Transcript profiling reveals new insights into the acclimation of *Synechococcus elongatus* PCC 7942 to iron starvation

Klaus-Peter Michel

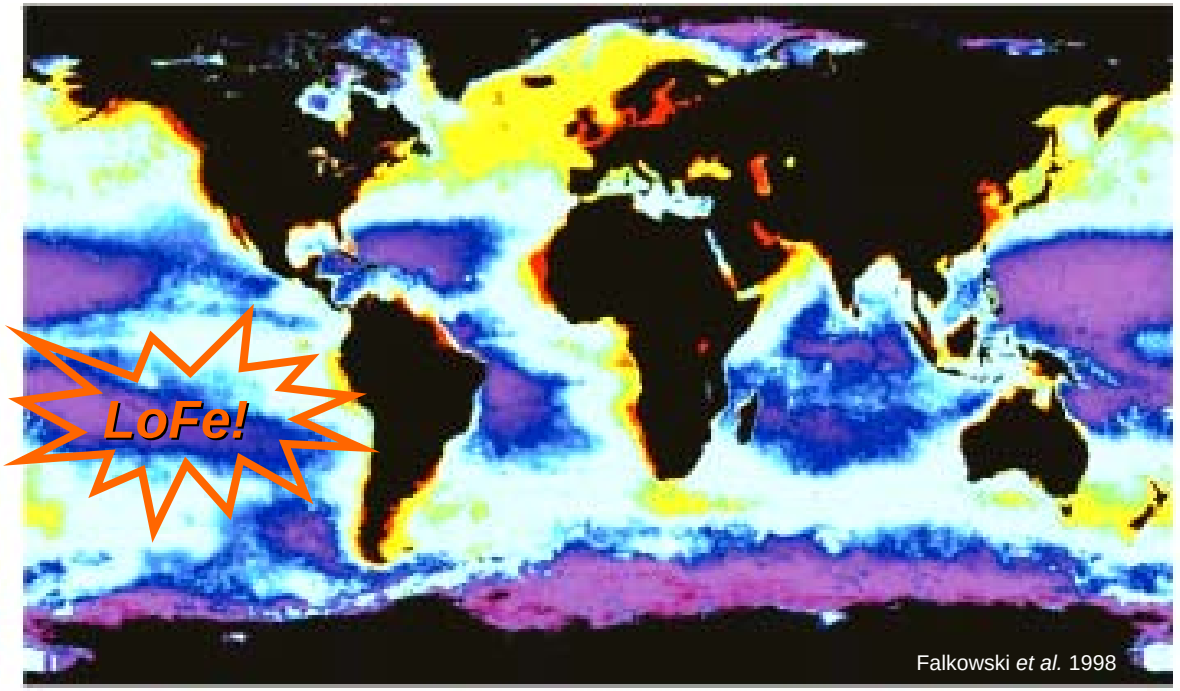
Molecular Cell Physiology
Department of Biology
Bielefeld University
D-33615 Bielefeld

Bielefeld University

Contents

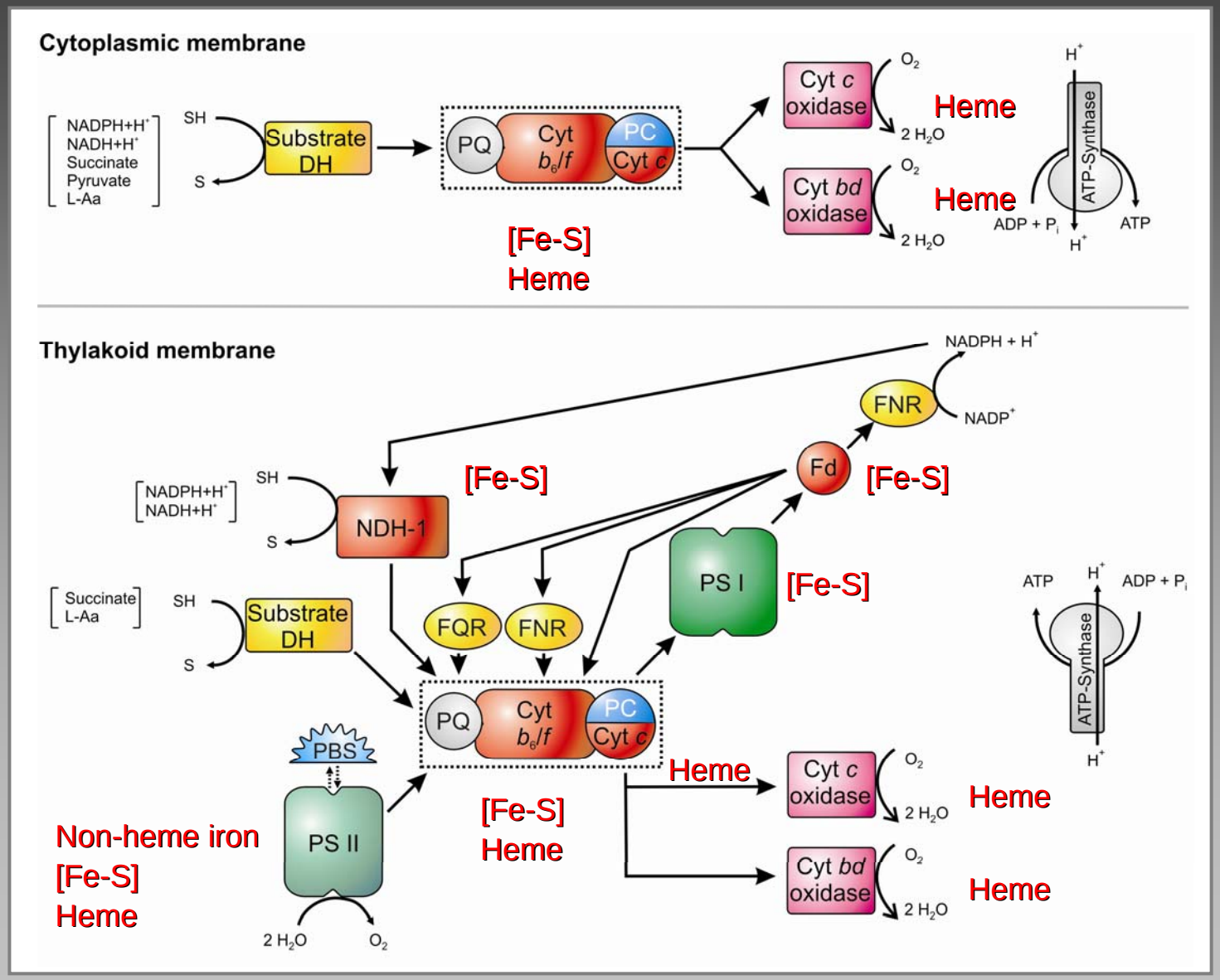
Iron deficiency severely limits biomass production in otherwise nutrient-rich habitats

Cyanobacteria still produce up to 40 % of the global biomass



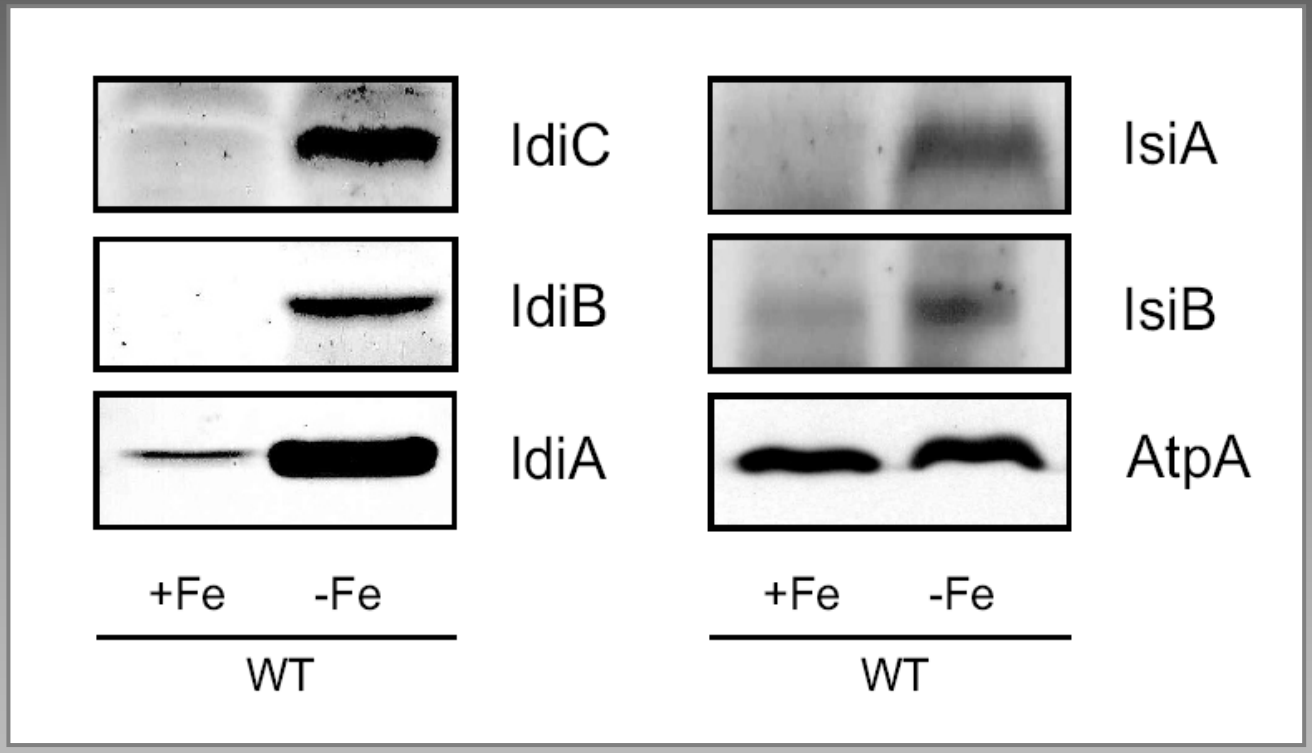
Iron deficiency often severely limits biomass production in otherwise nutrient-rich habitats.

