# *Why does the red algal plastid lineage lack plastocyanin?*

## Molecular evolution of metal transport mechanisms in unicellular algae

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# Metal homeostasis and tolerance

- metal cations are crucial for plant nutrition
  - copper, iron, zinc, and manganese act as important cofactors for many enzymes and are essential for both mitochondrial and chloroplast functions
- in excess, these essential cations become toxic
  - like heavy metals with no generally established function, such as cadmium, lead or mercury
- plants have developed a complex network of metal uptake, chelation, trafficking, and storage processes
  - metal transporters are required to maintain metal homeostasis and thus constitute important components of this network (Clemens 2001; Hall and Williams 2003)

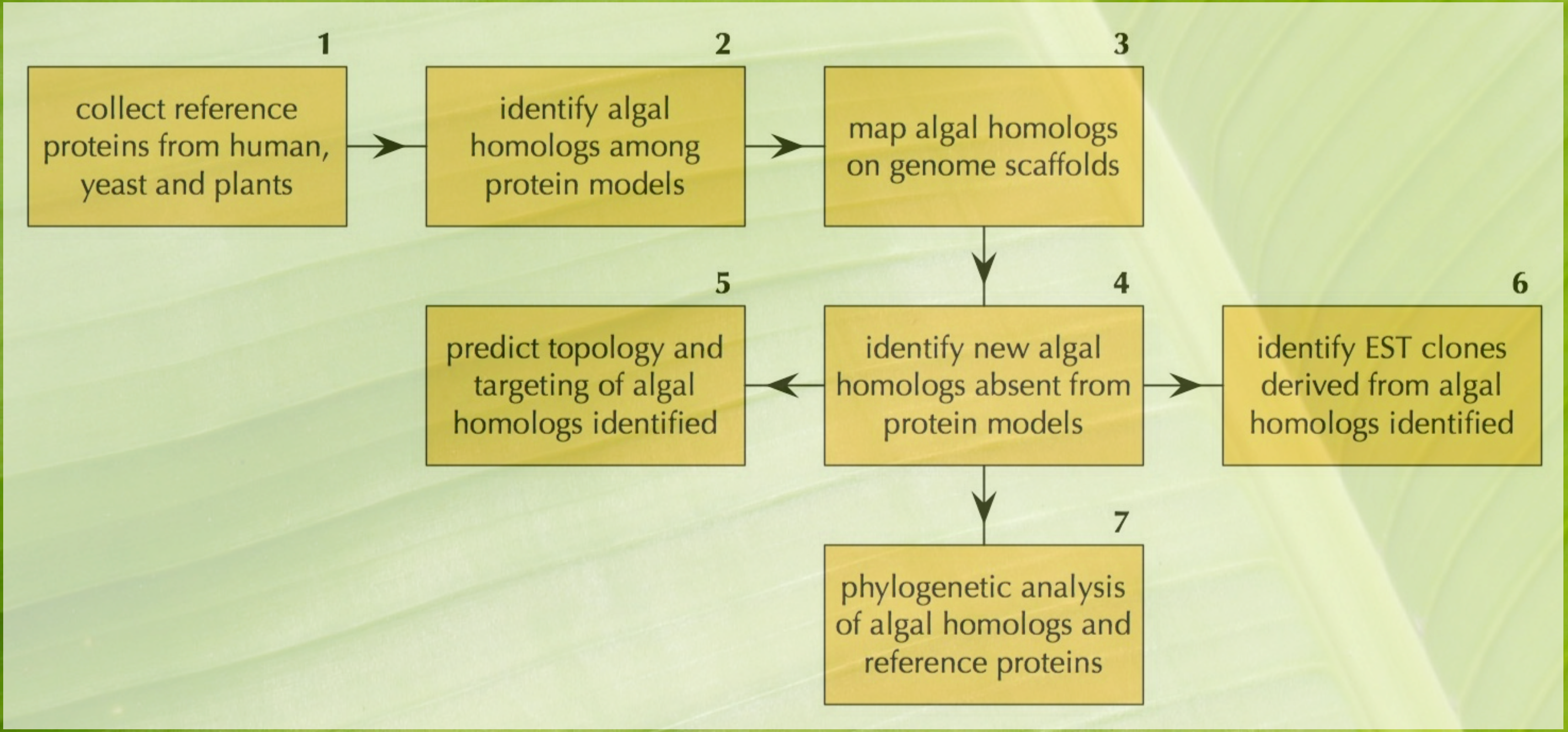
# Two unicellular algal models

- *Chlamydomonas reinhardtii* Dangeard — unicellular green alga (flagellate) living in water and soils; nuclear genome size: 125 Mbp (19,832 ORFs) (http://genome.jgi-psf.org/)

