Critical Roles of Iron and Other Metals in Algal Productivity
Metal requirements of the photosynthetic apparatus
Reactive oxygen species: production hot sports in the photosynthetic apparatus

**Mn:** *Synechocystis* 6803 1x10⁶, *Rhodobacter capsulatus* 1x10⁴, *E. coli* 1x10⁴ atoms/cell

(Keren et al. 2002, Finney and O'Halloran 2003, Shcolnick et al. 2007)

**Fe:** *Synechocystis* 6803 5x10⁶, *E. coli* 1x10⁵ atoms/cell

(Keren et al. 2004, Finney and O'Halloran 2003)
Iron is the forth most abundant element in the Earth’s crust. Fe(III) is virtually insoluble at neutral pH in the presence of oxygen. Fe(II) is soluble but prone to react with reactive oxygen species.

\[
\begin{align*}
H_2O_2 + \text{Fe(II)} & \rightarrow \text{Fe(III)} + \text{OH}^- + \text{OH}^- \quad \text{(Fenton reaction)} \\
\text{O}_2^- + \text{Fe(III)} & \rightarrow \text{Fe(II)} + \text{O}_2
\end{align*}
\]
Iron uptake from the environment
Iron uptake from the environment

![Graph showing iron uptake from the environment over time with different concentration levels.]
Uptake from the environment
Iron limitation by chelators

![Graphs showing biomass vs. [chelator]/[Fe] with different concentrations of Fe and chelators: EDTA, DPTA, and DFB.](image)
Uptake from the environment
Iron transport rates

Iron uptake rate $\sim 3 \times 10^{-19}$ mol Fe cell$^{-1}$ h$^{-1}$
or $1.8 \times 10^5$ atoms cell$^{-1}$ h$^{-1}$

*Based on these $^{55}$Fe uptake assays*

Enough to support one doubling every $\sim 24$ h.
Membrane transport

FutABC
Fe(II) transporter

Fe

Outer membrane
Inner membrane
Thylakoid membranes

Katoh et al. (2000); Katoh et al. (2001); Koropatkin (2007)
\[ 2\text{Fe(II)} + \text{O}_2 + 4\text{H}_2\text{O} \rightarrow 2\text{Fe(O)OH(s)} + \text{H}_2\text{O}_2 + 4\text{H}^+ \]

1BFR (Frolow et al., 1994) and 1MOJ (Zeth et al., 2004)

\[ 2\text{Fe(II)} + \text{H}_2\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{Fe(O)OH(s)} + 4\text{H}^+ \]
Intracellular iron trafficking
A working hypothesis

- FutABC Fe(II) transporter
- Bacterioferritin
- DPS
- Thylakoid membranes

Fe

Outer membrane
Inner membrane
Experimental design
Iron limitation by chelators
Microarray experiments
4 way comparison design

\[ \text{WT-DFB} \leftrightarrow \Delta mrgA-DFB \leftrightarrow \Delta mrgA+DFB \leftrightarrow \text{WT+DFB} \]
### Microarray experiments