Iron is an essential cofactor of the cyanobacterial electron transport



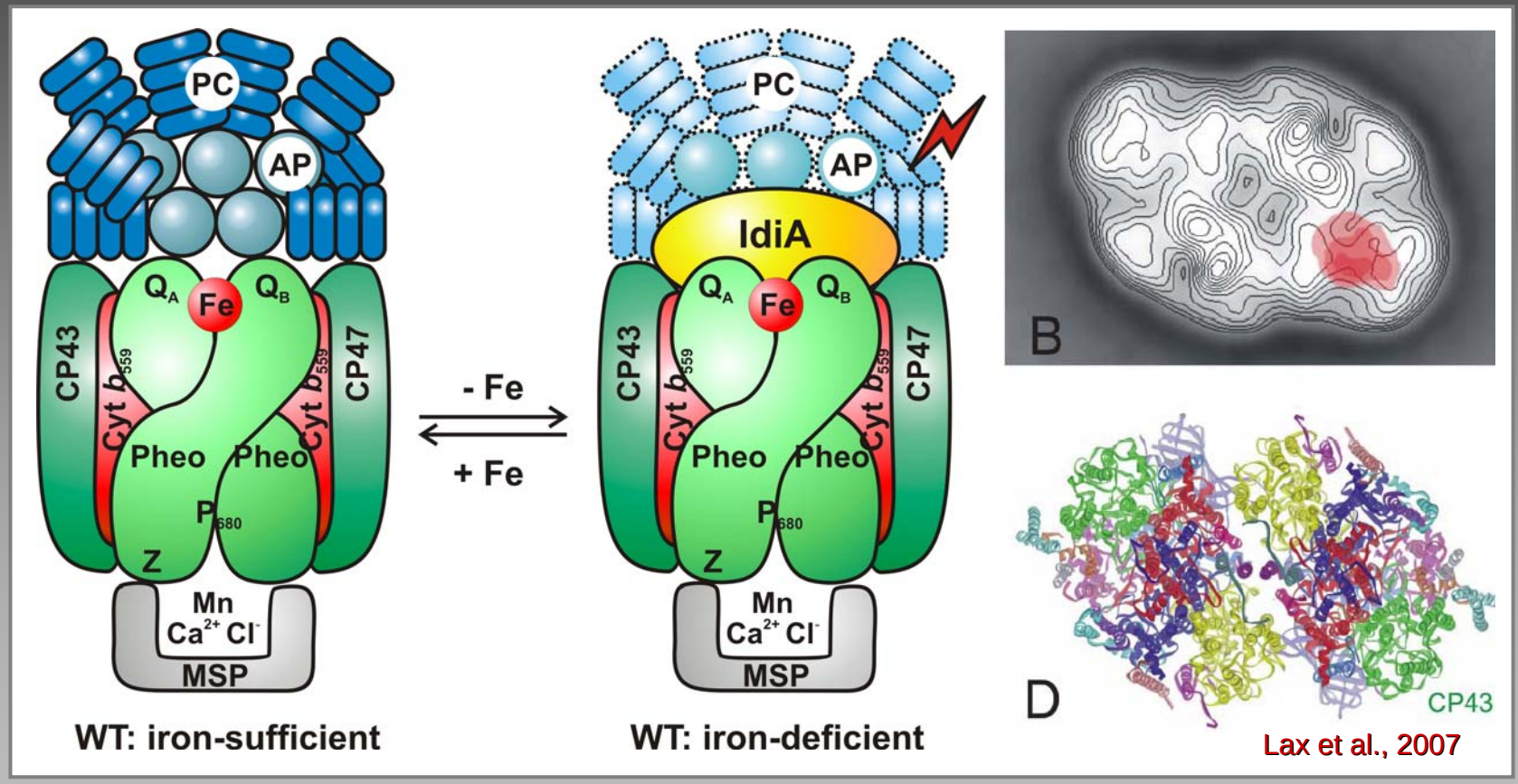
Idi and Isi proteins accumulate under LoFe growth conditions

Expression of Idi and Isi proteins in *S. elongatus* PCC 7942



Acclimation to iron starvation – Part I: IdiA

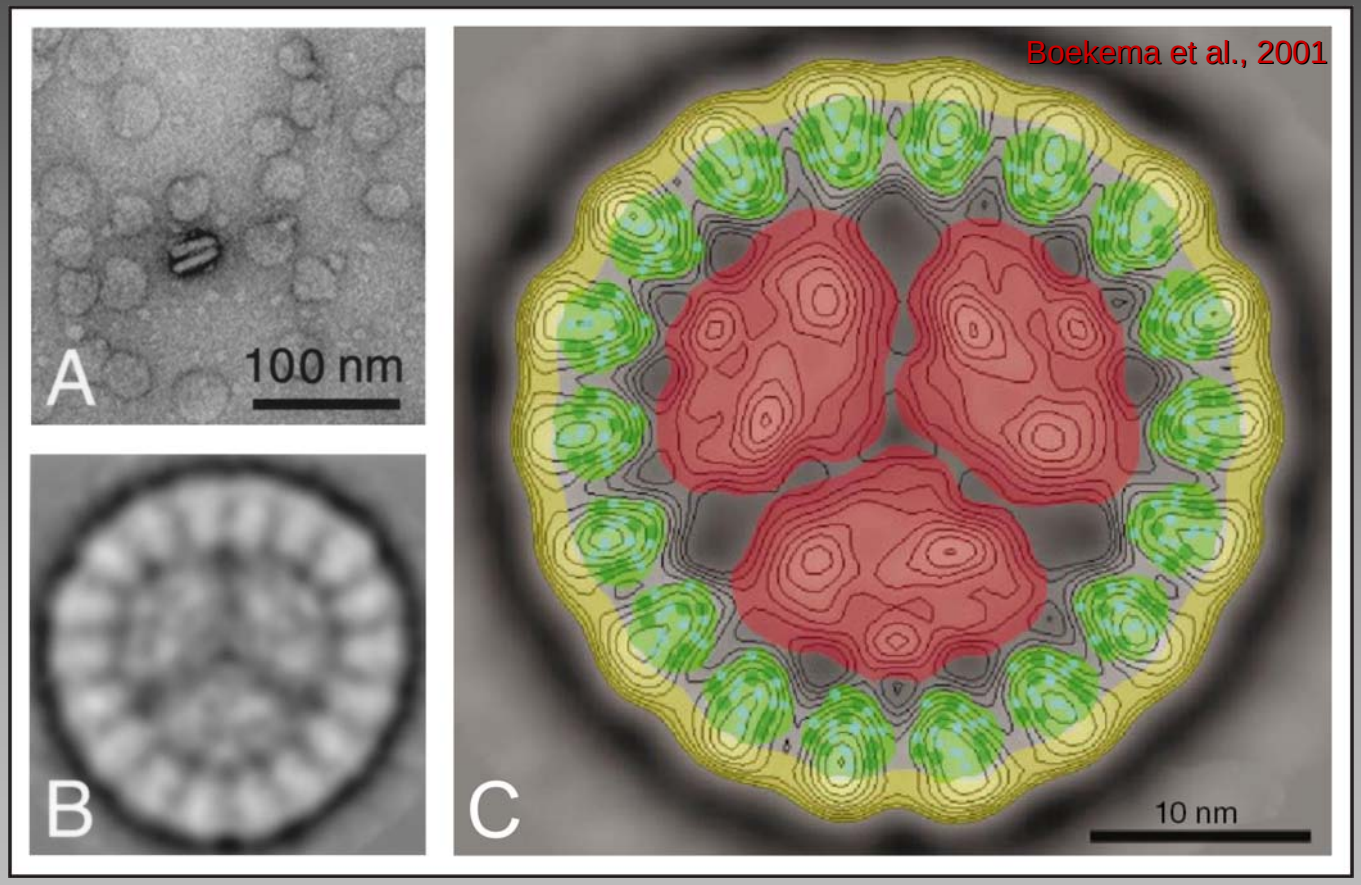
Model of IdiA function in *S. elongatus* PCC 7942



IdiA protects the acceptor side of PS II against oxidative stress, especially when this stress is caused by iron-deficient growth conditions.

Acclimation to iron starvation – Part II: IsiA

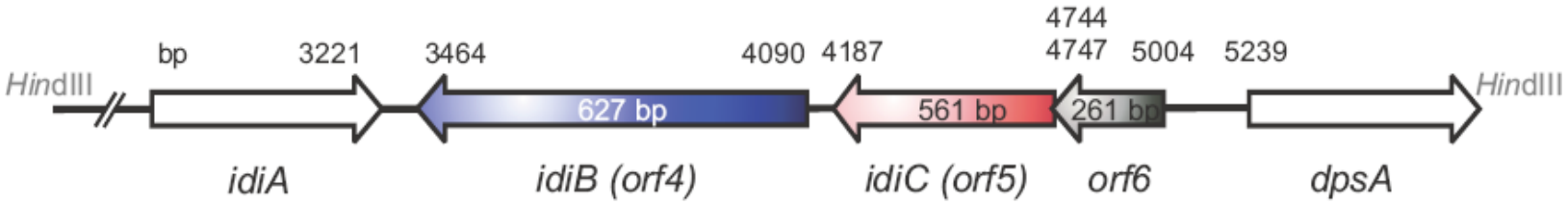
Contured version of a PS I-IsiA supercomplex from *S. elongatus* PCC 7942



IsiA has been assigned different functions besides the formation of an membrane-integral Chl a-containing light-harvesting antenna around trimeric PS I complexes of LoFe cells.