- *Cyanidioschyzon merolae* De Luca, Taddei, and Varano — unicellular red alga living in sulphur- and metal-rich acidic hot springs (pH 1.5 at 45°C); nuclear genome size: 16.5 Mbp (5,531 ORFs) (Matsuzaki et al. 2004)

# Efficient mining of multigene families

## Overview of the semi-automated analysis pipeline



1
collect reference proteins from human, yeast and plants

2
identify algal homologs among protein models

3
map algal homologs on genome scaffolds

4
identify new algal homologs absent from protein models

5
predict topology and targeting of algal homologs identified

6
identify EST clones derived from algal homologs identified

7
phylogenetic analysis of algal homologs and reference proteins

Application to 11 families or subfamilies of metal transporters in *Chlamydomonas* and *Cyanidioschyzon*

# Two lifestyles, two different pictures

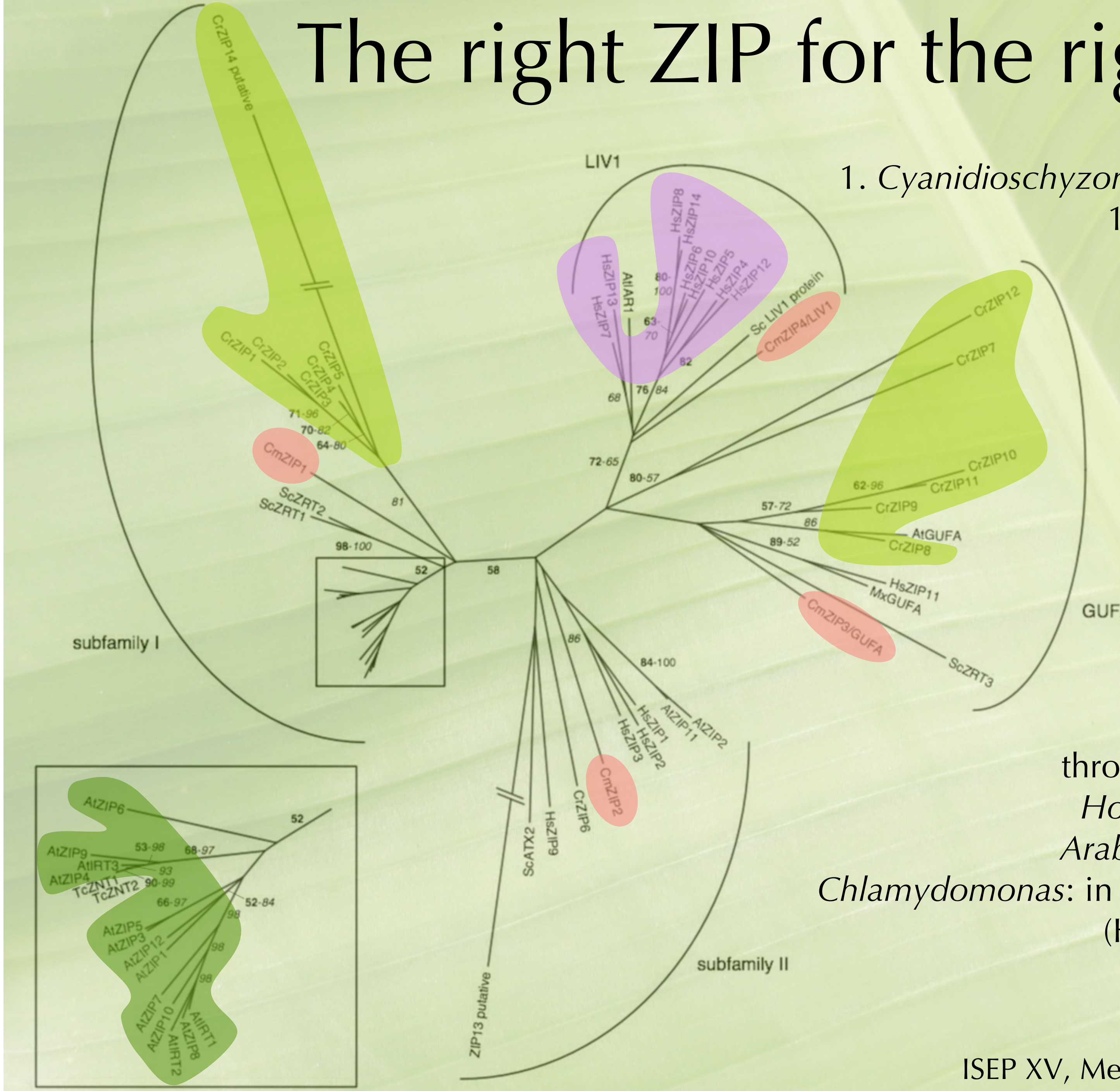
## Summary of metal transporter family sizes

