Physiologia Plantarum lecture:

Mechanisms of iron homeostasis in plants and their regulations

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Mechanisms of iron homeostasis in plants and their regulations
Iron is an essential element for life

- Transition metal
- 4th element on earth

\[ \begin{array}{ccc}
\text{Fe} & 26 & +2 \\
\text{Fe} & 3 & +3 \\
55.85 & & \\
\end{array} \]

\( \text{Fe}^{2+} \) Ferrous iron, reduced
\( \text{Fe}^{3+} \) Ferric iron, oxidised

Abundant... but

- Low iron availability in 1/3 of the cultivated soils (calcaraceous)
- Diet for 2/3 of the world population is based on plant food (100 g of cereal flour contain less than 10% of the recommended daily iron supply)

As a consequence

3 billion human beings are iron deficient (increased susceptibility for infections and retardation of mental and psychomotor development and of growth)

Iron entrance in the food chain is linked to a major public health problem

http://www.who.int/nutrition/topics/ida/en/index.html
**Plant Iron Deficiency**

- Iron abundant in soils, BUT poorly available for plants ($10^{-10}M$ soluble/ $10^{-8}M$ required)

- Iron fertilization expensive and poorly efficient

- Phenotype of iron deficient plants
  - Growth decrease and lower yields
  - Heavy metals (Zn, Mn, Cd...) accumulation
  - Decreased chlorophyll content
Iron Deficiency Alters Chloroplast Structure and Functions

Chloroplasts = plant leaf specific organelles containing chlorophyll
+ photosynthesis: CO2 assimilation ---> C skeleton of organic molecules
+ N and S assimilation ---> Amino Acid and Vitamin synthesis

These reactions depend upon iron proteins (electron transport chain, nitrite and sulfite reductases...etc)
Iron within the chloroplast photosynthetic apparatus

stroma

LHC

Lumen

PSII

2 Fe

PSI

14 Fe

ATP synthetase

(from FA Wollman)
Iron is essential ... but toxic

Fenton Reaction

Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{-}  
(Superoxide ion)

Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{-} + HO^{-}  
(Hydroxyl radical)

Humans:
Hemachromatosis

Plants:
Rice « bronzing »
Molecular principle to control iron homeostasis in eukaryotic cells

Iron excess

Uptake: Iron transporters

Storage: Ferritin

Iron deficiency

Uptake: Iron transporters

Storage: Ferritin
Two different iron uptake strategies between graminaceous and non-graminaceous plants correlate with a differential sensitivity to iron availability in calcaraceous soils.
**Arabidopsis (non graminaceous) IRT1 from the ZIP family**

- Eight predicted trans-membrane domains (I-VIII)
- WT transports Fe, Zn, Cd and Mn
- E103 (red) --> Ala = transports Fe, Cd, Mn but not Zn
- D100 (blue) --> Ala = transports Zn, Cd but not Fe and Mn
- D136 (green) --> Ala = transports Zn and reduced level of Cd
- H96, H197, S198, H224 and E228 (grey) --> Ala = no transport activity
- C109, Y295, D300 and E305 (yellow) --> Ala = no change with WT

(from Rogers et al [2000] PNAS 97 : 12356-12360)
Localisation of *IRT1* expression territories

*pIR1::GUS* fusions expressed at the root periphery in the epidermal cell layer in response to iron deficiency

*IRT1* mRNA detected in the root periphery by *in situ* hybridization

*IRT1*-GFP fusion localized at the plasma membrane
**IRT1 is involved in iron uptake**

<table>
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<th><strong>WT</strong></th>
<th><strong>irt1-1</strong></th>
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<tr>
<td>Fe</td>
<td>R</td>
<td>L</td>
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</table>

55Fe feeding for 48 hours indicates that *irt1-1* is deficient in iron uptake.

**IRT1-KO mutant is chlorotic and altered in its development.**

Conclusion

IRT1 is the Major Essential Root Iron Transporter in Dicots to Take Up Iron from the Soil in case of Deficiency

Varotto et al. (2002) Plant J 31:589-599
Henriques et al. (2002) Plant Mol Biol 50:587-597

IRT2 shares the same territories than IRT1, but its function is not redundant to IRT1

Vert et al. (2001) The Plant J, 26:181-189
Maize (graminaceous) YS1

YS1 for Yellow Stripe 1 (Beadle, 1929)
Interveinal leaf yellowing
Deficient in Fe(III)-mugineic acid root uptake (von Wiren et al., 1994, Plant Physiol 106: 71-77)

Ac transposon tagging of ys1 by Elsbeth Walker (University of Massachussetts at Ahmerst)

2.8 kb ys1 cDNA cloned from our root library originated from iron-starved plants
Maize YS1 and 8 YS-Like from *Arabidopsis* are very similar

*Arabidopsis* YSL are highly conserved with maize YS1:
- 70 to 80% similarities
- 12 putative transmembrane domains
- E residues enrichment at the NH2-terminal extremity
- REGLE-like motif conservation at the NH2-terminal extremity
- both belongs to the Oligo Peptide Transporter (OPT) family

Conclusions
- YS1 is the major graminaceous Fe(III)-phytosiderophores uptake transporter
- YS1 is also able to transport Fe(II)-NA when expressed in heterologous systems (yeast ans Xenopus oocytes)
- Eight Arabidopsis thaliana genes (ysl 1-8) are homologous to maize Ys1, raising the questions of their role in non graminaceous plants, and of the nature of the substrate they could transport (Iron-Nicotianamine?)