Identification of *idiC*/IdiC in *S. elongatus* PCC 7942

Properties of *idiC*/IdiC



- The gene *idiC* is part of the iron-regulated *idiB* operon together with *idiB* and *orf6*. *IdiB* encodes the transcription factor of *idiA* and *orf6* encodes a yet uncharacterized protein.
- The gene *idiC* consists of 561 bps and overlaps by 3 bps with the upstream *orf6*.
- The gene *idiC* contains no detectable transcriptional start or termination sites.
- All attempts to insertionally inactivate *idiC* by interposon mutagenesis created merodiploid mutants.
- The gene *idiC* encodes a soluble protein of 20.5 kDa, a pI of 9.17 (18 D/E and 24 R/K).

IdiC is a thioredoxin-like [2Fe-2S] ferredoxin

IdiC has similarity to NuoE of the *E. coli* NDH-1 complex

```
ORF5   S. PCC 6301   AVLEVCTRGTCRRRGALELCDRLQA----EAKTC----AVEVRTRGCLGRCKQGINVRRS
NuoE   E. coli       HHIRVCTGTACHLKGSEALLKALEKKLGIKPGETTADGKFTLEPVECLGACGQAPVVMIN
PetF2  S. PCC 7942   ----ICCHHTCPKQGSTAILAAFQA----QAPA-----DVEVRQAGCFGECGNGPLVR--
PetF   H. NRC-1      ----VCTNQTCAAEGAPAVLERLRQ----EARDADE-DSLRVTRTSCLGQCGDGPNVAVY
HoxF   S. PCC 6301   IRLRCCTATGCRANGAEAVFKAVQQ----TIADQNLGDRCEAVSVGCLGLCGAGPLVQCD
NuoF   B. VPI-5482   ----ICGGTGCKASSSQGITENLQK----AIERNGITDKVDVITVGCFGFCEKGPIVKII

IdiC             MKLPFYLEGYFLGLQDPDTPDNIRFIHVQSD-GGNRYTLKLAKPLRHLPWQQLTVGQPLR 59
NuoE             -------------MHENQQPQTEAFELSAAEREAIEHEMHHYEDPRAASIEALKIVQKQR 47

IdiC             -IEGQQSFQGLDLPPKLKAERVLFDPAGLPAFIASEEPPKPQPAVLEVCTRGTCRRRGAL 118