| **Organisms** | **Protein families/subfamilies** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **CDF** | **ZIP** | **CAX** | **COPT** | **P-Type ATPases** | **ABC transporters** | | **YSL** | **FTR** | **NRAMP** | **IREG1** |
| | | | | | **HMA** | **MRP** | **ATM/HMT** | | | | |
| *Homo* | 9 | 14 | – | 2 | 2 | 12 | 2 | – | – | 2 | 1 |
| *Saccharomyces* | 5 | 5 | 1 | 3 | 2 | 6 | 1 | 1 | 1 | 3 | – |
| *Arabidopsis* | 12 | 17 | 12 | 5 | 8 | 15 | 3 | 9 | – | 6 | 3 |
| *Chlamydomonas* | 5 | 14 | 4 | 1 | 3 | 7 | 3 | – | 1 | 3 | – |
| *Cyanidioschyzon* | 3 | 4 | 2 | 1 | 2 | 2 | 3 | – | 4 | 3 | 1 |

*Chlamydomonas* has 41 putative metal transporters
while *Cyanidioschyzon* has 25 putative metal transporters

(Hanikenne et al. 2005)

# The right ZIP for the right metal



1. *Cyanidioschyzon* is the **ancestral state**:
1 protein = 1 subfamily

2. **ZIP diversification**
through massive radiation:
*Homo*: in subfamily LIV1
*Arabidopsis*: in subfamily I
*Chlamydomonas*: in subfamily I and GUFA
(Hanikenne et al. 2005)

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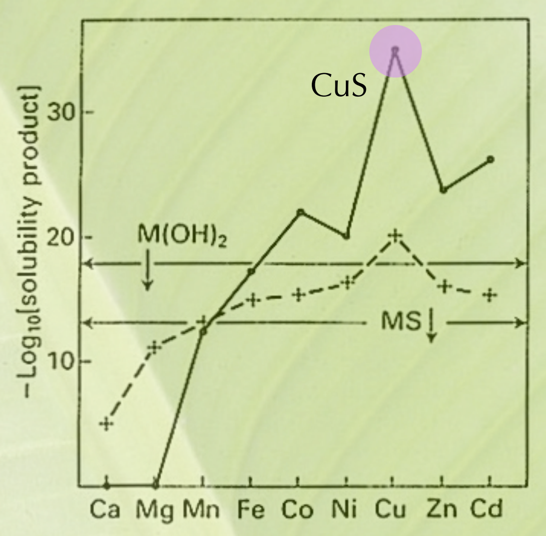
# With or without iron and copper

- before photosynthesis (–4,000 MYA)
  - no $O_2$ (reducing environment)
  - Fe (II) available
  - Cu (II) unavailable [CuS ↓]
- after photosynthesis (–2,700 MYA)
  - $O_2$ (oxidizing environment)
  - Fe (III) unavailable [$Fe(OH)_3$ ↓]
  - Cu (II) available



(Fraústo da Silva and Williams 2001)

