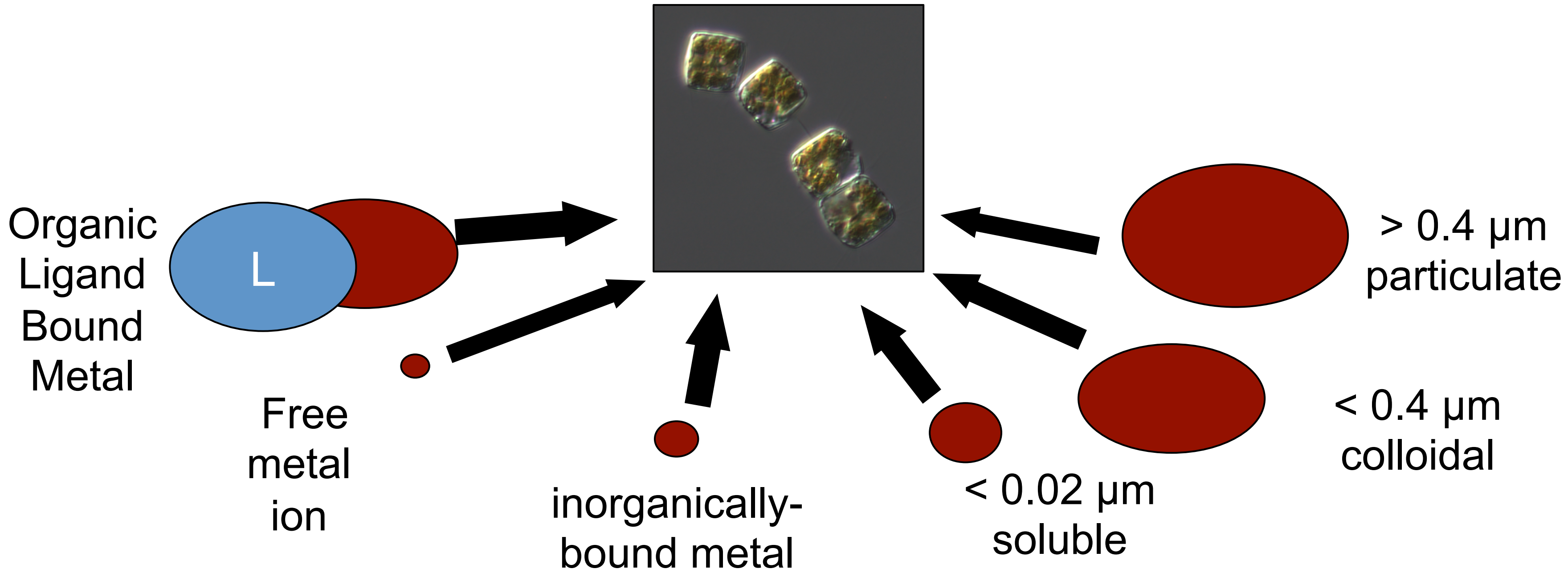


Organic Ligand Bound Metal

L

Free metal ion

inorganically-bound metal

< 0.02 µm soluble

< 0.4 µm colloidal

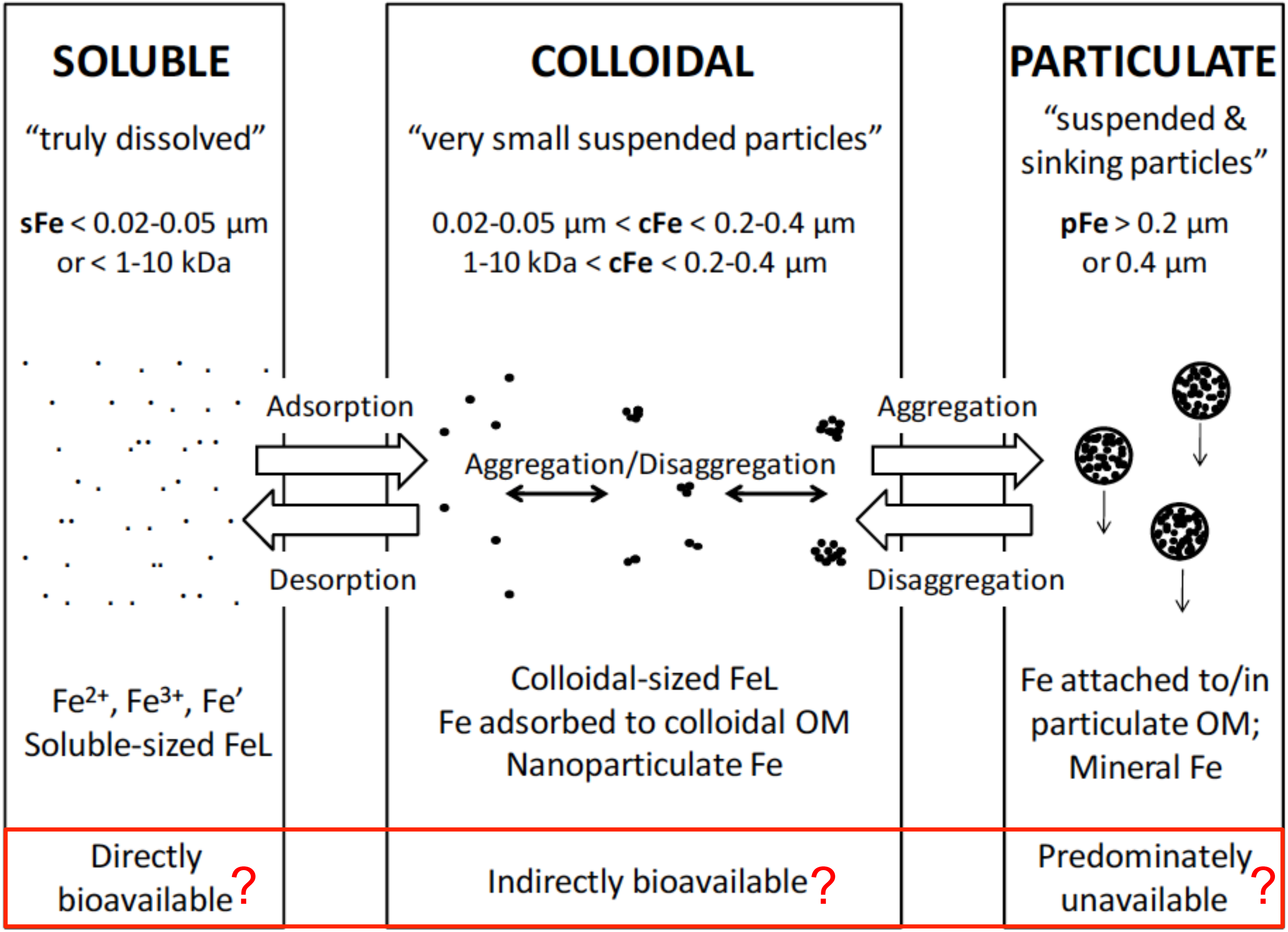
> 0.4 µm particulate

Molecular Indicators of Trace Element Bioavailability

P. Dreux Chappell

OCEAN, EARTH & ATMOSPHERIC SCIENCES
OLD DOMINION UNIVERSITY

NSF



SOLUBLE	COLLOIDAL	PARTICULATE
"truly dissolved"	"very small suspended particles"	"suspended & sinking particles"
sFe < 0.02-0.05 µm or < 1-10 kDa	0.02-0.05 µm < **cFe** < 0.2-0.4 µm 1-10 kDa < **cFe** < 0.2-0.4 µm	**pFe** > 0.2 µm or 0.4 µm
Fe^{2+}, Fe^{3+}, Fe' Soluble-sized FeL	Colloidal-sized FeL Fe adsorbed to colloidal OM Nanoparticulate Fe	Fe attached to/in particulate OM; Mineral Fe
Directly bioavailable?	Indirectly bioavailable?	Predominately unavailable?