#### Results summary

<table>
<thead>
<tr>
<th>General pathway</th>
<th>No. of genes</th>
<th>ΔmrgA/WT</th>
<th>WT DFB/WT</th>
<th>ΔmrgA DFB/ΔmrgA</th>
<th>ΔmrgA DFB/WT DFB</th>
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</thead>
<tbody>
<tr>
<td>Amino acid biosynthesis</td>
<td>83</td>
<td>1↓ 2↑</td>
<td>4↓ 1↑</td>
<td>16↓ 14↑</td>
<td>16↓ 6↑</td>
</tr>
<tr>
<td>Biosynthesis of cofactors, prosthetic groups, and carriers</td>
<td>116</td>
<td>3↓ 5↑</td>
<td>5↓ 8↑</td>
<td>10↓ 25↑</td>
<td>23↓ 18↑</td>
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<tr>
<td>Cell envelope</td>
<td>63</td>
<td>0↓ 8↑</td>
<td>3↓ 1↑</td>
<td>12↓ 5↑</td>
<td>9↓ 4↑</td>
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<tr>
<td>Cellular processes</td>
<td>61</td>
<td>3↓ 3↑</td>
<td>4↓ 1↑</td>
<td>8↓ 19↑</td>
<td>3↓ 13↑</td>
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<tr>
<td>Central intermediary metabolism</td>
<td>31</td>
<td>2↓ 3↑</td>
<td>0↓ 2↑</td>
<td>3↓ 3↑</td>
<td>4↓ 1↑</td>
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<tr>
<td>DNA replication, restriction, modification, recombination, and repair</td>
<td>51</td>
<td>3↓ 1↑</td>
<td>2↓ 0↑</td>
<td>5↓ 10↑</td>
<td>8↓ 6↑</td>
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<td>Energy metabolism</td>
<td>86</td>
<td>1↓ 3↑</td>
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<tr>
<td>Fatty acid, phospholipid and sterol metabolism</td>
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<td>2↓ 1↑</td>
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<tr>
<td>Hypothetical</td>
<td>449</td>
<td>41↓ 45↑</td>
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<td>Other categories</td>
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<td>Photosynthesis and respiration</td>
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<tr>
<td>Purines, pyrimidines, nucleosides, and nucleotides</td>
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<td>Regulatory functions</td>
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<td>Transcription</td>
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<td>8↓ 3↑</td>
<td>33↓ 15↑</td>
<td>56↓ 11↑</td>
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<tr>
<td>Transport and binding proteins</td>
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<td>7↓ 17↑</td>
<td>18↓ 38↑</td>
<td>34↓ 14↑</td>
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<tr>
<td>Unknown</td>
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<td>10↓ 11↑</td>
<td>58↓ 89↑</td>
<td>45↓ 64↑</td>
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<tr>
<td><strong>Total</strong></td>
<td>3165</td>
<td>127↓ 128↑</td>
<td>97↓ 92↑</td>
<td>342↓ 572↑</td>
<td>458↓ 321↑</td>
</tr>
</tbody>
</table>

**Table 1:** Differentially regulated genes in response to the DFB treatment or to deletion of *mrgA*, according to their functional category:

Genes were considered deferentially regulated if \(|\text{fold change}| > 1.4\) and \(p < 0.05\). The number of transcripts that were down regulated by the treatment (DFB, ΔmrgA or both) is marked by \(↓\), the number of unregulated genes by \(↑\). Categories which are discussed in the manuscript appear in bold typeface.

- NADH dehydrogenase genes ↓
- Phycobiliprotein genes ↓
- Nitrogen assimilation ↓
- Detoxification genes ↑
- **futC** ↑
- **feoB** ↑
- **slr1406** ↑
- **slr1316–slr1319** ↑
Microarray experiments
Results summary

Wild-type +DFB/Wild-type:
- slr1736
- slr1737
- PerR
- Psb28-2 Oligopeptide binding protein of ABC transporter
- sll1621
- AhpC peroxiredoxin
- sll1620
- slr1739
- slr1740
- trnD-GUC
- sll1618

DmrgA +DFB/DmrgA:
- slr1736
- slr1737
- PerR
- Psb28-2 Oligopeptide binding protein of ABC transporter
- sll1621
- AhpC peroxiredoxin
- sll1620
- slr1739
- slr1740
- trnD-GUC
- sll1618

Fold change:
-5  -4  -3  -2  -1  0  3  6  9  12  15

1 KB
Integration of iron homeostasis and ROS chemistry

% difference from corresponding wild type sample
Integration of iron homeostasis and ROS chemistry

Cells grown in 10 μM Fe and exposed to H$_2$O$_2$ for 24 h in a 0 μM Fe medium.
Integration of iron homeostasis and ROS chemistry

C. WT  
D. ΔmrgA  
E. ΔbfrAΔbfrB  
F. ΔbfrAΔbfrBΔmrgA

Low Fe grown cells
High Fe grown cells

[H2O2] (mM)
Integration of iron homeostasis and ROS chemistry
Role of PerR

![Graph showing cell growth over time with different conditions and labels for each condition](Image)
Integration of iron homeostasis and ROS chemistry
Updated working hypothesis
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