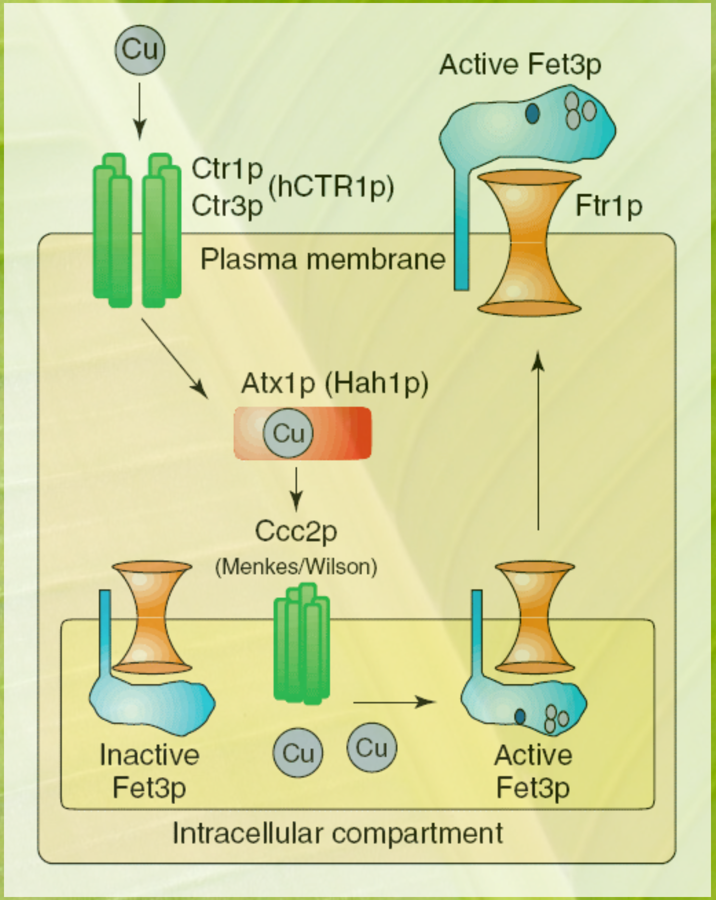
| Element | Reducing environment | Oxidizing environment |
|---|---|---|
| iron | Fe (II) (high) | as hydroxide (low) |
| copper | as sulphide (low) | Cu (II) (moderate) |
| sulphur | $HS^-$ (high) | $SO_4^{2-}$ (high) |

element availabilities (Fraústo da Silva and Williams 2001)

being Fe- and S-rich, acidic hot springs could mimick the primitive reducing environment and deplete the water from its soluble Cu (Brock 1978; Teasdale et al. 1996; Fraústo da Silva and Williams 2001)

# Fe / Missing and spare puzzle pieces

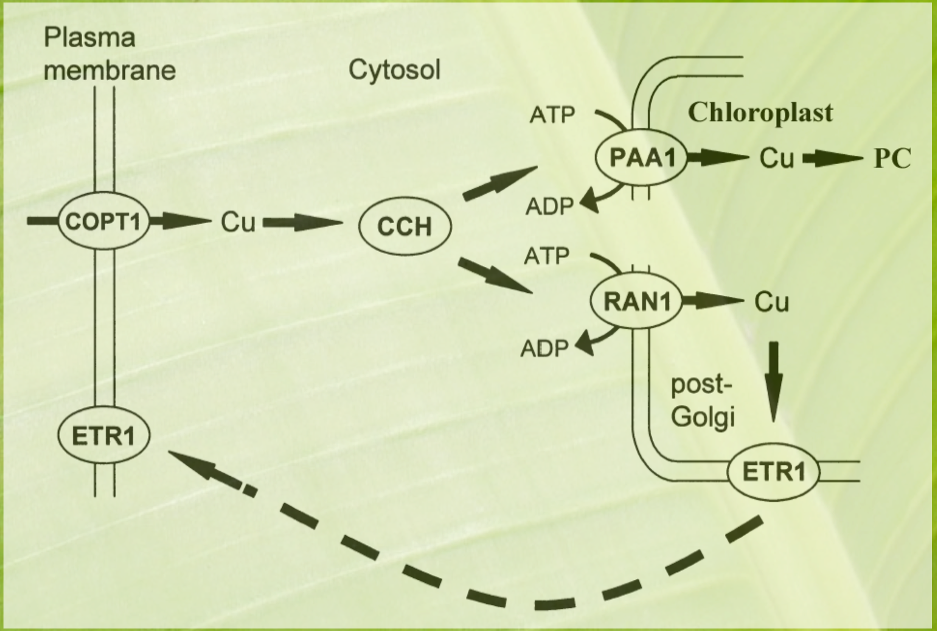
- IREG1 (Fe efflux)
  - first described in human enterocytes (McKie et al. 2000)
  - found in Arabidopsis, but not in yeast nor in *Chlamydomonas*
  - one gene in *Cyanidioschyzon*
- FTR (Cu-dependent Fe uptake)
  - first described in yeast (Radisky and Kaplan 1999)
  - *Chlamydomonas* has the complete pathway, along with another uncharacterized Cu-independent pathway (Lafontaine et al. 2002)
  - *Cyanidioschyzon* lacks the Cu-oxidase, but has four FTRs, among which two are very highly expressed, while the others would be targeted to the mitochondrion