NuoE             GWVPDGAIHAIADVLGIPAS----DVEGVATFYSQIFRQPVGRHVIRYCDSVVCHINGYQ 103
IdiC             ELCDRLQAEAKTCAVEVRTRG--------CLGRCKQGINVRRSSDNQILSQLSPQAAAEL 170
NuoE             GIQAALEKKLNIKPGQTTFDGRFTLLPTCCLGNCDKGPNMMIDEDT--HAHLTPEAIPEL 161

IdiC             LSPWRTPAVVSGTAVG 186
NuoE             LERYK----------- 166
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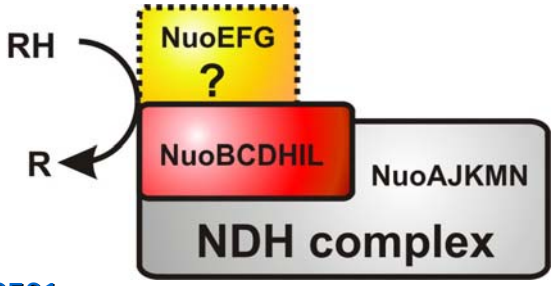
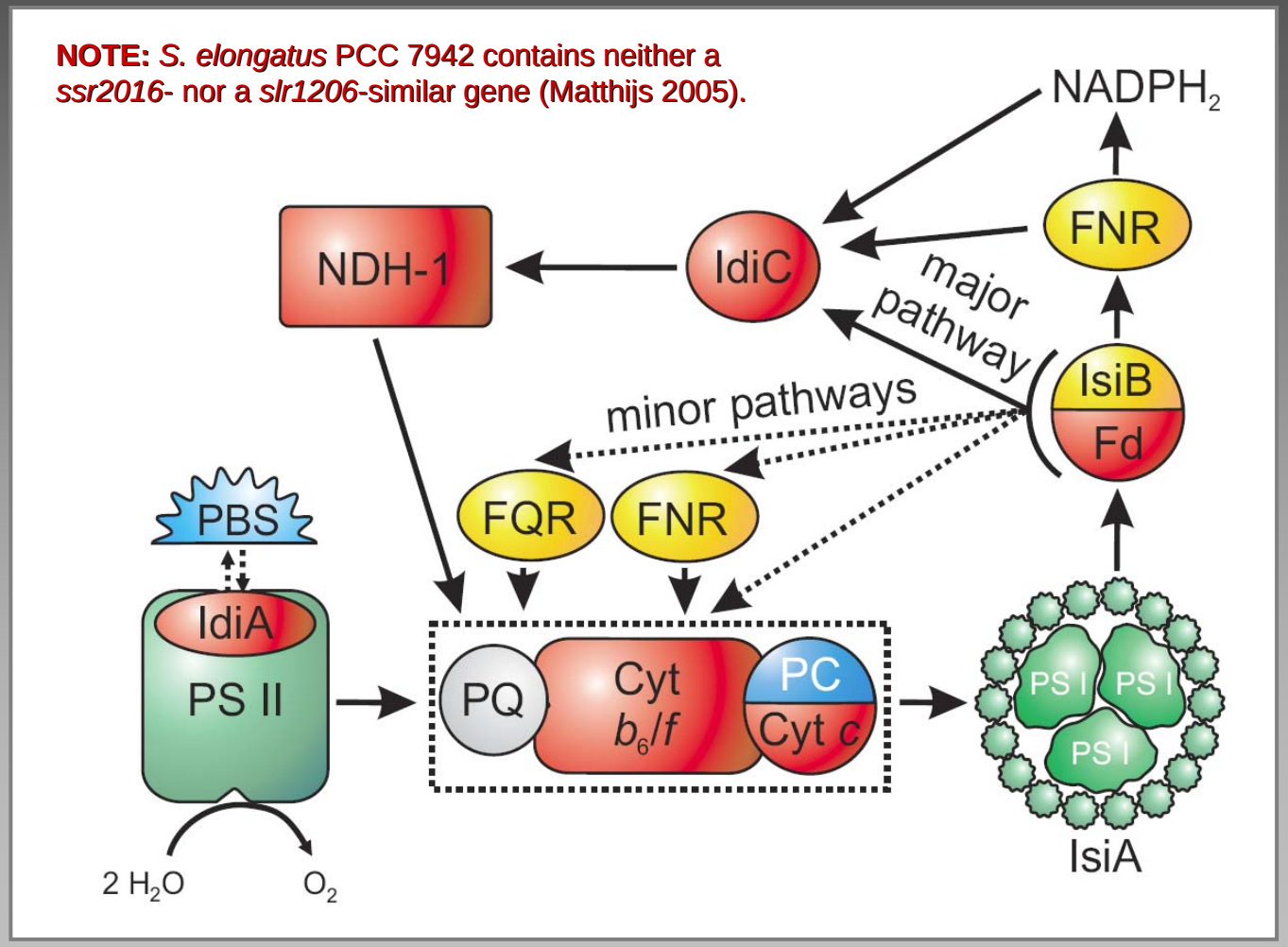


IdiC-similar proteins are present in:

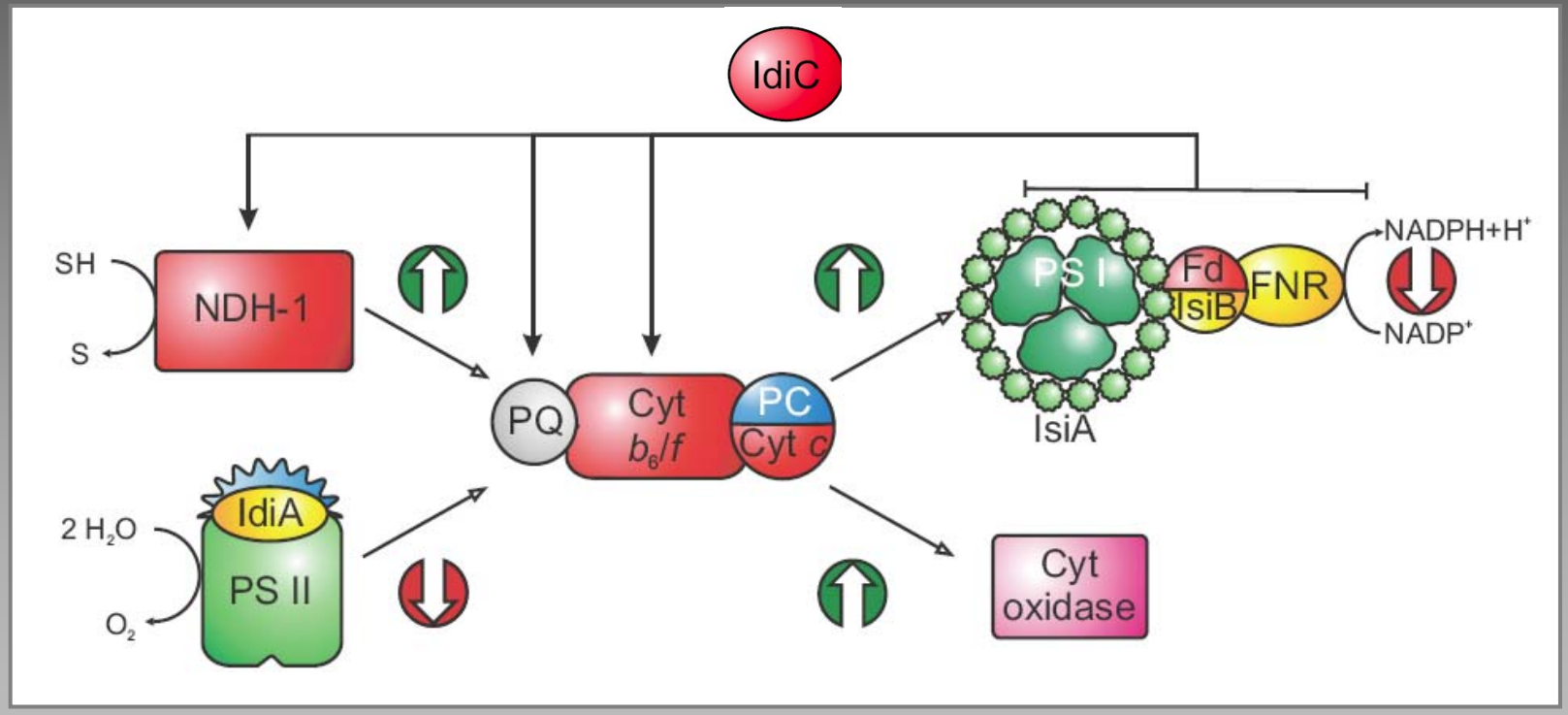
- *S. elongatus* PCC 6301
- *S. elongatus* JA-3-3AB
- *S. elongatus* JA-2-3BA (2-13)
- *Anabaena variabilis*
- *Anabaena* PCC 7120
- *Trichodesmium erythraeum*
- *Nodularia spumigena*
- *Lyngbia* sp. PCC 8106
- *Nostoc punctiforme*
- *Crocosphera watsonii* WH 8501
- *Gloeobacter violaceus* PCC 7421
- *Thermosynechococcus elongatus* BP-1

Model of IdiC-mediated photosynthetic cyclic electron transport around PS I in *S. elongatus* PCC 7942



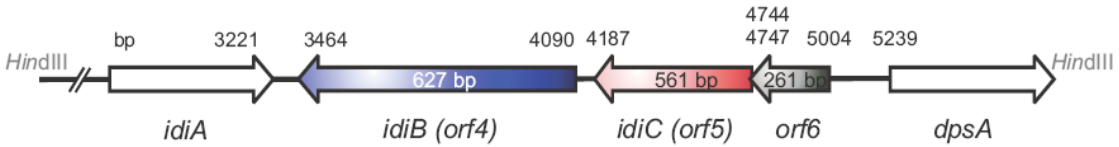
Physiological consequences of acclimation to LoFe

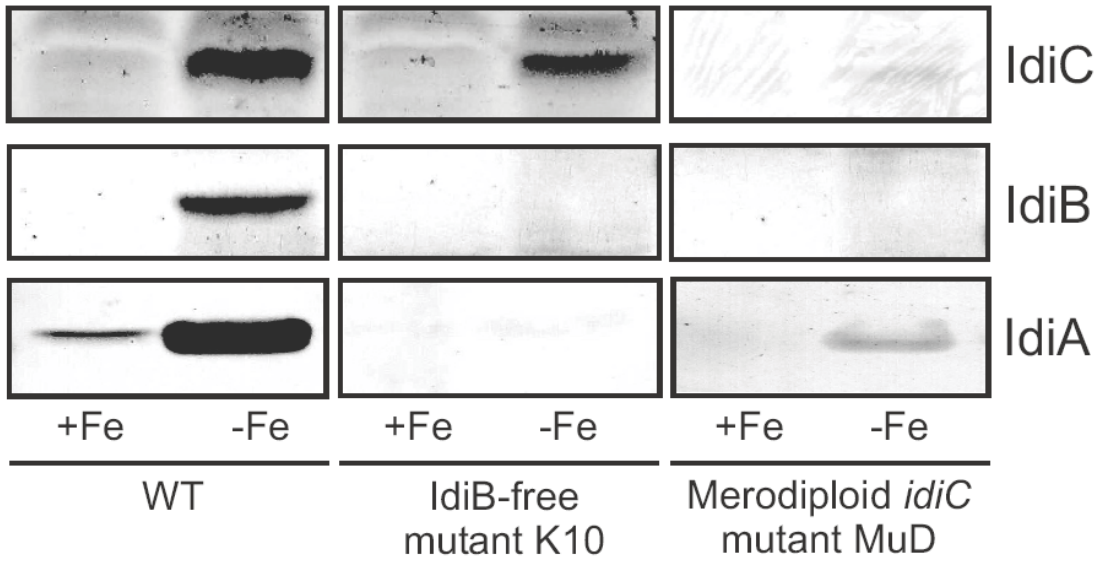
Acclimation to iron starvation significantly alters cellular electron transport activities



Contents

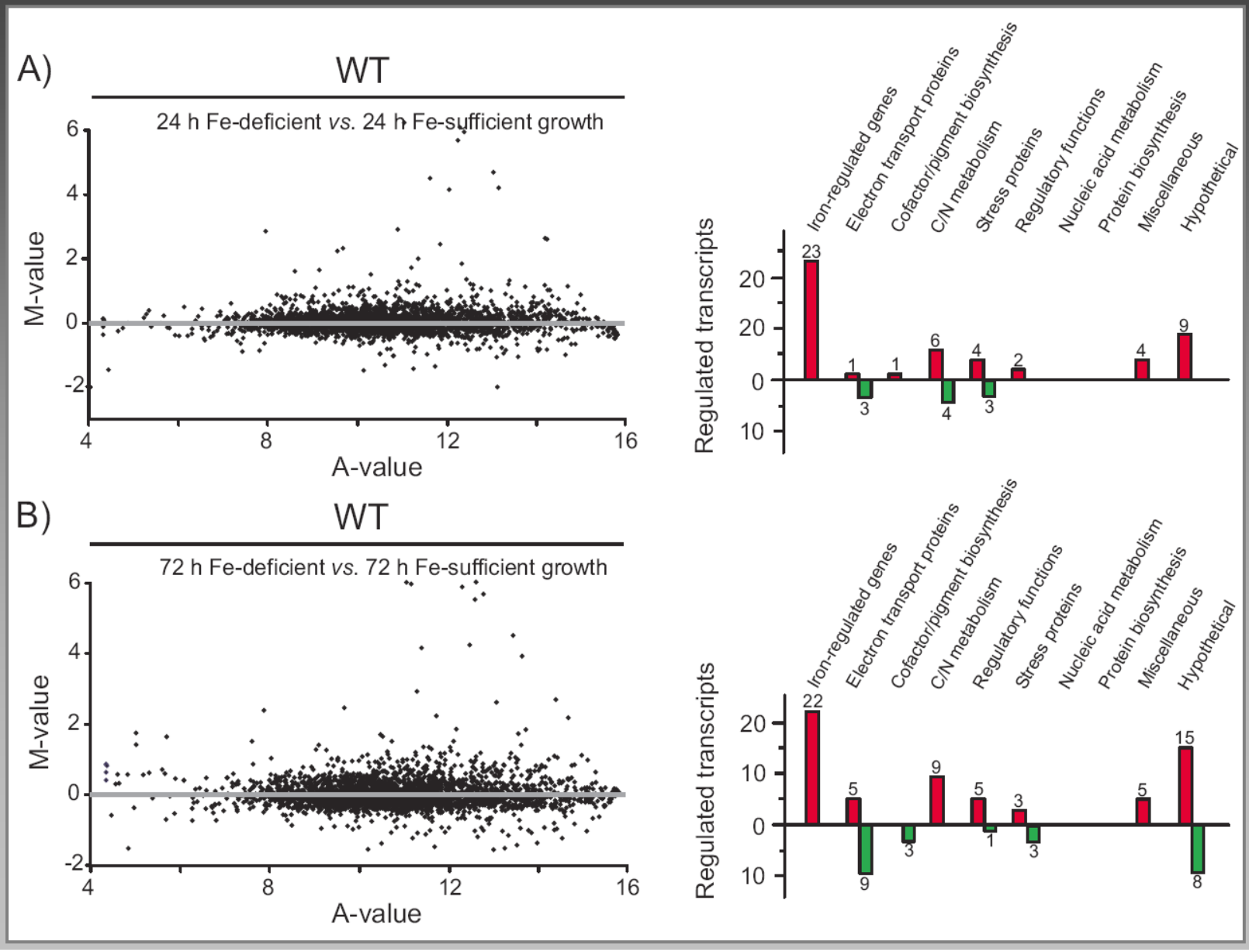
Transcript profiling with *S. elongatus* PCC 7942 WT, the IdiB-free mutant K10, and the *idiC*-merodiploid mutant MuD



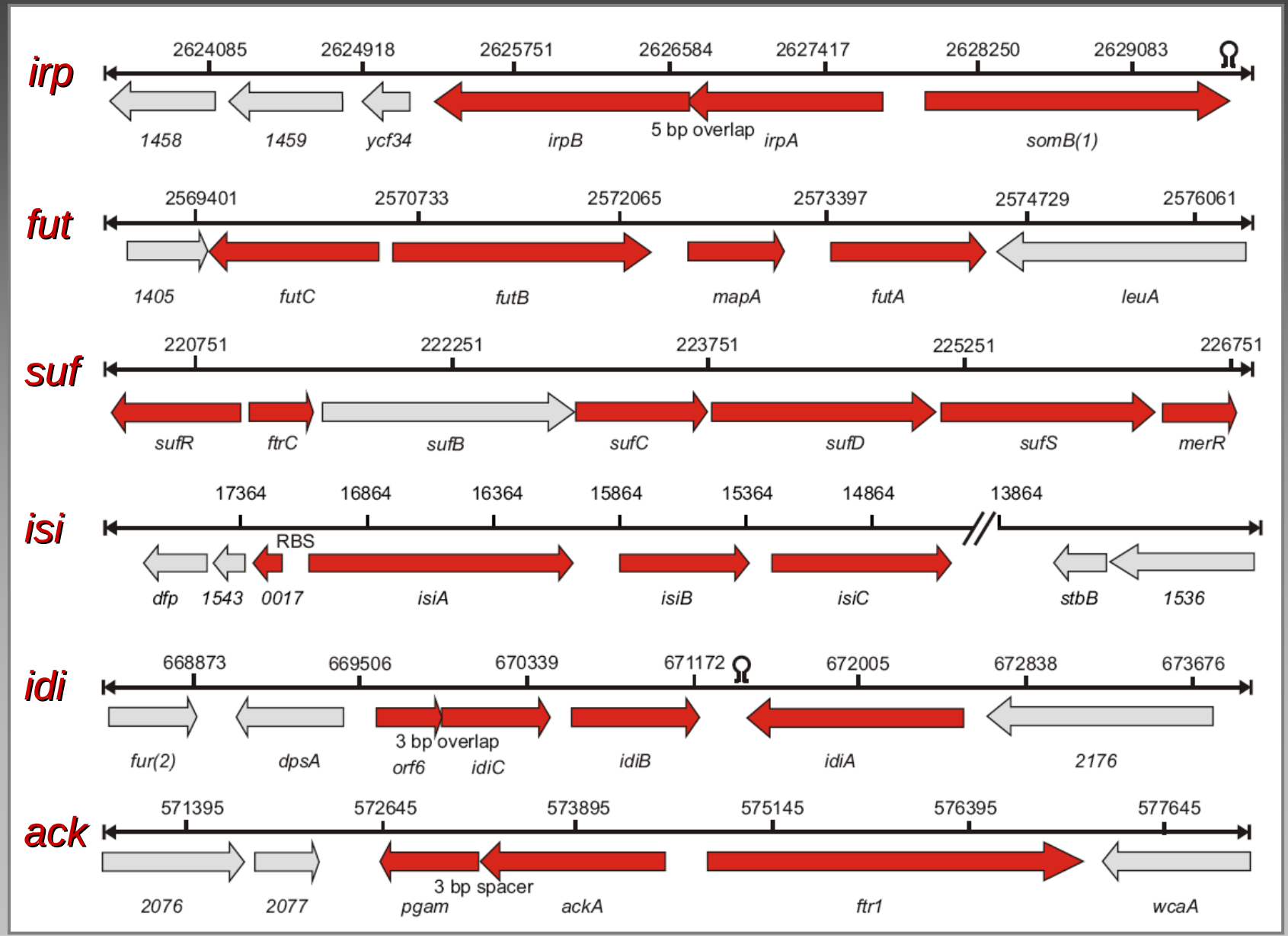


- The IdiB-free mutant does not express IdiB but still contains high amounts of IdiC under iron-limiting growth conditions.