J.N. Fitzsimmons Ph.D. Thesis, 2013

How Can Molecular Tools Help?

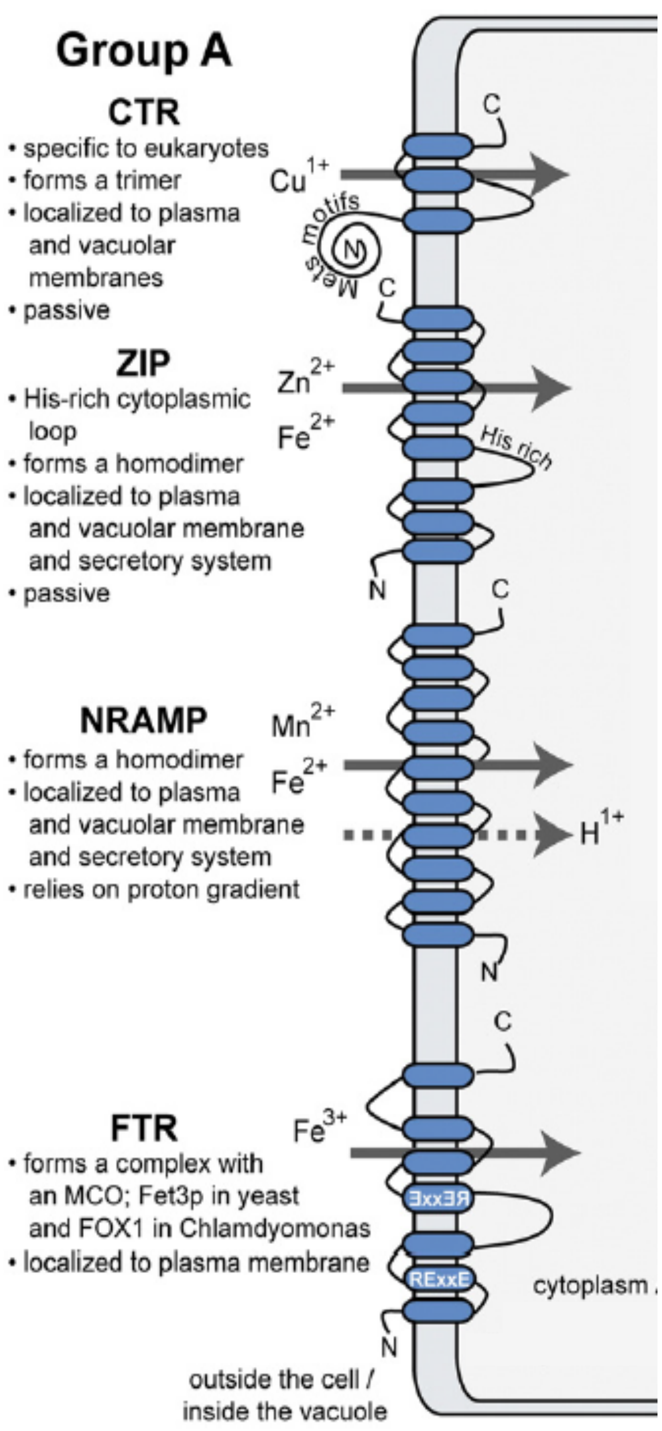
- Genomics & transcriptomics can be used to identify genetic capacity for specific types of metal uptake
  - This can be done on individual genomes/transcriptomes or in mixed communities (meta-omics)
- “-omic” techniques can be used to identify molecular markers indicative of limitation/stress
  - Could include genes/proteins in addition to those involved in uptake
- We can use these stress markers to evaluate bioavailability of different forms of metals

The ins and outs of algal metal transport

Crysten E. Blaby-Haas *, Sabeeha S. Merchant

Biochimica et Biophysica Acta 1823 (2012) 1531–1552

- CTR = Copper TRansporter
  - Passive transporter of Cu^{+1} dependent on copper reduction in periplasm
  - Specific to eukaryotic algae (absent in *Aureococcus anophagefferrens*)
- ZIP/IRT/ZRT
  - Passive divalent metal transporters found in eukaryotic and prokaryotic genomes
  - Different ZIP/IRT genes are responsive to Fe, Cu, and Zn limitation in *Chlamydomonas reinhardtii*



Group A

CTR

- specific to eukaryotes
- forms a trimer
- localized to plasma and vacuolar membranes
- passive

ZIP

- His-rich cytoplasmic loop
- forms a homodimer
- localized to plasma and vacuolar membrane and secretory system
- passive

NRAMP

- forms a homodimer
- localized to plasma and vacuolar membrane and secretory system
- relies on proton gradient

FTR

- forms a complex with an MCO; Fet3p in yeast and FOX1 in Chlamdyomonas
- localized to plasma membrane