(Askwith and Kaplan 1998)

# Cu / More missing pieces

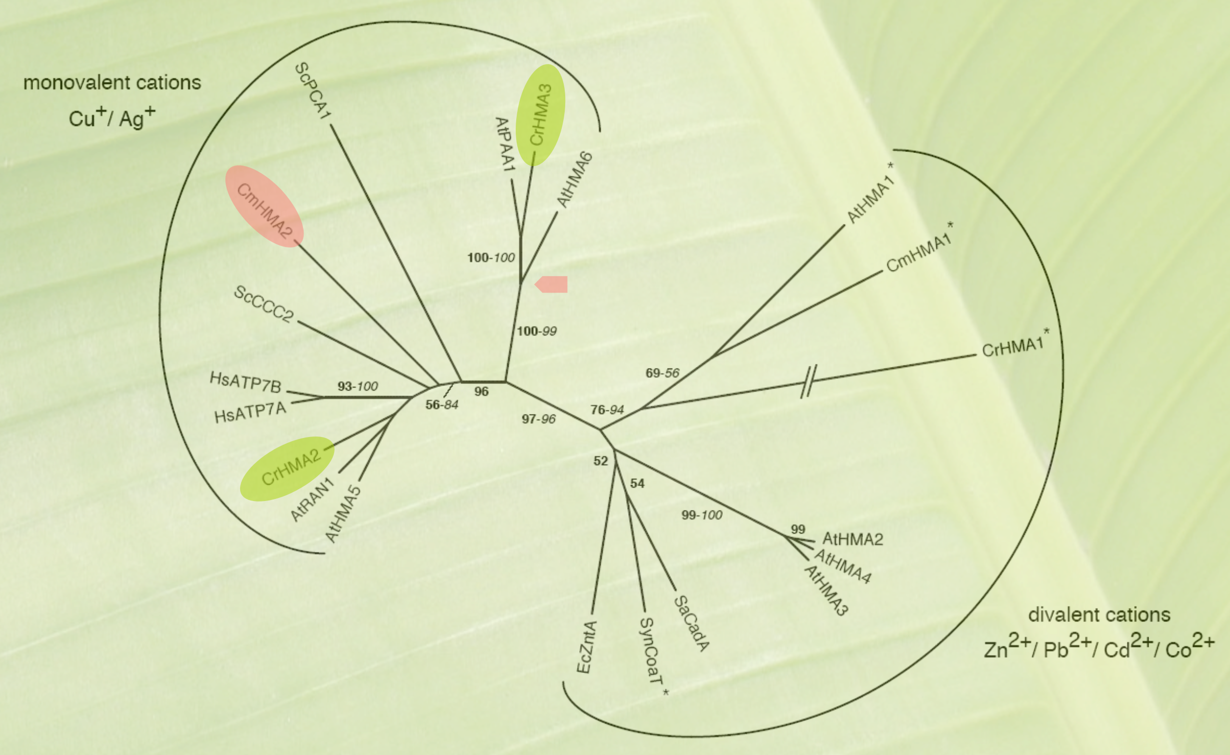
- Cu uptake in *Arabidopsis*
  - similar to yeast, but with one additional compartment, the plastid
  - COPT1 is homologous to CRT1/3, CCH (chaperon) to ATX1, and RAN1 (P-type ATPase/HMA) to CCC2; ETR1 is an ethylene receptor (another Cu-protein) assembled in Golgi
  - PAA1 (also HMA) import Cu into the plastid



(Williams et al. 2000)

# Cu / More missing pieces *(continued)*

- *Chlamydomonas* has the complete pathway for both compartments (Lafontaine et al. 2002)
- *Cyanidioschyzon* has a RAN1-like (Golgi) protein but lacks the PAA1 (plastid) homolog (as well as PC)



(Hanikenne et al. 2005)