- The absence of IdiB prevents expression of IdiA.
- The *idiC*-merodiploid mutant MuD has severely reduced concentrations of IdiA, IdiB, and IdiC.

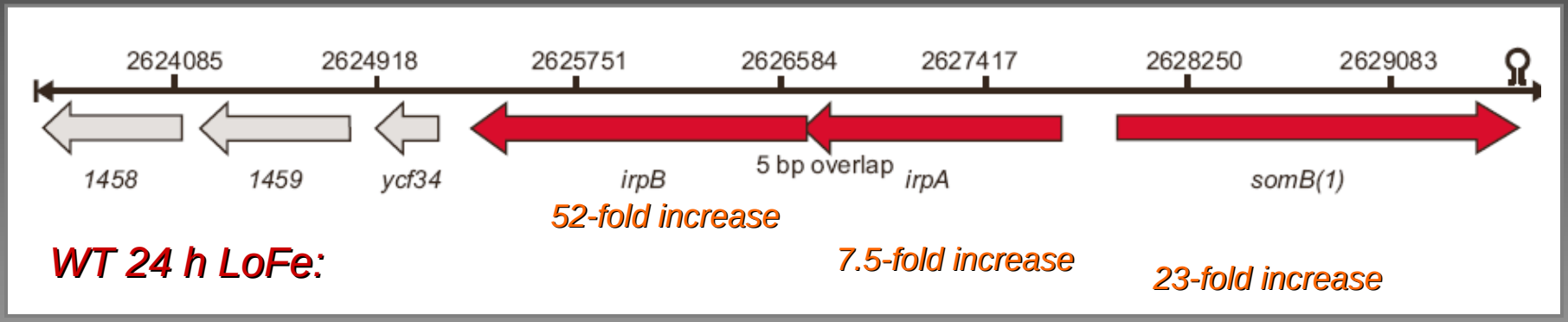
Transcript profiling of *S. elongatus* PCC 7942 with an oligonucleotide-based DNA microarray approach



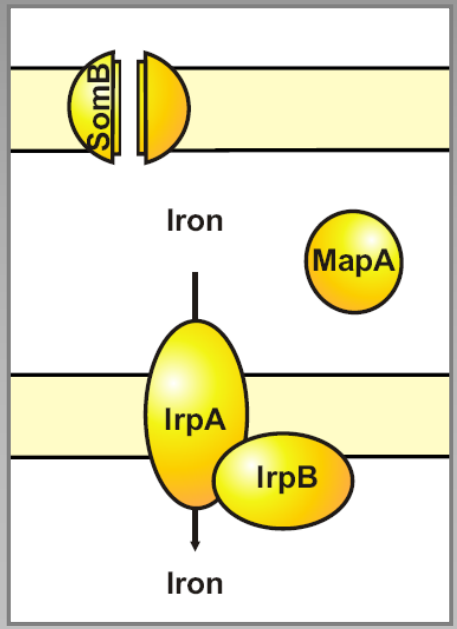
Transcript profiling of *S. elongatus* PCC 7942 *vs.* LoFe cells identified six iron-reponsive genome regions



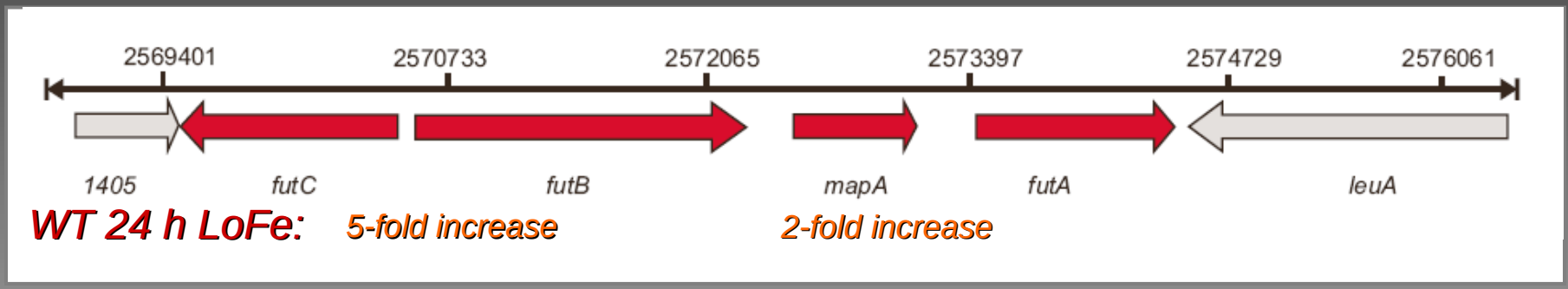
The *irpAB* operon and *somB(1)* are transcribed in elevated amounts under LoFe



- *irpA*: Membrane-associated iron-regulated protein A with miscellaneous function.
- *irpB*: Multiheme *c*-type cytochrome with two heme-binding sites. Heterologous expression causes immediate growth arrest!
- *somB*: Outer membrane protein, which probably forms porin-like ß-barrel structures and which might also connect to the S-layer.

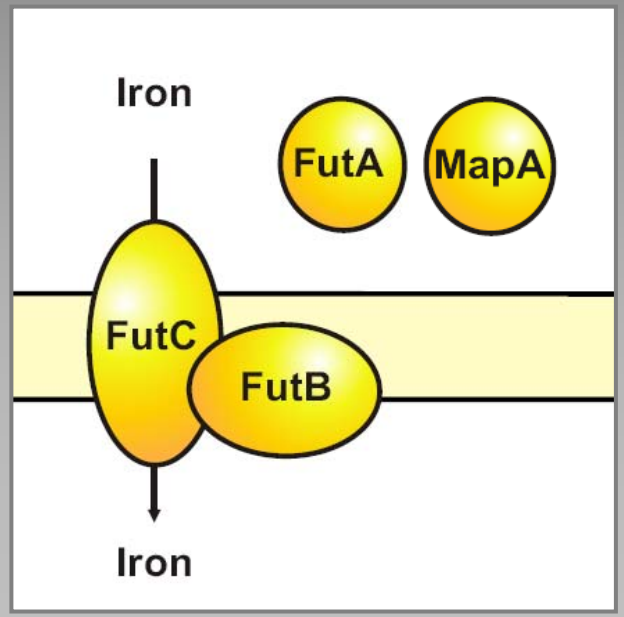


The *fut* operon is expressed in elevated amounts under LoFe

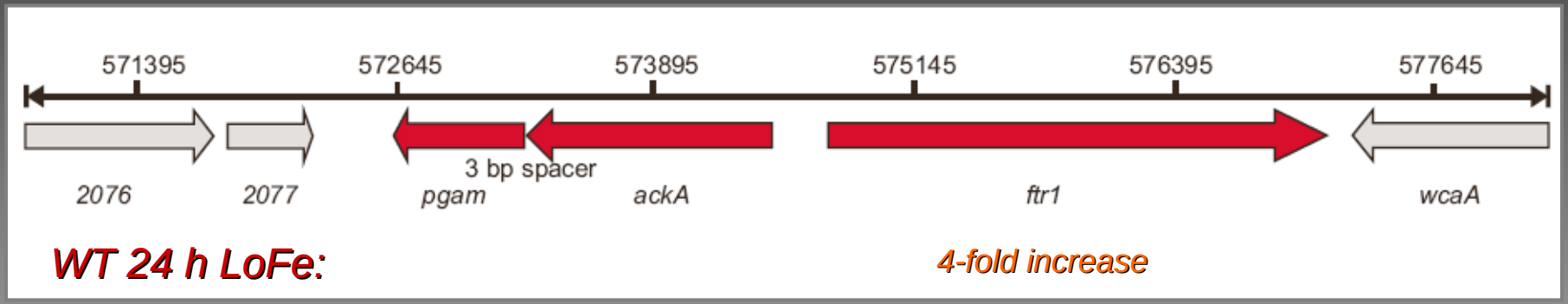


- ***fut* operon**: Ferric iron uptake transport system and is widely distributed in different genera of cyanobacteria.

- ***mapA***: MapA partly resembles periplasmic solute-binding proteins and thus, is likely to belong to the Fut system or another iron uptake system.



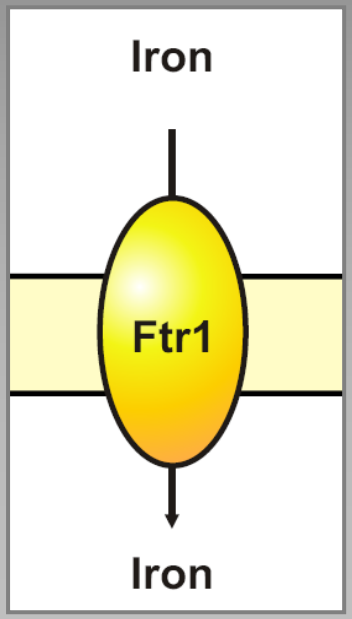
LoFe induces expression of a novel iron transporter