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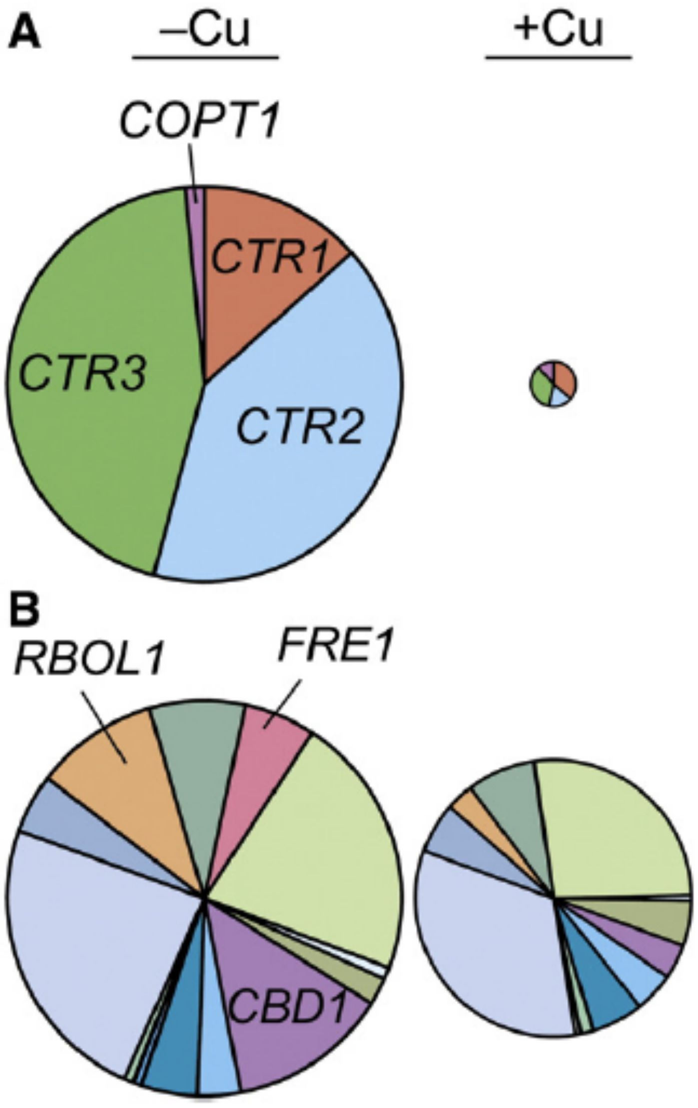
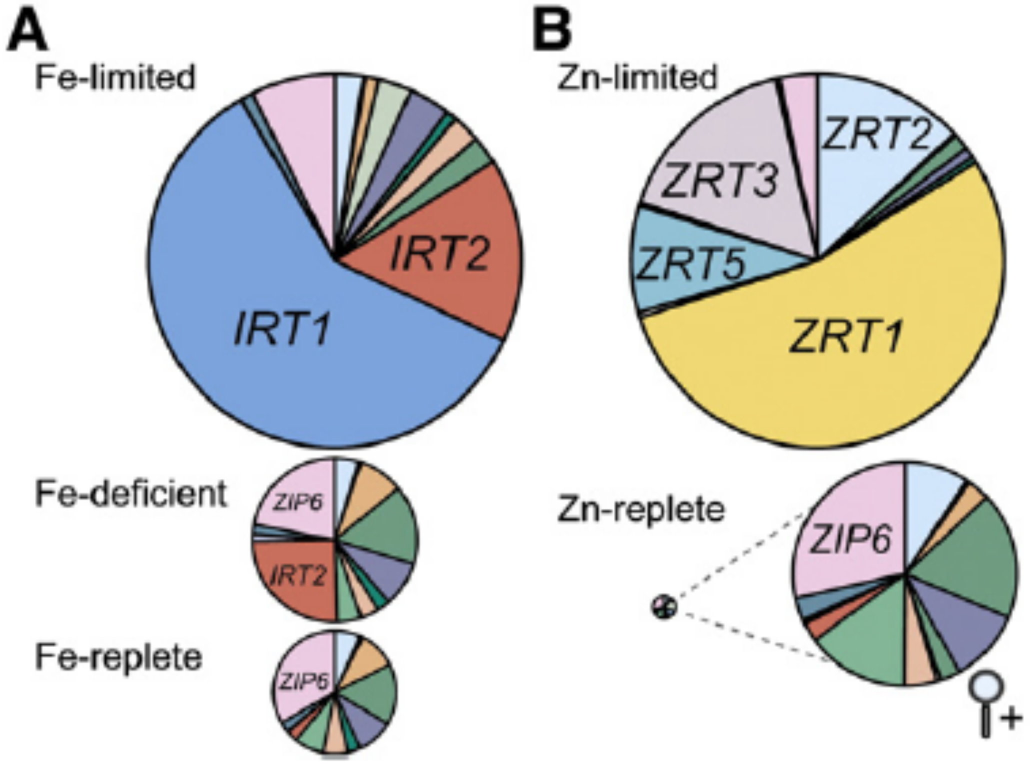


- Cu limitation transcriptomic work in *Chlamydomonas* reveals link between CTR and reductive pathways:
  - CTR expression
  - Expression of putative Ferric Reductases

The ins and outs of algal metal transport

Crysten E. Blaby-Haas *, Sabeeha S. Merchant

Biochimica et Biophysica Acta 1823 (2012) 1531–1552



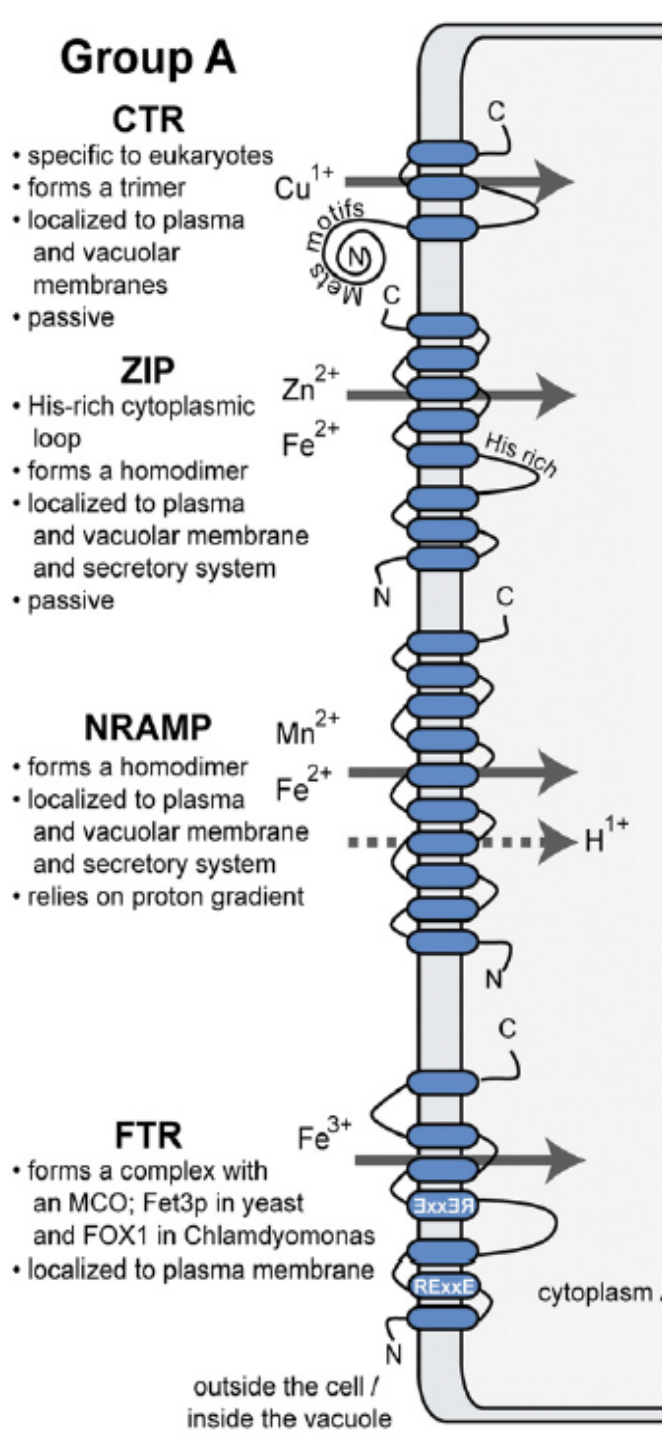
- Transcriptomic analysis of ZIP/ IRT/ZRT genes under different limitation stressors suggests specific roles for individual genes