# Lacking PC and doing well

- in some photosynthetic organisms, PC may be replaced by a soluble *c*-type cytochrome known as Cyt $c_6$ (or Cyt $c_{553}$) (Wood 1978; Merchant 1998)
- although phylogenetically unrelated, Cyt $c_6$ and PC actually do share a number of crucial physicochemical parameters (De la Rosa et al. 2002)
  - size (8–10 kDa)
  - redox potential (340–370 mV)
  - isolectric point (variable but similar within each organism)
  - functional areas (charged patches and hydrophobic residues)
- 3 types of organisms (Wood 1978; Sandmann et al. 1983)
  - Cyt $c_6$ only
  - PC only
  - both Cyt $c_6$ and PC (expression according to Cu availability)

# Distribution of PC and Cyt $c_6$

## Green algae

| Organism | PC | Cyt $c_6$ | Organism | PC | Cyt $c_6$ |
|---|---|---|---|---|---|
| **Chlorophyceae (i)** | | | **Chlorophyceae (ii)** | | |
| *Haematococcus pluvialis* | ✓ | | *Scenedesmus obliquus* | ✓ | ✓ |
| *Dunaliella parva* | ✓ | | *Scenedesmus armatus* | ✓ | ✓ |
| *Chlamydomonas reinhardtii* | ✓ | ✓ | *Pediastrum boryanum* | ✓ | ✓ |
| *Gonium sociale* | ✓ | ✓ | *Monoraphidium braunii* | ✓ | ✓ |
| *Eudorina elegans* | ✓ | ✓ | *Eremosphaera viridis* | ✓ | ✓ |
| *Pandorina morum* | ✓ | ✓ | **Prasinophyceae** | | |
| **Charophyceae** | | | *Platymonas subcordiformis* | ✓ | |
| *Netrium digitus* | ✓ | ✓ | **Chlorophyceae (iii)** | | |
| *Micrasterias thomasiana* | ✓ | ✓ | *Chlorella* (genus) | ✓ | (✓) |

(Sandmann et al. 1983)

## Land plants

PC only (but see later)

# Distribution of PC and Cyt $c_6$ *(continued)*

## Red algae and red plastid lineage

| Organism | PC | Cyt $c_6$ | Organism | PC | Cyt $c_6$ |
|---|---|---|---|---|---|
| **Rhodophyta** | | | **Xhantophyceae** | | |
| *Cyanidium caldarium* | | ✓ | *Bumilleriopsis filiformis* | | ✓ |
| *Porphyridium cruentum* | | ✓ | *Vischeria stellata* | | ✓ |
| *Porphyridium aerugineum* | | ✓ | *Tribonema aequale* | | ✓ |
| *Polysiphonia* sp. | | ✓ | *Bumilleria sicula* | | ✓ |
| **Chrysophyceae** | | | **Phaeophyceae** | | |
| *Phaeodactylum tricornutum* | | ✓ | *Ectocarpus siliculosus* | | ✓ |
| *Skeletonema costatum* | | ✓ | *Cutleria multifida* | | ✓ |

(Sandmann et al. 1983)