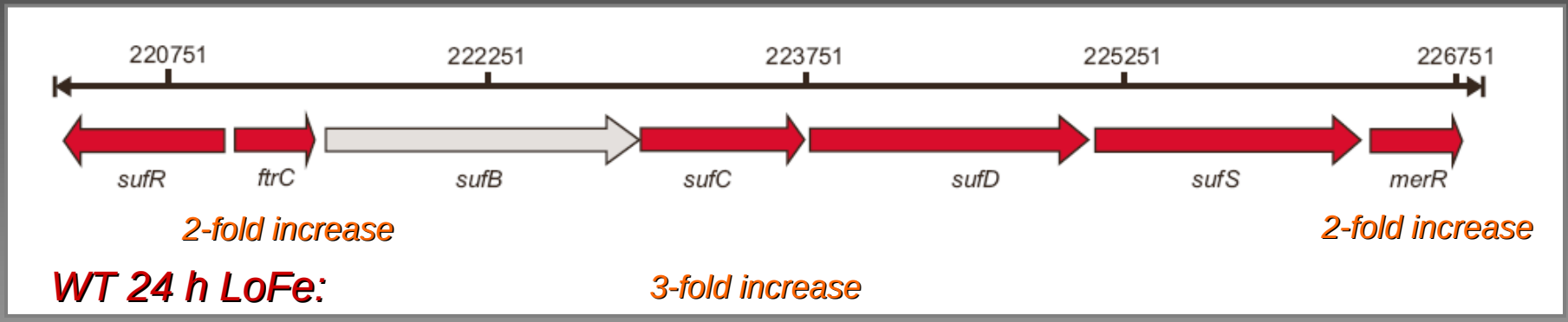
WT 24 h LoFe:

4-fold increase

- ***ftr1***: Ftr1 is part of a high-affinity ferrous iron transporter first identified in yeast.

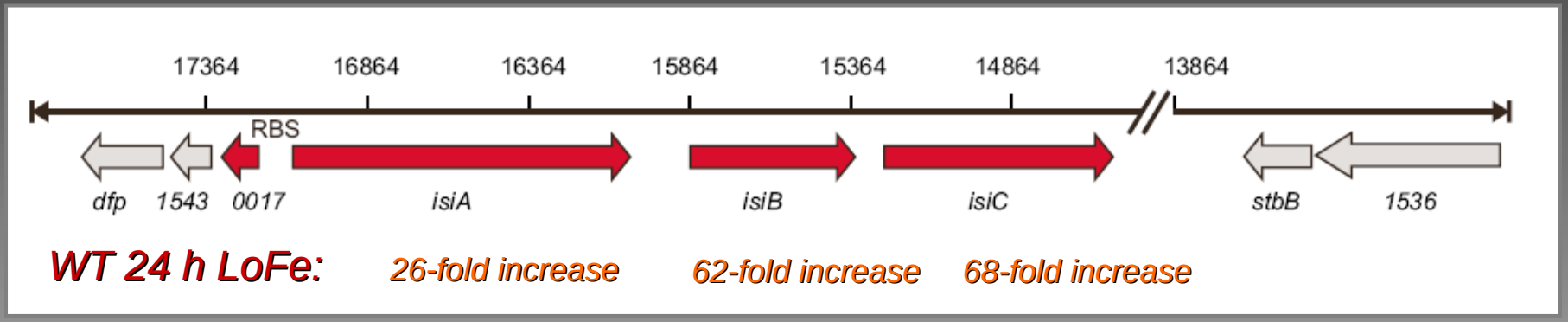


The *suf* operon is transcribed in elevated amounts under LoFe



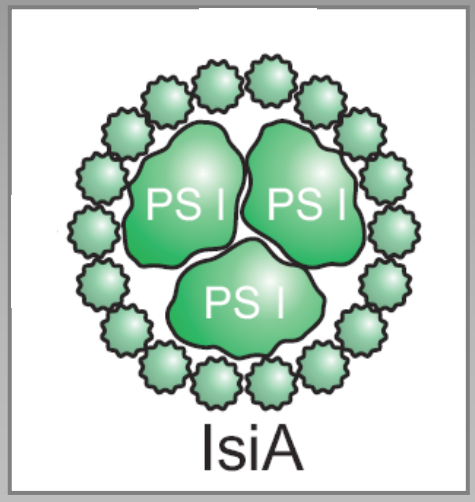
- ***suf* operon:** The *suf* operon encodes an [Fe-S] assembly system. SufR is the transcriptional repressor of the *suf* operon.
- ***ftrC*:** FtrC corresponds to the catalytic ß-SU of the Fdx:Trx reductase.
- ***merR*:** MerR is the transcriptional regulator of the mercury resistance operon.

isiABC are transcribed in elevated amounts under LoFe



WT 24 h LoFe: *26-fold increase* *62-fold increase* *68-fold increase*

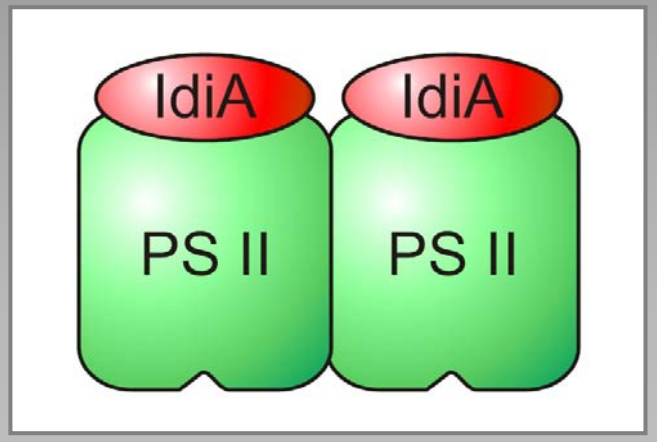
- ***isiA***: IsiA, the iron starvation-inducible CP43' analog protein modifying PS I complexes during selected stress conditions.
- ***isiB***: Flavodoxin, which substitutes for the loss of ferredoxin in the course of iron starvation.
- ***isiC***: IsiC, a putative hydrolase with a typical ß-fold. Its function remains enigmatic.



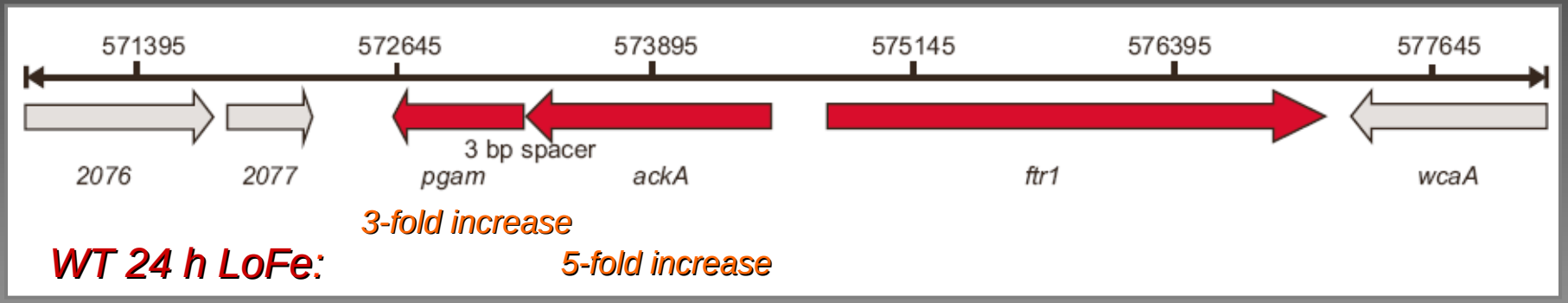
The *idi* genes are transcribed in elevated amounts under LoFe

WT 24 h LoFe: *18-fold increase* *19-fold increase* *75-fold increase* *12-fold increase*

- ***idiA***: Iron deficiency-induced protein A, which protects the acceptor side of PS II against ROS.
- ***idiB***: HTH transcriptional activator of *idiA* and the IdiB regulon.
