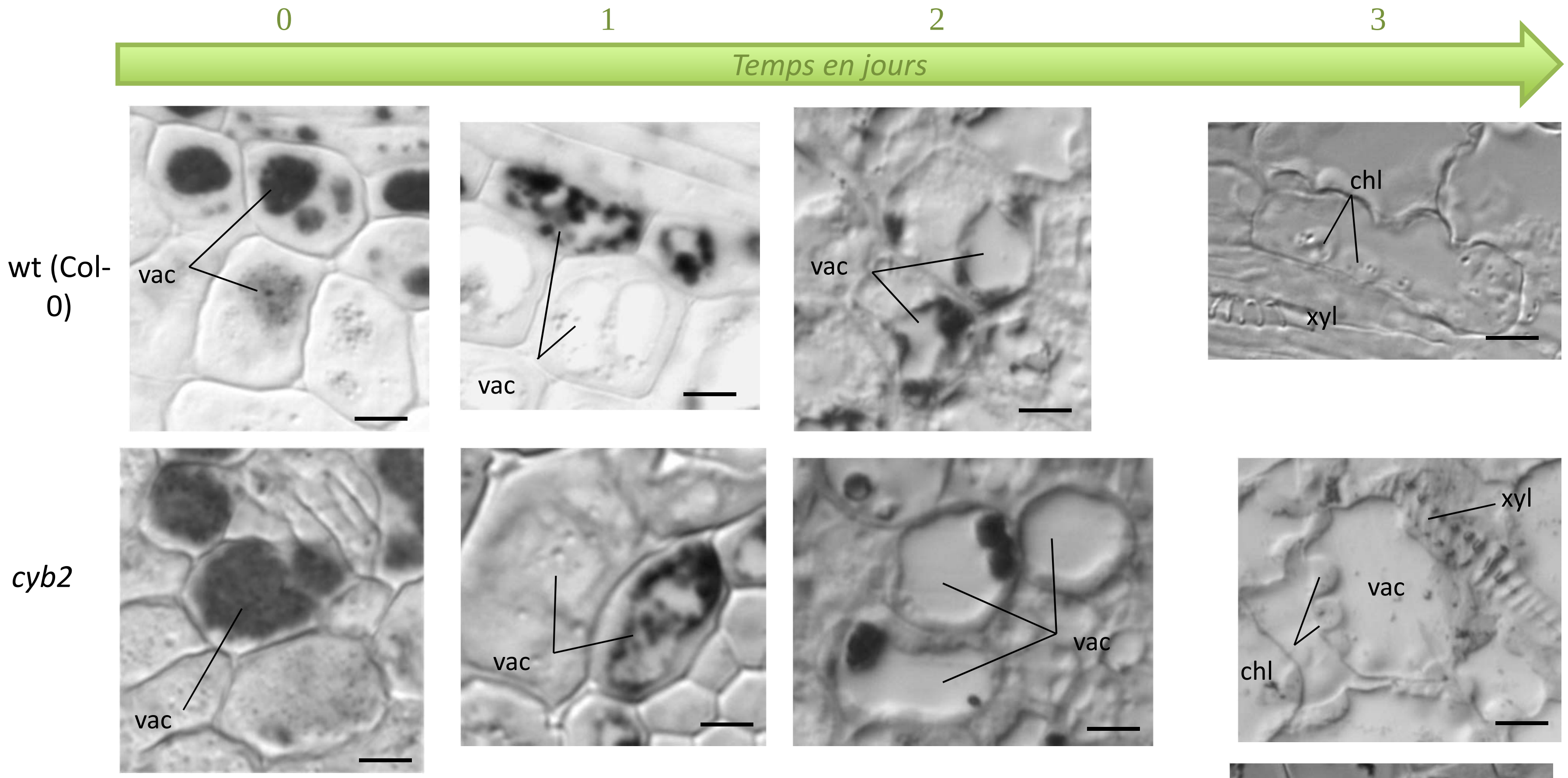
Ex : NRAMP : comparaison fonction et séquences entre Chlorella et Ostreococcus

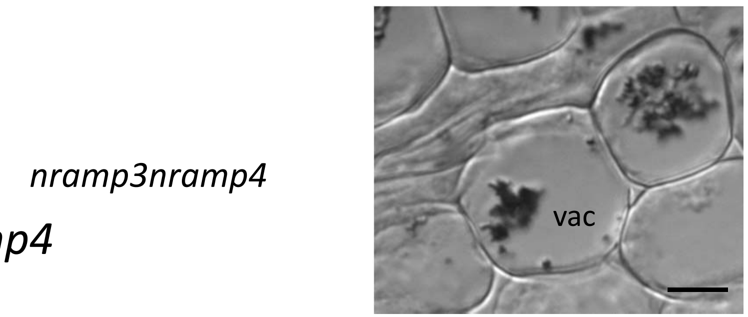
A/Evolution de séquences NRAMP

B/ Caractérisation fonction

régulation	suivi expression des gènes (QPCR sous carence ou excès métaux) suivi protéine (pOtLUC en cours @ Banyuls)
mutant KO	Ostreococcus (en cours @ Banyuls) Chlorella : tout est à faire (mise au point transformation…)
localisation	Ostreococcus (construction GFP en cours @ Banyuls)

Cellules endoderme
Perls/DAB





Le mutant *cyb2* ne phénocopie pas *nramp3nramp4*