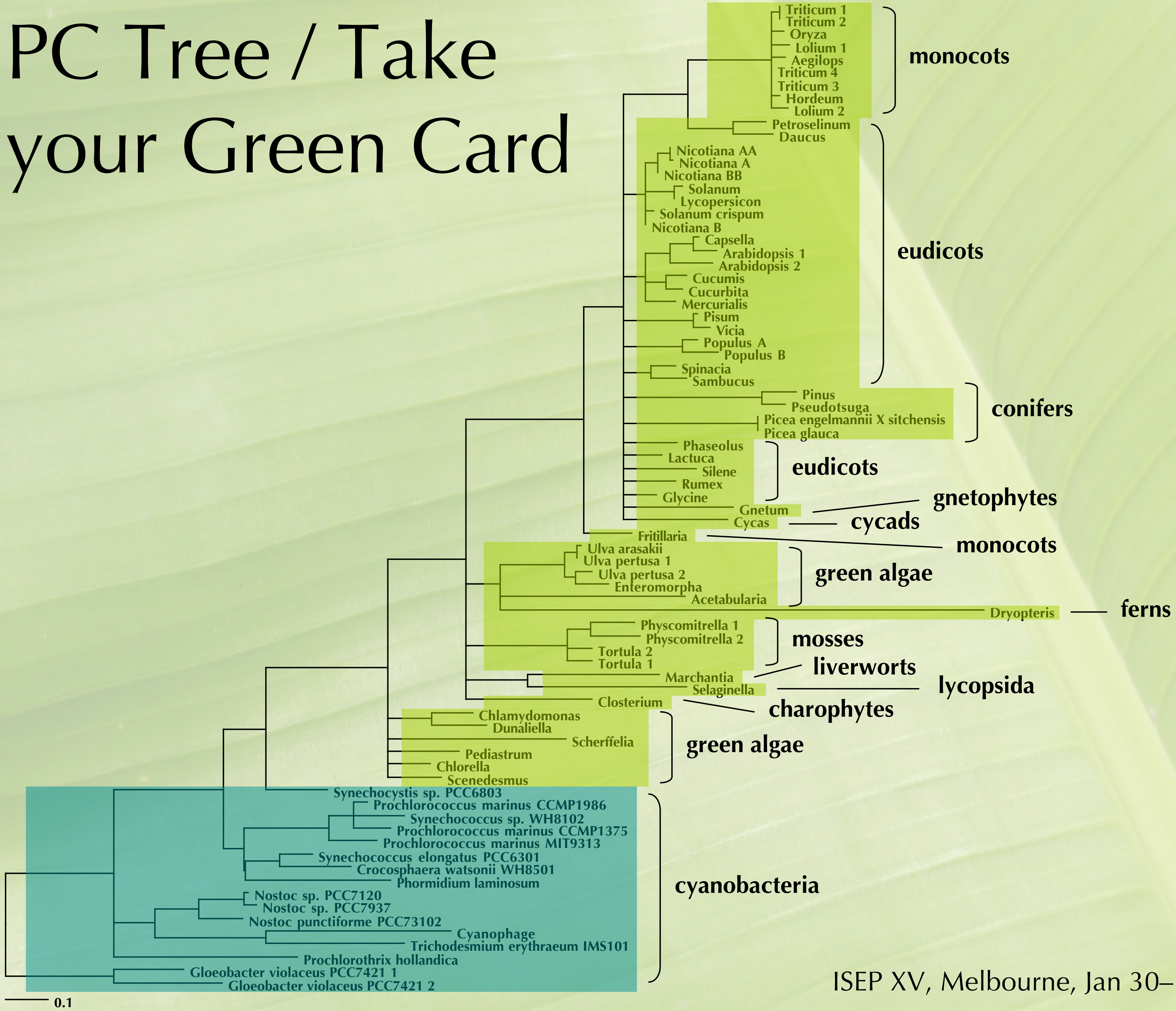
## Cyanobacteria

Cyt $c_6$ predominant (but some cyanobacteria have both PC and Cyt $c_6$)

# PC and Cyt $c_6$ in the genomic era

1. **collection** of PC and Cyt *c* protein sequences by BLASTP and TBLASTN
    - complete genomes of 9 cyanobacteria
    - complete genomes of 3 eukaryotic algae (*Chlamydomonas*, *Cyanidioschyzon*, and *Thalassiosira pseudonana*)
    - ESTs from GenBank (as of January 16, 2005)
2. **alignments** based on structural information whenever available (Redinbo et al. 1994; Navarro et al. 2004; Brayer and Murphy 1996; Kerfeld and Krogman 1998; Wastl et al. 2004
3. **tree** building
    1. topology: MP (ratchet)
    2. branch lengths: ML (WAG)