Electrophysiology of YS1 = proton coupled phytosiderophore symporter

bHLH transcription factor networks for regulation of iron uptake transport systems


Iron circulation within the plant

Fe(III)-citrate and Fe(II) NA are two major chelates for iron distribution throughout the plant via xylem (blue) and phloem (pink) saps.

Frd3 (MATE type) involved in citrate loading of xylem.

Fe(II)-NA is transported by YSLs.

Nicotianamine (NA) structure

YSL transporters

Overview of sub-cellular iron utilisation in plant cells

- Vacuole iron influxer (VIT1) and effluxers (Naramp3 / Nramp4) have been identified.
- Transporters for mitochondria and Chloroplasts iron uptake are still uncharacterized.
- Both mitochondria and chloroplasts are able to synthesize heme and Fe-S clusters.

Arabidopsis thaliana ferritins

- Ferritins are located in plastids

- 4 genes in Arabidopsis AtFer1 to -4

  Gaymard et al., 1996 Biochem. J. 318, 67-73
  Petit et al., 2001 Biochem. J. 359, 575-582

<table>
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<tr>
<th>shoots</th>
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<td>AtFer3</td>
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<td>AtFer4</td>
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<td>25 S</td>
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AtFer1, -3 and -4 transcriptional regulation in response to iron excess

AtFer1 = most expressed ferritin gene in response to iron excess

Model to study iron homeostasis regulation
**WORKING MODEL**

**A - Low iron**

- **REPRESSOR**

  5' → **AtFer1 promoter** → 3'

**B - High iron**

- **Plastid**
  - NOS activity enzyme
  - NO
  - Proteasome dependent degradation

- **REPRESSOR**

  5' → **AtFer1 promoter** → 3'

**References**

Ferritin gene expression in various *Arabidopsis* organs

*Arabidopsis* *AtFer*2 expressed specifically in mature seeds

*Arabidopsis* *AtFer*1-3-4 expressed in vegetative organs (leaves, roots, floral stalk), in flowers, and in germinating seeds.

Petit *et al.*, 2001 Biochem. J. 359, 575-582
What do we learn concerning ferritin function \textit{in planta}, using a reverse genetic approach in \textit{Arabidopsis}?

Ravet \textit{et al.}, 2009 Plant. J. 57 : 400-412

- Ferritin function in seeds
- Ferritin function in leaves
- Lack of ferritins and flower development
- Role of ferritins in iron mediated oxidative stress
What do we learn concerning ferritin function *in planta*, using a reverse genetic approach in *Arabidopsis*?

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T-DNA insertion mutation in the \textit{AtFer2} Ferritin Gene

- T-DNA insertion in the \textit{AtFer2} gene leads to mutant seeds lacking ferritins.
- Iron content is unchanged in ferritinless \textit{fer2} seeds.
- Seed ferritins are not a major iron store in \textit{Arabidopsis} (Iron ferritin = 5\% of total seed iron).
Role of Fer2 seed ferritin in protection against oxidative stress

Germination of ferritinless fer2 seeds is more sensitive to methylviologen treatment than wild type Col seeds

Seed ferritins participate to protection against oxidative stress during the germination process
Iron storage subcellular compartmentation in seeds

- **VIT1** is necessary for iron loading of vacuoles in seeds (Kim et al 2006 Science 314:1295-1298)

- **NRAMP3** and **NRAMP4** are required for Fe remobilization from vacuoles during germination (Lanquar et al 2005 EMBO J 24 :4041-4051)

- **Seed ferritin FER2** not a major storage form in plastids (Ravet et al, 2009 Plant. J. 57:400-412)

However, is there any crosstalk between vacuoles and plastids to establish seed iron homestasis?
Vacuoles / Plastids crosstalk to establish Seed Iron Homeostasis

Seed ferritin Fer2 protein abundance decreased in nramp3nramp4 KO mutants and in VIT1 overexpressors

(Ravet et al, 2009 Molecular Plant, in press)
Vacuoles / Plastids crosstalk to establish Seed Iron Homeostasis

Seed ferritin Fer2 transcript abundance unaffected nramp3nramp4 KO mutants

Post-transcriptional regulation of ferritin AtFer2 in seeds requires a functional iron storage vacuolar compartment

(Ravet et al, 2009 Molecular Plant, in press)
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- **Ferritin function in leaves**
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T-DNA insertion mutant in the *AtFer1-3 and -4* Ferritin Genes

T-DNA insertion in *AtFer1* and *AtFer4* genes leads to knock-out null single *fer1* and *fer4* mutants