The ins and outs of algal metal transport

Crysten E. Blaby-Haas *, Sabeeha S. Merchant

Biochimica et Biophysica Acta 1823 (2012) 1531–1552

- NRAMP
  - Metal permease that transports divalent cations
  - Mn^{2+} and Fe^{2+} believed to be the main targets
  - Present in prokaryotes and most eukaryotes (not found in *Phaeodactylum tricornutum*)
- FTR= Fe Transporter
  - Fe^{3+} transporter that forms a complex with multi-copper oxidases (MCOs:FET/FOX)
  - Found in most algal genomes (not praesinophytes or some chromalveolates)



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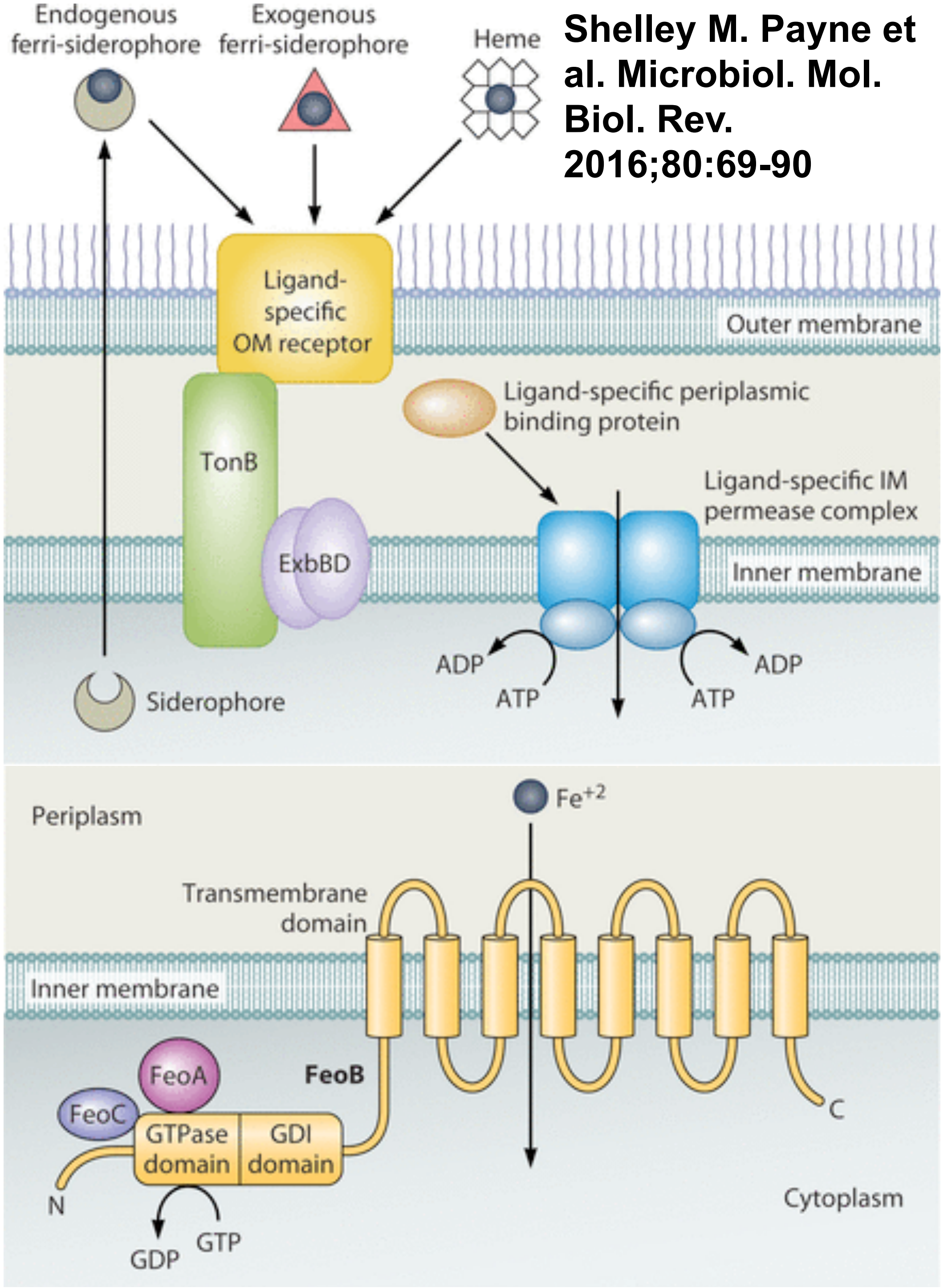
- forms a complex with an MCO; Fet3p in yeast and FOX1 in Chlamdyomonas
- localized to plasma membrane