# PC Tree / Take your Green Card



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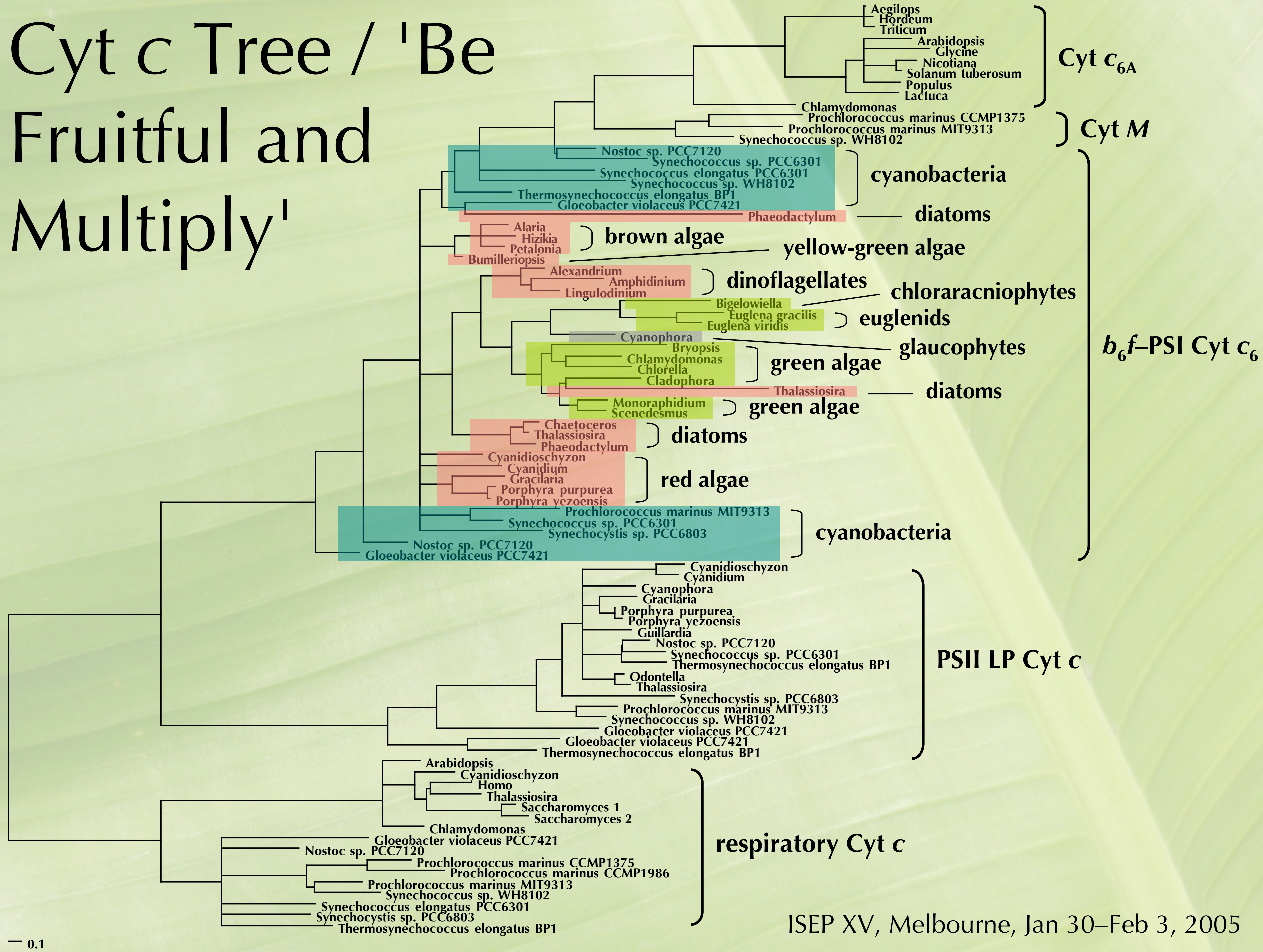
# Cyt *c* Tree / 'Be Fruitful and Multiply'



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# Red lineage / *Cyanidium* forever?

1. both PC and Cyt $c_6$ do exist in Cyanobacteria, but among Eukaryotes, PC is limited to primary green lineage; Cyt $c_6$ and $c_{6A}$ are found in both green and red lineages, as well as in Glaucophytes
2. Cyanidiales do live in an ecological niche notably Fe-rich and probably Cu-limited
3. since Cyanidiales appear to be basal to present red algae (Yoon et al. 2004), the PC loss (a Cu-protein) in favor of Cyt $c_6$ (an Fe-protein) in the red plastid lineage could be a relict of an era during which red algae were experiencing an evolutionary bottleneck as Cyanidiales in acidic hot springs

# Seeking the truth

in order to support our hypothesis, we should

1. apply our analysis pipeline to the complete genome and ESTs of the diatoms *Thalassiosira pseudonana* (Armbrust et al. 2004) and *Phaeodactylum tricornutum* (Montsant et al. 2005), as well as to *Emiliana huxleyi* (Haptophyceae) sequences as soon as they will be available
2. look for other Cu-proteins (mainly oxidases) that could be lacking in the red plastid lineage; Cu/Zn SOD would have been a good candidate but while it is found in fungi, it is missing in many lower Eukaryotes (Kitayama et al. 1999, Merchant 2005), including *Chlamydomonas* and *Cyanidioschyzon* (data not shown)

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**Reference**

Hanikenne M, Krämer U, Demoulin V, and Baurain D (2005) A comparative inventory of metal transporters in the green alga *Chlamydomonas reinhardtii* and the red alga *Cyanidioschyzon merolae*. *Plant Physiology* 137 (in press)