- ***idiC***: (Fe-S) thioredoxin-like ferredoxin with similarity to NuoE.
- ***orf6***: 10 kDa hypothetical protein with miscellaneous function.

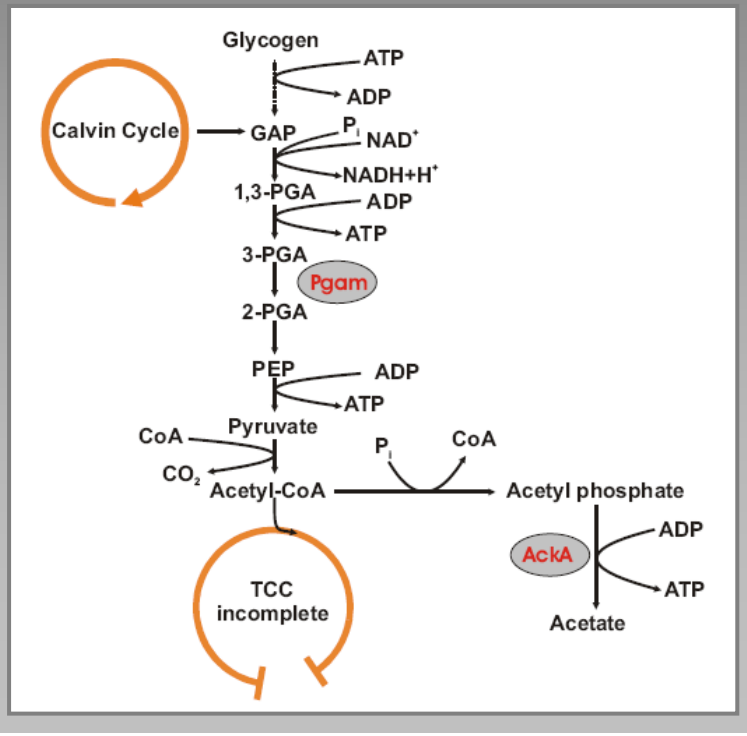


Acclimation to LoFe changes acetate metabolism



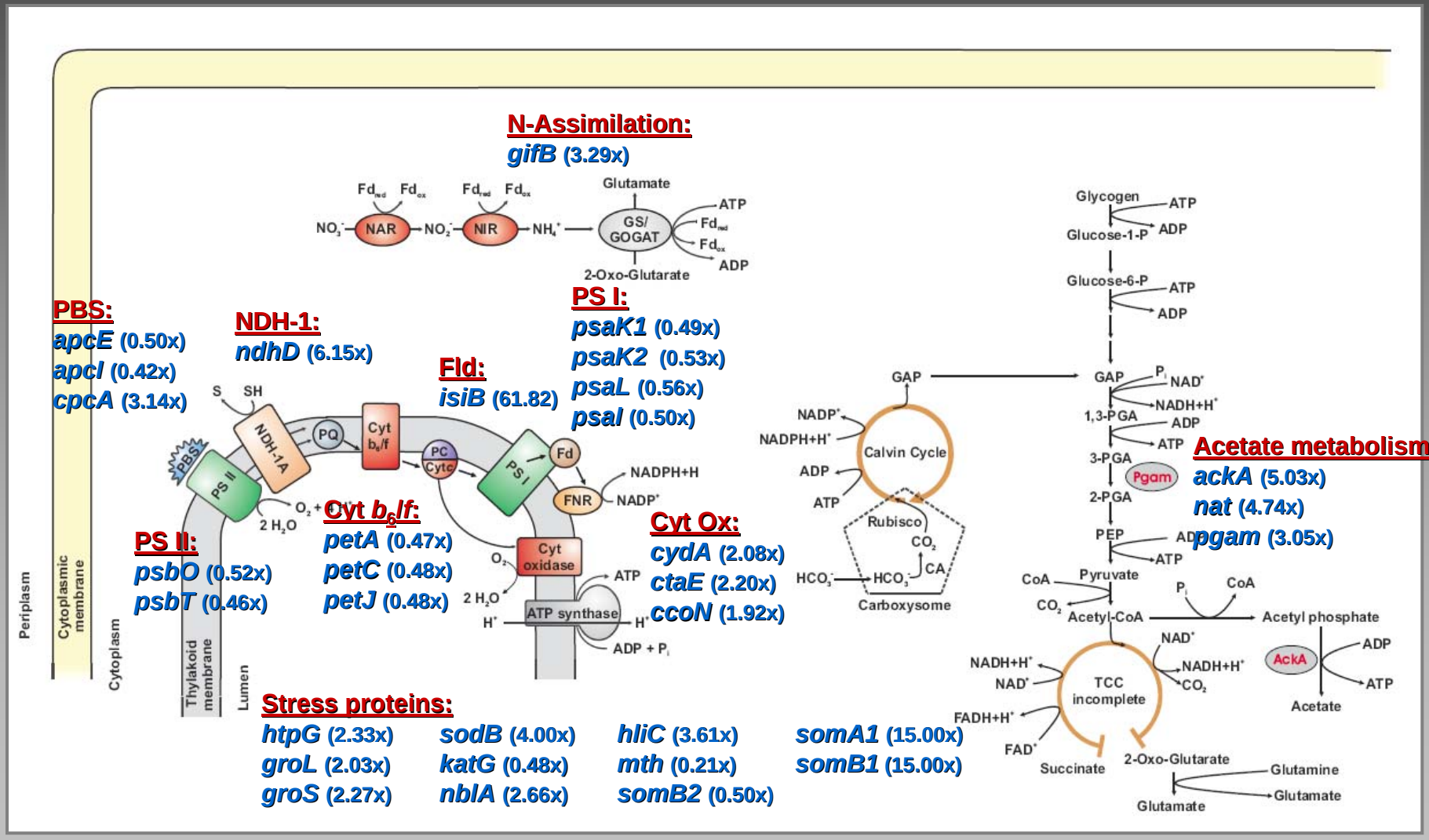
- ***pgam:*** Phosphoglycerate kinase catalyzes the conversion of 3-phosphoglycerate (3-PGA) to 2-phosphoglycerate (2-PGA).

- ***ackA:*** Acetate kinase A catalyzes the formation acetate and ATP from acetyl phosphate and ADP.



Acclimation to LoFe affects major components of electron transport

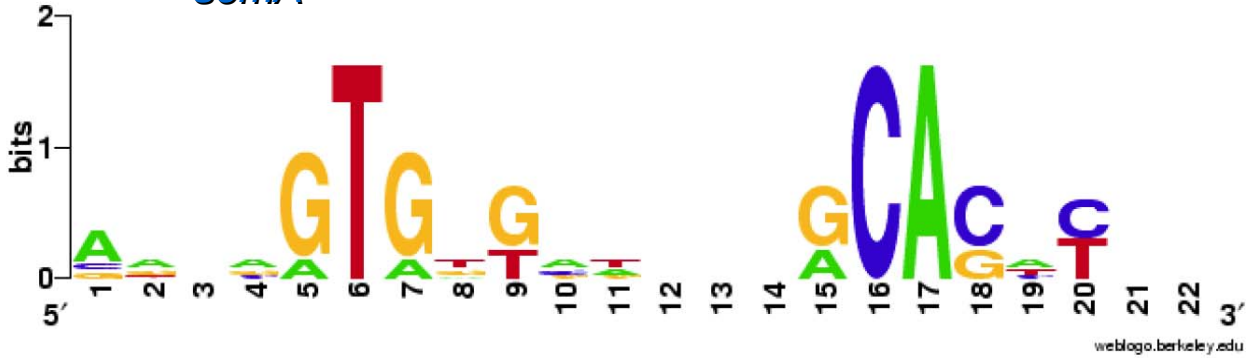
Selected differentially-regulated transcripts in WT grown under LoFe



Comparative transcript profiling of the IdiB-free mutant *vs.* WT identified novel members of an IdiB regulon

Members of the IdiB regulon:

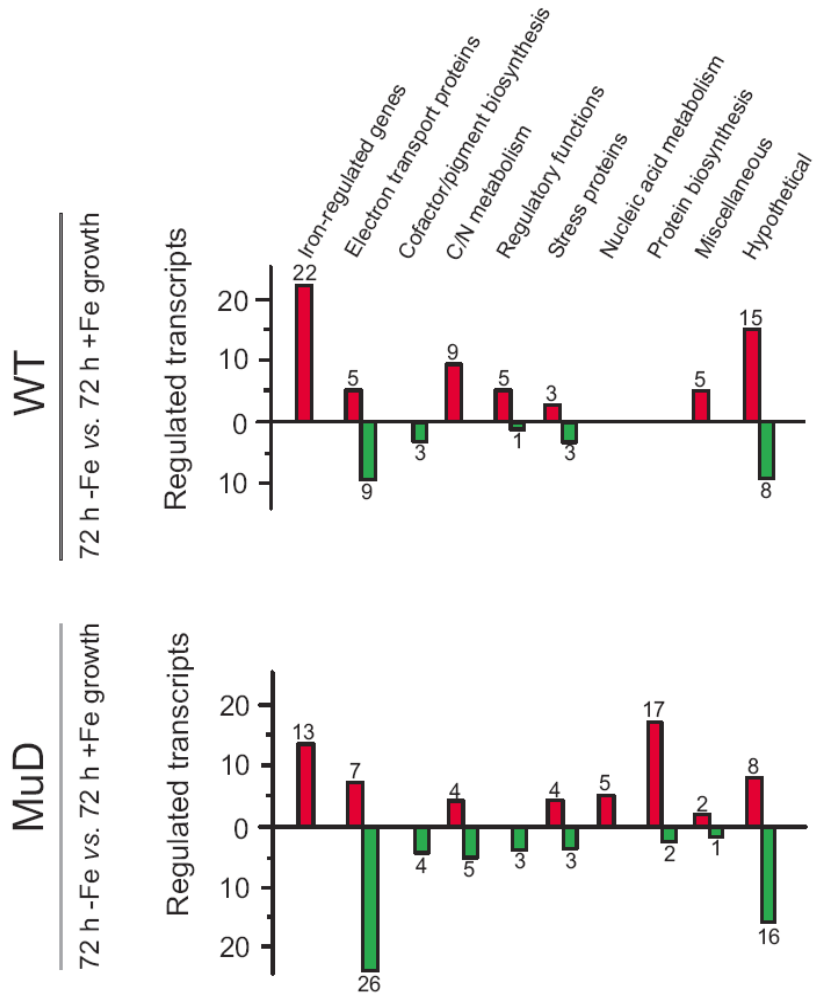
irpA	*ftr1*	*idiA*	*pgam*
irpB			*ackA*
somB			
somA			



IdiB-binding site consensus sequence

Reduced cellular IdiC concentrations predominantly affect transcripts encoding proteins of the photosynthetic apparatus

Transcript profiling of MuD *vs*. WT grown for 72 h under LoFe



Transcripts specifically affected by the reduced amounts of IdiC in mutant MuD

psbB (0.54x)
psbC (0.46x)
psbT (0.46x)

psaA (0.43x)
psaB (0.38x)
psaC (0.35x)
psaJ (0.29x)
psaL (0.30x)

petD (0.50x)

ctaA (2.17x)
rub (2.04x)

apcA (0.26x)
apcB1 (0.48x)
apcB2 (0.37x)
cpcA (0.46x)
cpcB1 (0.28x)
cpcB2 (0.29x)

cpcI1 (0.23x)
cpcI2 (0.36x)