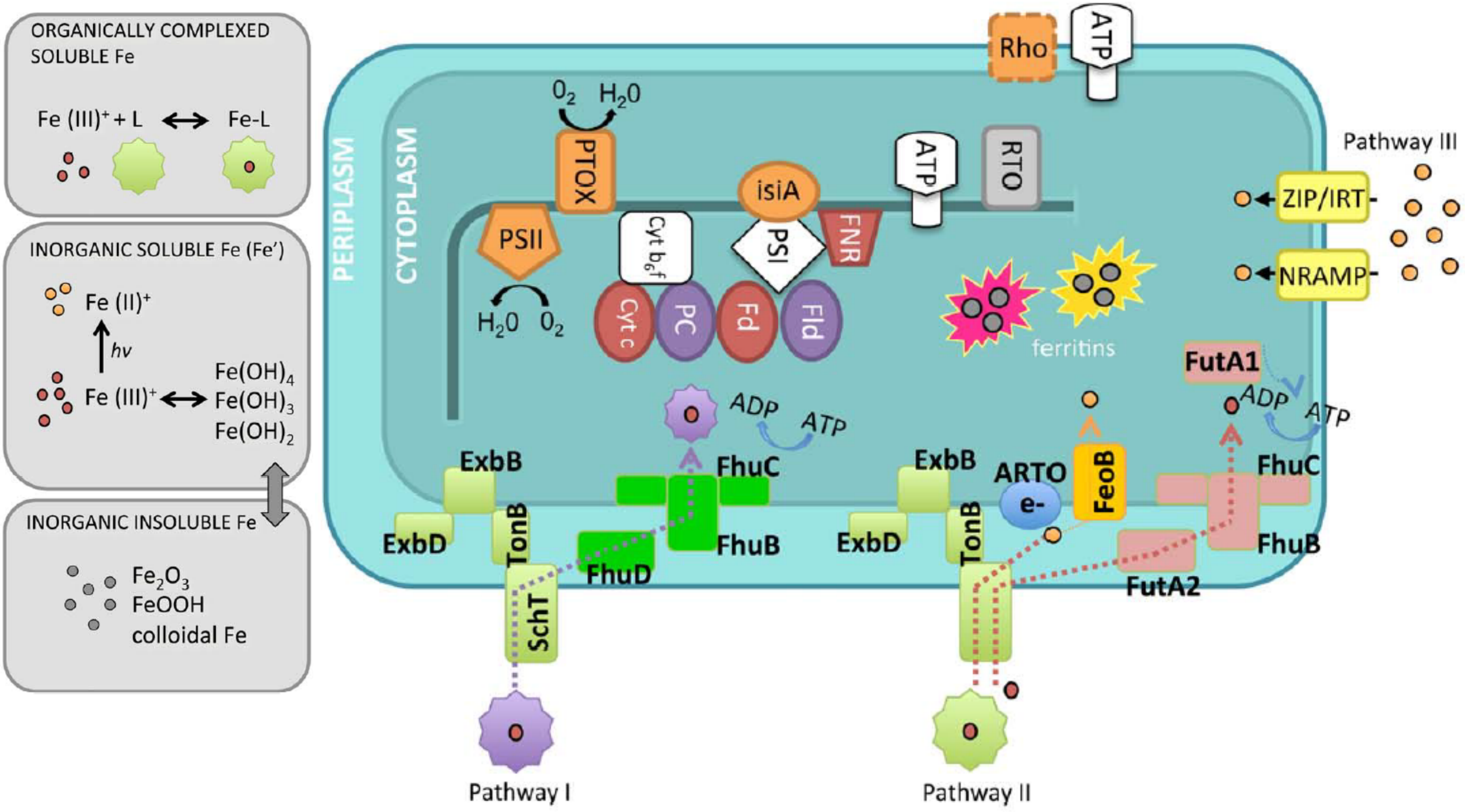
Additional Fe Uptake Pathways

- TonB dependent siderophore transport
  - Found in many marine bacteria and cyanobacteria and select eukaryotic algae
- ABC-type transporters
  - Active transport pathway
- FeoB
  - Fe^{2+} transporter common in cyanobacteria (except picocyanos). Not in eukaryotic algae.



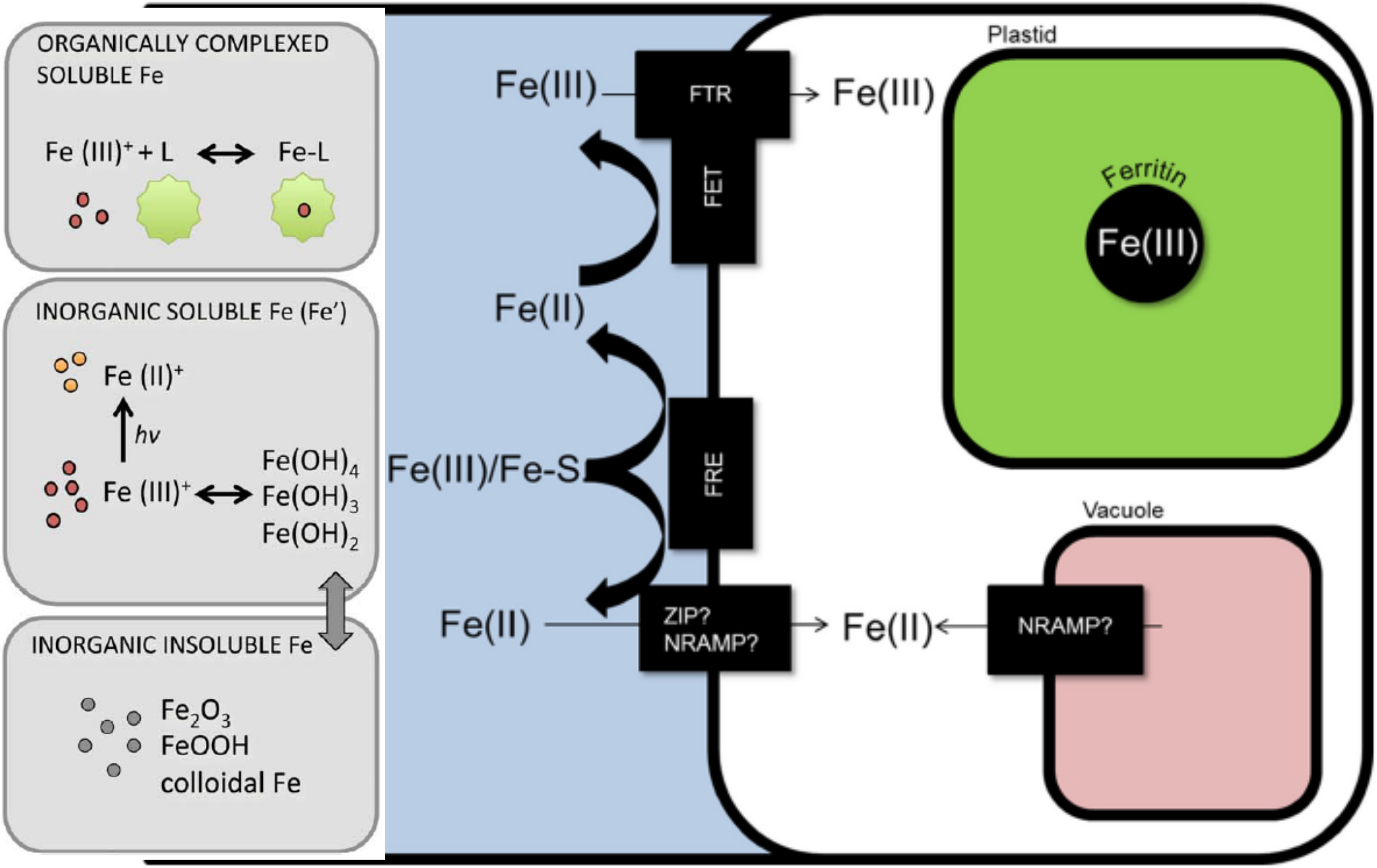
Shelley M. Payne et al. Microbiol. Mol. Biol. Rev. 2016;80:69-90

Iron homeostasis in marine cyanobacteria as predicted by genomic analyses



Marchetti A and Maldonado MT. (2016) Iron, In: Borowitzka MA, Beardell J and Raven J (ed.) The Physiology of Microalgae. Springer Publishing. 233-279.