T-DNA insertion in promoter region of *AtFer3* leads to knock-down single *fer3* mutant

Obtention of the triple *fer1*-3-4 mutants by succesive crossing using single *fer1*, *fer3* and *fer4* mutants give
Ferritin protein subunit content in leaves of various ferritin mutants

Confirmation that Fer2 protein is absent in leaves

Fer1 is the major ferritin protein in leaves present as a 28 kDa subunit, whereas Fer3/4 are subunits of 26.5 kDa

No ferritin subunits are detected in leaves of the fer1-3-4 triple mutant
Arabidopsis rosette biomass in wild type Arabidopsis and in the fer1-3-4 triple mutant lacking leaf ferritins

No biomass difference between Col (wild type plants) and the fer1-3-4 mutant devoided of leaf ferritin when irrigated with tap water

Irrigating Col with a Fe-EDDHA solution increases its biomass

Irrigating fer1-3-4 with a Fe-EDDHA solution decreases its biomass
Analysis of various components of the *fer1-3-4* mutant photosynthetic apparatus

Amounts of plastidial electron transfer chain and photosynthesis related polypeptides, and chlorophyll content are unchanged in the *fer1-3-4* mutant.
Quantum yield of PSII ($\Phi_{PSII}$) is unaffected in the $fer1-3-4$ mutant under Fe or water irrigation.

Net Photosynthesis rate is decreased in the $fer1-3-4$ mutant when irrigated with 2 mM Fe-EDDHA.
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Pleiotropic alterations during fruit formation in the *fer1-3-4* mutant

Impaired flower and silique development in Fe-irrigated *fer1-3-4* mutant

Seed production decreases in the *fer1-3-4* mutant irrigated with iron
Reciprocal grafting between Col and *fer1-3-4* bolted plants

*fer1-3-4* flowers phenotype is independent of leaves.
Iron content in Col and *fer1-3-4* floral stalks

Under water irrigation conditions, Fe content is unchanged between Col and *fer1-3-4* plants in floral stem, and in unfertilized or fertilized flowers.

Under iron irrigation conditions, Fe content decreases in floral stems of *fer1-3-4*, and increases in unfertilized or fertilized flowers, comparatively to Col.
Metal transporters expression in Col and fer1-3-4

<table>
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<th>Gene</th>
<th>AGI</th>
<th>Stem Col</th>
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</tr>
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</table>

Fold change of the expression of various metal transporter genes between iron irrigated or water irrigated plants are altered in fer1-3-4 comparatively to Col

Alteration of iron allocation between stems of floral stalks and flowers in fer1-3-4 plants could be related to the deregulation of expression of various metal transporter genes
What do we learn concerning ferritin function *in planta*, using a reverse genetic approach in *Arabidopsis*?

Ravet *et al.*, 2009 *Plant. J.* 57: 400-412

- Ferritin function in seeds
- Ferritin function in leaves
- Lack of ferritins and flower development
- Role of ferritins in iron mediated oxidative stress
ROS imaging in *Col* and *fer1-3-4* Arabidopsis flowers

**GUS staining**

- Col Fe - +
- pAtFer1:GUS
- pAtFer3:GUS
- pAtFer4:GUS

**ROS imaging**

- DCFDA ROS imaging
- Chlorophyll
- Overlay

**ROS quantification**

Reactive Oxygen Species (ROS) overaccumulate in *fer1-3-4* flowers from water irrigated plants (and even more from iron irrigated plants, not shown)
ROS detoxifying enzyme activities in Col and *fer1-3-4* Arabidopsis leaves and flowers

> Catalase
> Ascorbate peroxidase
> Glutathione reductase

ROS detoxifying enzyme activities are enhanced in *fer1-3-4* leaves and flowers from water irrigated plants (and even more from iron irrigated plants, not shown)
Conclusions (I)

- Fer2 is the only seed ferritin in *Arabidopsis* with a:
  - limited function in iron storage
  - role for protecting germinating seedlings against oxidative stress

- A *fer1-3-4* mutant has no leaf ferritin and consequently
  - its biomass is affected when irrigated with iron
  - its CO2 fixation is decreased, without alteration of the thylakoid electron transfer chain

- *fer1-3-4* mutant has pleiotropic flower defects when irrigated with iron
  - independently of the lack of ferritins in leaves
  - leading to seed yield decrease
Conclusions (II)

Expression of various metal transporters is deregulated in *fer1-3-4*, consistent with an alteration of iron allocation between stems of floral stalk and flowers.

ROS scavenging mechanisms are enhanced in *fer1-3-4* plants:
- sufficient to avoid oxidative damages under water irrigation
- insufficient under Fe excess, explaining phenotypes (decreased biomass, flower and siliques alterations) under these conditions.
Iron nutrition and homeostasis is a very dynamic process involving many molecular actors which are integrated at the whole plant level through complex regulatory pathways (hormones, long distance signaling, transcription factor networks, post-transcriptional controls...etc) ................

.... It is another story which is starting to emerge ....

Than you for your attention!