cpcH (0.25x)
cpcG (0.48x)

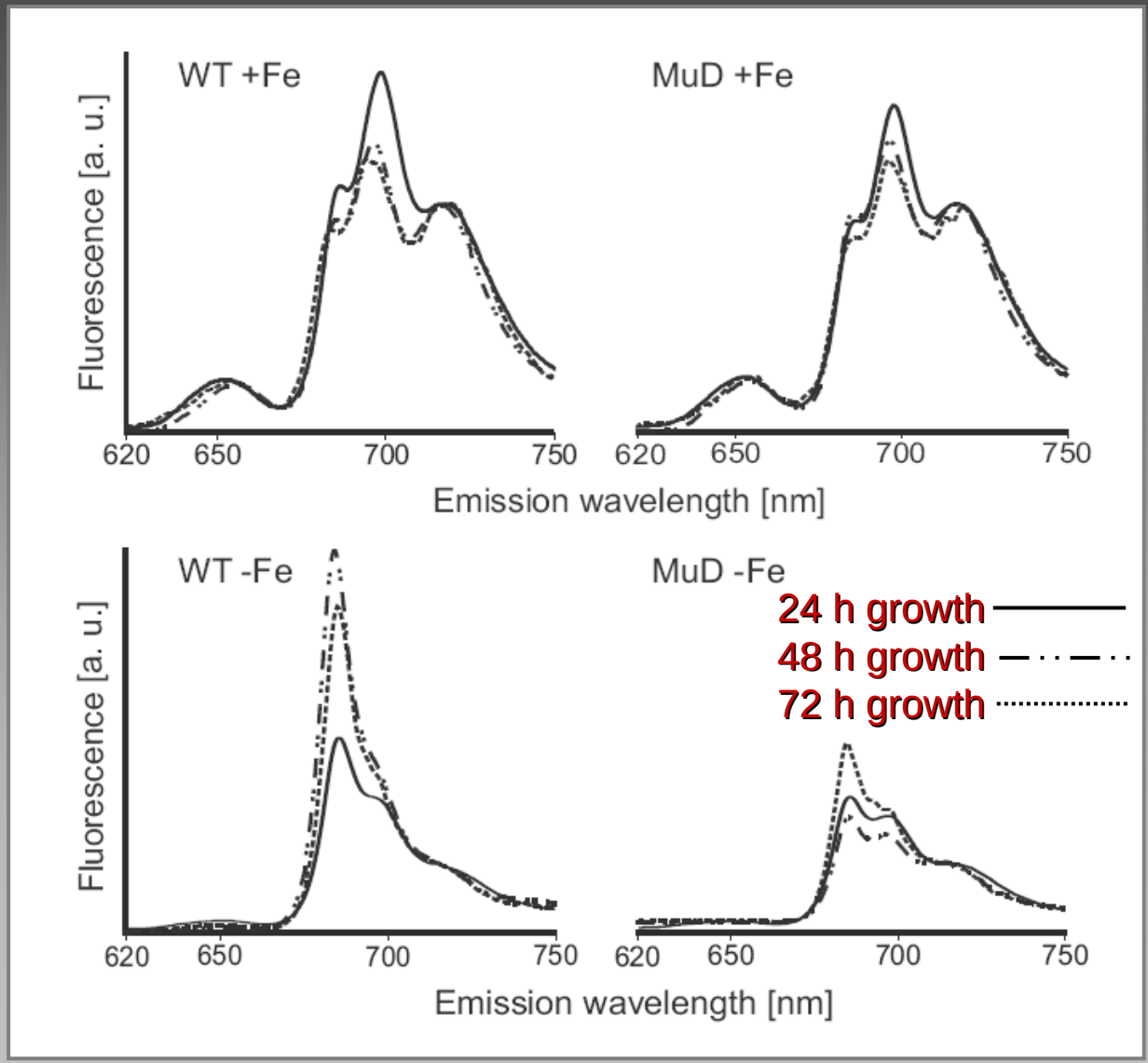
dxr (0.48x)

isiA (11.00x)
isiB (5.06x)
isiC (8.64x)

- The PS I-modifying reactions are likely to be altered in the *idiC*-merodiploid mutant.

Reduced amounts of IdiC affect regulation of IsiA expression

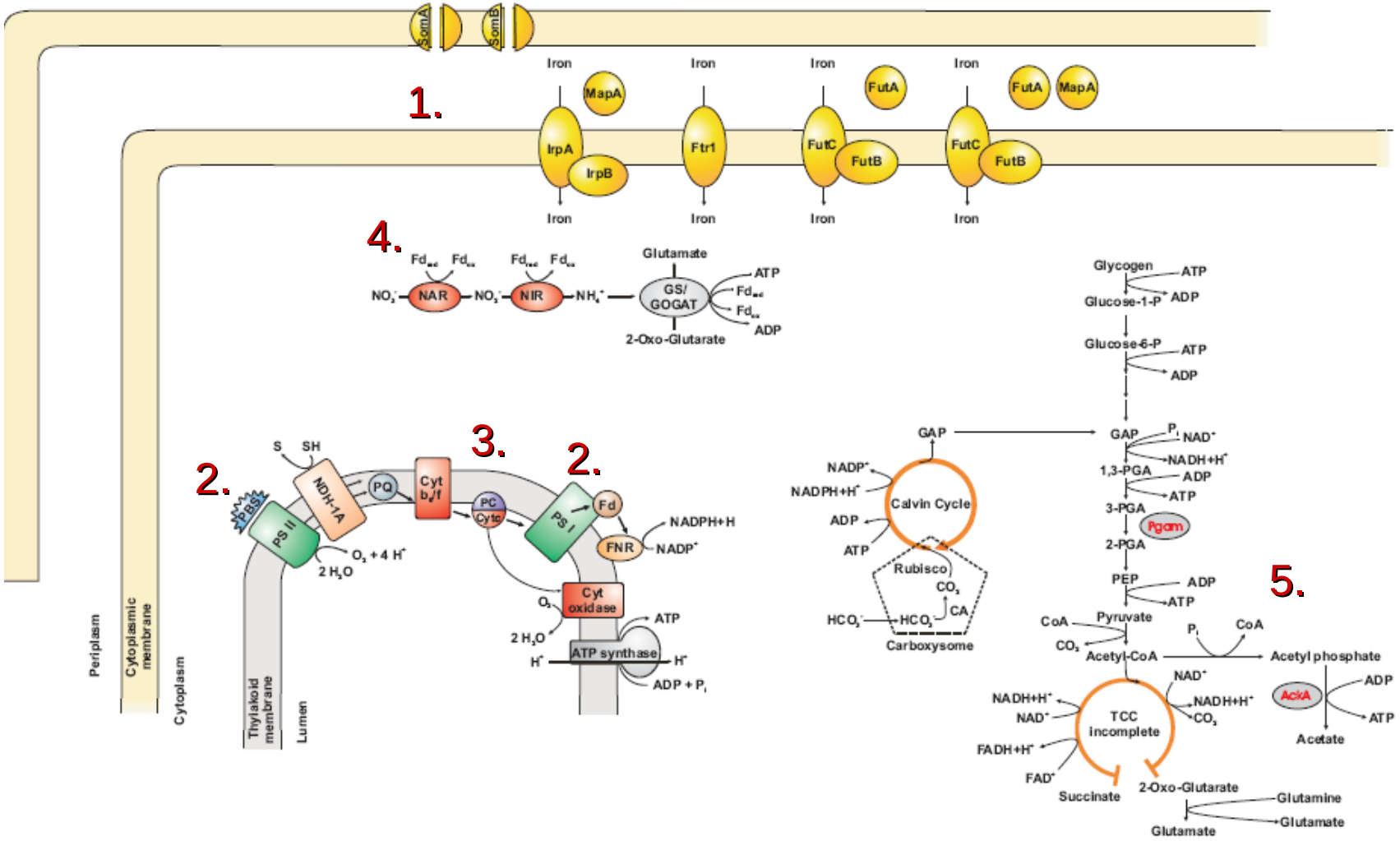
77 K pigment fluorescence of WT and the *idiC*-merodiploid mutant MuD



Contents

Summary – Part I

The adaptational response to iron starvation



Summary – Part II

- Transcript profiling of WT identified six chromosomal regions with genes arranged in sequence that are up-regulated under LoFe:

  irpA/B region: encoding an iron uptake system
  fut region: encoding an iron uptake system
  suf region: encoding an [Fe-S] assembly system
  isiA/B/C region: encoding electron transport modifying proteins
  idiA/B/C region: encoding electron transport modifying proteins and a transcription factor
  ackA/pgam region: encoding enzymes of acetate synthesis (ATP synthesis site)

- Transcript profiling of the IdiB-free mutant identified of novel members of an IdiB regulon:

  idiA, *irpA*, *irpB*, *somB(1)*, *somA*, *ftr1*, *ftrC*, *ackA*, and *pgam*.

- Transcript profiling of the *idiC*-merodiploid mutant MuD showed that LoFe combined w/ low IdiC concentrations predominantly resulted in a higher decrease of the steady-state level of transcripts encoding proteins of the photosynthetic apparatus – especially of PS I.

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