Potential iron homeostasis systems in marine diatoms as predicted by genomic analyses



ORGANICALLY COMPLEXED SOLUBLE Fe

$Fe(III)^+ + L \leftrightarrow Fe\text{-}L$

INORGANIC SOLUBLE Fe (Fe′)

$Fe(II)^+$

$h\nu$

$Fe(III)^+ \leftrightarrow Fe(OH)_4$, $Fe(OH)_3$, $Fe(OH)_2$

INORGANIC INSOLUBLE Fe

Fe_2O_3

FeOOH

colloidal Fe

Morrissey and Bowler 2012 Frontiers in Microbiology

RESEARCH ARTICLE

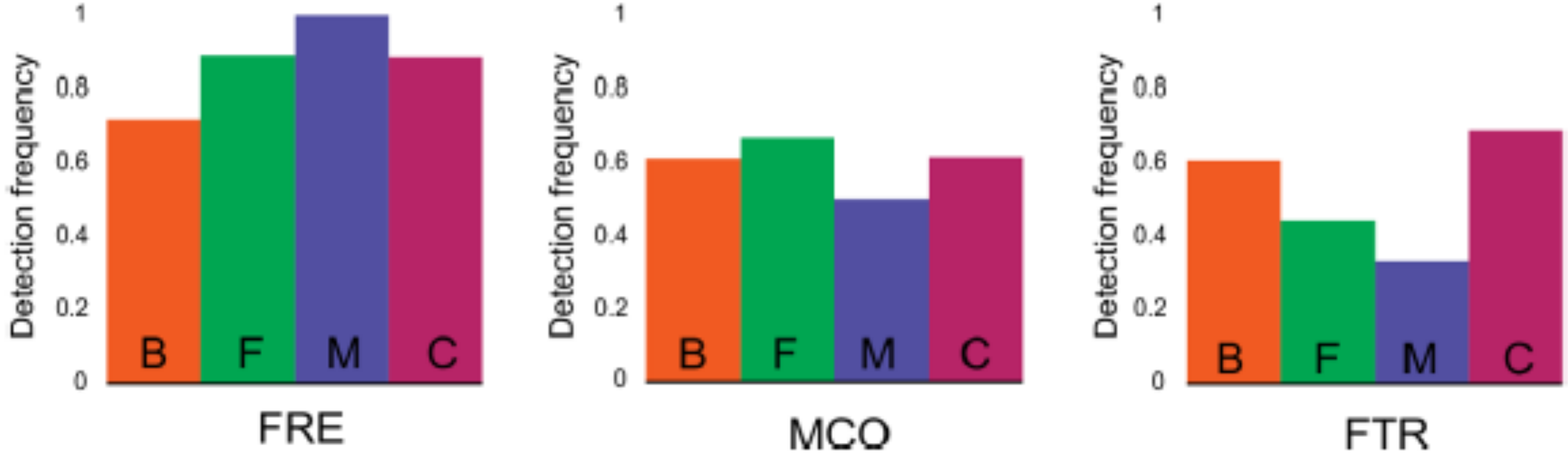
Diversity and Evolutionary History of Iron Metabolism Genes in Diatoms

Ryan D. Groussman, Micaela S. Parker, E. Virginia Armbrust*

School of Oceanography, University of Washington, Seattle, Washington, United States of America

doi:10.1371/journal.pone.
0129081.g002

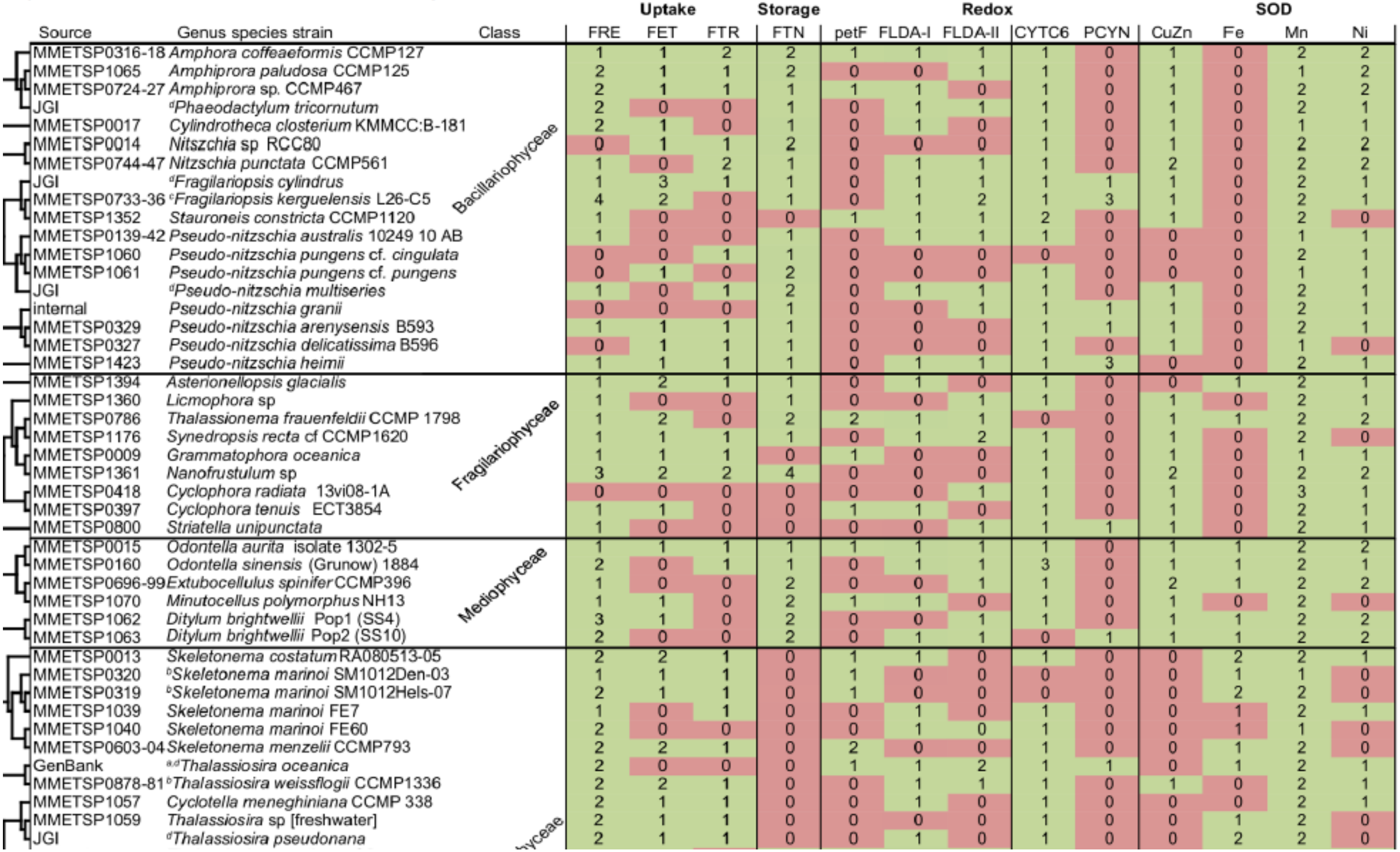
transcriptome sequencing enabled analysis



- Frequency of detected transcripts for: ferric reductase (FRE), multi-copper oxidase (MCO), and iron(III) permease (FTR) in diatom transcriptomes from different phylogenetic groups

Diversity and Evolutionary History of Iron Metabolism Genes in Diatoms

Ryan D. Groussman, Micaela S. Parker, E. Virginia Armbrust*



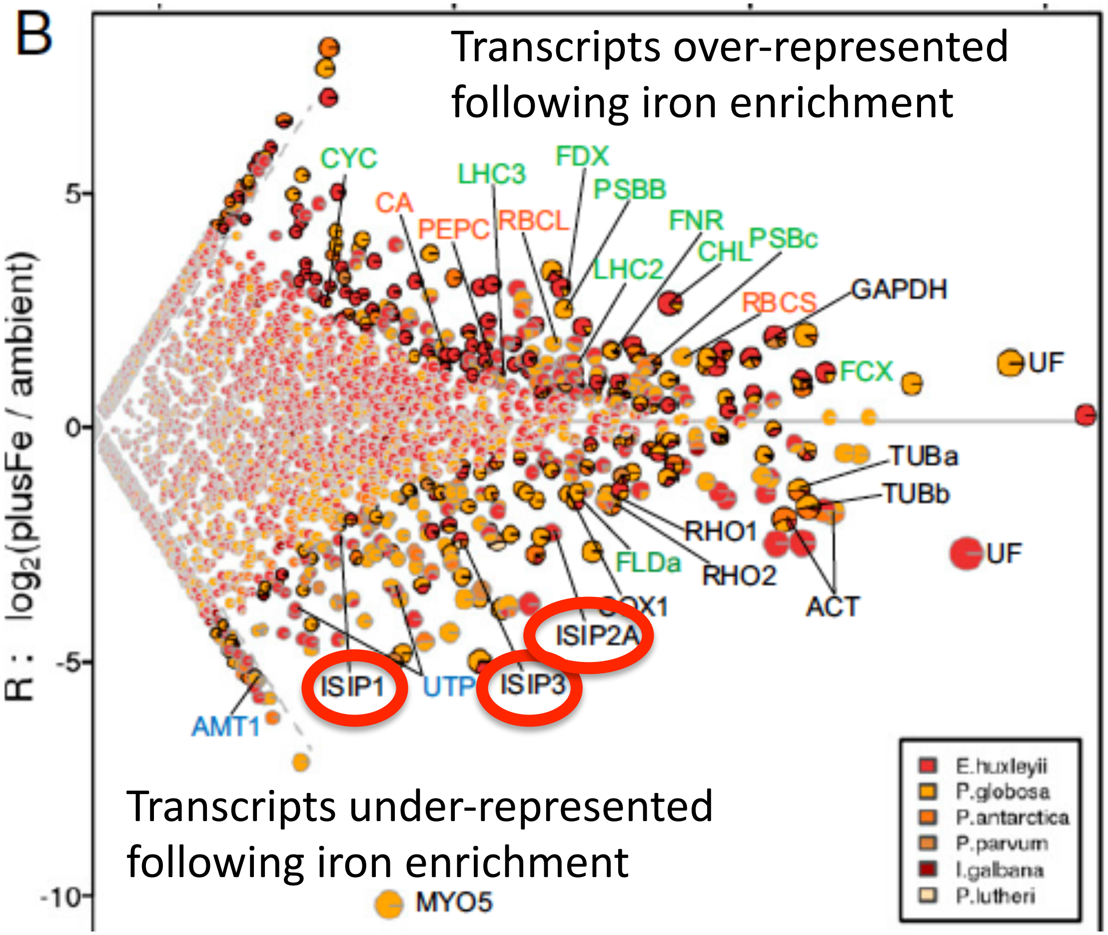
			Uptake			Storage	Redox					SOD			
Source	Genus species strain	Class	FRE	FET	FTR	FTN	petF	FLDA-I	FLDA-II	CYTC6	PCYN	CuZn	Fe	Mn	Ni
MMETSP0316-18	*Amphora coffeaeformis* CCMP127	Bacillariophyceae	1	1	2	2	1	1	1	1	0	1	0	2	2
MMETSP1065	*Amphiprora paludosa* CCMP125		2	1	1	2	0	0	1	1	0	1	0	1	2
MMETSP0724-27	*Amphiprora* sp. CCMP467		2	1	1	1	1	1	0	1	0	1	0	2	2
JGI	[d]*Phaeodactylum tricornutum*		2	0	0	1	0	1	1	1	0	1	0	2	1
MMETSP0017	*Cylindrotheca closterium* KMMCC:B-181		2	1	0	1	0	1	0	1	0	1	0	1	1
MMETSP0014	*Nitszchia* sp RCC80		0	1	1	2	0	0	0	1	0	1	0	2	2
MMETSP0744-47	*Nitzschia punctata* CCMP561		1	0	2	1	0	1	1	1	0	2	0	2	2
JGI	[c]*Fragilariopsis cylindrus*		1	3	1	1	0	1	1	1	1	1	0	2	1
MMETSP0733-36	[c]*Fragilariopsis kerguelensis* L26-C5		4	2	0	1	0	1	2	1	3	1	0	2	1
MMETSP1352	*Stauroneis constricta* CCMP1120		1	0	0	0	1	1	1	2	0	1	0	2	0
MMETSP0139-42	*Pseudo-nitzschia australis* 10249 10 AB		1	0	0	1	0	1	1	1	0	0	0	1	1
MMETSP1060	*Pseudo-nitzschia pungens* cf. *cingulata*		0	0	1	1	0	0	0	0	0	0	0	2	1
MMETSP1061	*Pseudo-nitzschia pungens* cf. *pungens*		0	1	0	2	0	0	0	1	0	0	0	1	1
JGI	[d]*Pseudo-nitzschia multiseries*		1	0	1	2	0	1	1	1	0	1	0	2	1
internal	*Pseudo-nitzschia granii*		0	0	0	1	0	0	1	1	1	1	0	2	1
MMETSP0329	*Pseudo-nitzschia arenysensis* B593		1	1	1	1	0	0	0	1	1	1	0	2	1
MMETSP0327	*Pseudo-nitzschia delicatissima* B596		0	1	1	1	0	0	0	1	0	1	0	1	0
MMETSP1423	*Pseudo-nitzschia heimii*		1	1	1	1	0	1	1	1	3	0	0	2	1
MMETSP1394	*Asterionellopsis glacialis*	Fragilariophyceae	1	2	1	1	0	1	0	1	0	0	1	2	1
MMETSP1360	*Licmophora* sp		1	0	0	1	0	0	1	1	0	1	0	2	1
MMETSP0786	*Thalassionema frauenfeldii* CCMP 1798		1	2	0	2	2	1	1	0	0	1	1	2	2
MMETSP1176	*Synedropsis recta* cf CCMP1620		1	1	1	1	0	1	2	1	0	1	0	2	0
MMETSP0009	*Grammatophora oceanica*		1	1	1	0	1	0	0	1	0	1	0	1	1
MMETSP1361	*Nanofrustulum* sp		3	2	2	4	0	0	0	1	0	2	0	2	2
MMETSP0418	*Cyclophora radiata* 13vi08-1A		0	0	0	0	0	0	1	1	0	1	0	3	1
MMETSP0397	*Cyclophora tenuis* ECT3854		1	1	0	0	1	1	0	1	0	1	0	2	1
MMETSP0800	*Striatella unipunctata*		1	0	0	0	0	0	1	1	1	1	0	2	1
MMETSP0015	*Odontella aurita* isolate 1302-5	Mediophyceae	1	1	1	1	1	1	1	1	0	1	1	2	2
MMETSP0160	*Odontella sinensis* (Grunow) 1884		2	0	1	1	0	1	1	3	0	1	1	2	1
MMETSP0696-99	*Extubocellulus spinifer* CCMP396		1	0	0	2	0	0	1	1	0	2	1	2	2
MMETSP1070	*Minutocellus polymorphus* NH13		1	1	0	2	1	1	0	1	0	1	0	2	0
MMETSP1062	*Ditylum brightwellii* Pop1 (SS4)		3	1	0	2	0	0	1	1	0	1	1	2	2
MMETSP1063	*Ditylum brightwellii* Pop2 (SS10)		2	0	0	2	0	1	1	0	1	1	1	2	2
MMETSP0013	*Skeletonema costatum* RA080513-05	yceae	2	2	1	0	1	1	0	1	0	0	2	2	1
MMETSP0320	[b]*Skeletonema marinoi* SM1012Den-03		1	1	1	0	1	0	0	0	0	0	1	1	0
MMETSP0319	[b]*Skeletonema marinoi* SM1012Hels-07		2	1	1	0	1	0	0	0	0	0	2	2	0
MMETSP1039	*Skeletonema marinoi* FE7		1	0	1	0	0	1	0	1	0	0	1	2	1
MMETSP1040	*Skeletonema marinoi* FE60		2	0	0	0	0	1	0	1	0	0	1	1	0
MMETSP0603-04	*Skeletonema menzelii* CCMP793		2	2	1	0	2	0	0	1	0	0	1	2	0
GenBank	[a,d]*Thalassiosira oceanica*		2	0	0	0	1	1	2	1	1	0	1	2	1
MMETSP0878-81	[b]*Thalassiosira weissflogii* CCMP1336		2	2	1	0	0	1	1	1	0	1	0	2	1
MMETSP1057	*Cyclotella meneghiniana* CCMP 338		2	1	1	0	0	1	0	1	0	0	0	2	1
MMETSP1059	*Thalassiosira* sp [freshwater]		2	1	1	0	0	0	0	1	0	0	1	2	0
JGI	[d]*Thalassiosira pseudonana*		2	1	1	0	0	1	0	1	0	0	2	2	0

doi:10.1371/journal.pone.0129081.g002

Comparative metatranscriptomics identifies molecular bases for the physiological responses of phytoplankton to varying iron availability

Adrian Marchetti[a,1,2,3], David M. Schruth[a,1], Colleen A. Durkin[a], Micaela S. Parker[a], Robin B. Kodner[a], Chris T. Berthiaume[a], Rhonda Morales[a], Andrew E. Allen[b], and E. Virginia Armbrust[a,2]

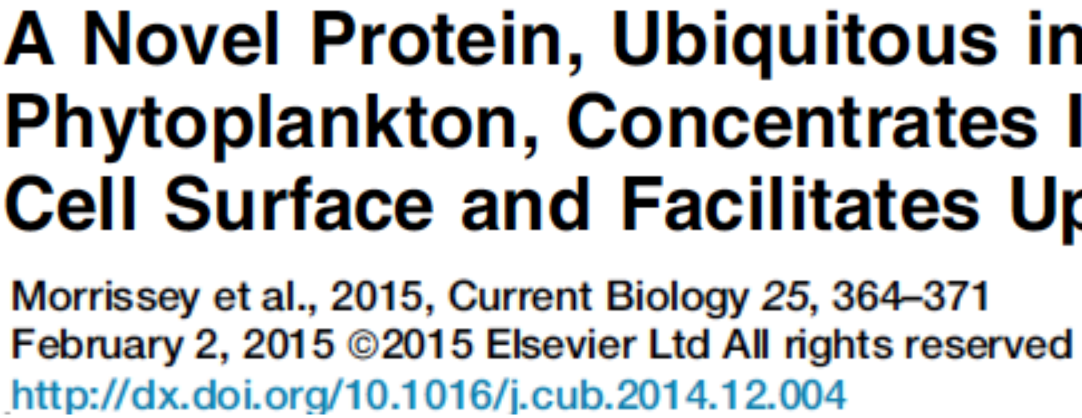
- Fe limited transcriptomes and metatranscriptomes identified genes likely involved in previously uncharacterized additional Fe uptake pathways



Transcripts over-represented following iron enrichment

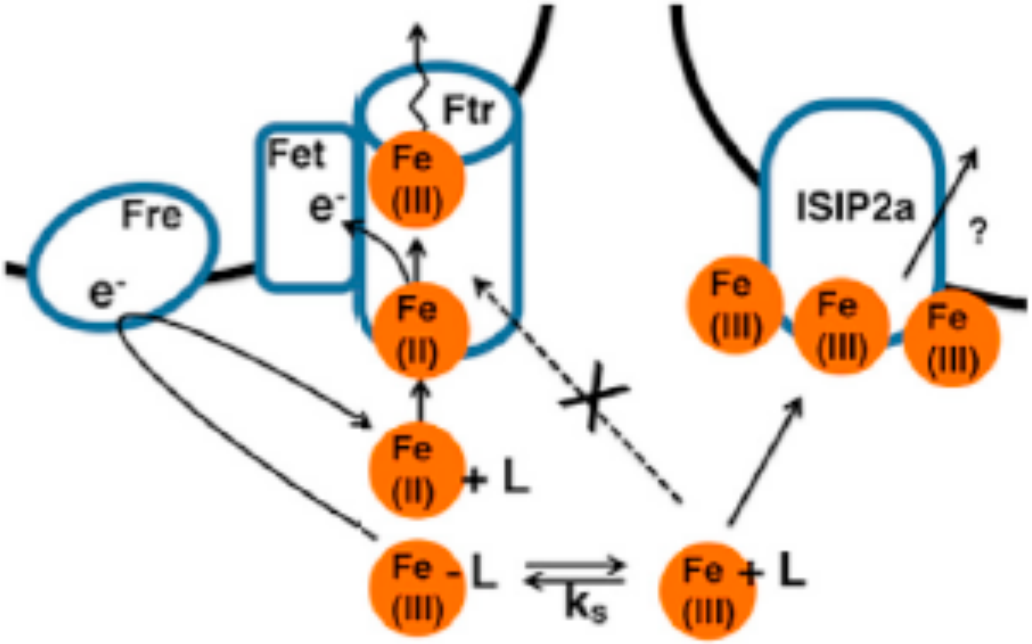
Transcripts under-represented following iron enrichment

PNAS 109:E317-E325 (2012)



A Novel Protein, Ubiquitous in Marine Phytoplankton, Concentrates Iron at the Cell Surface and Facilitates Uptake

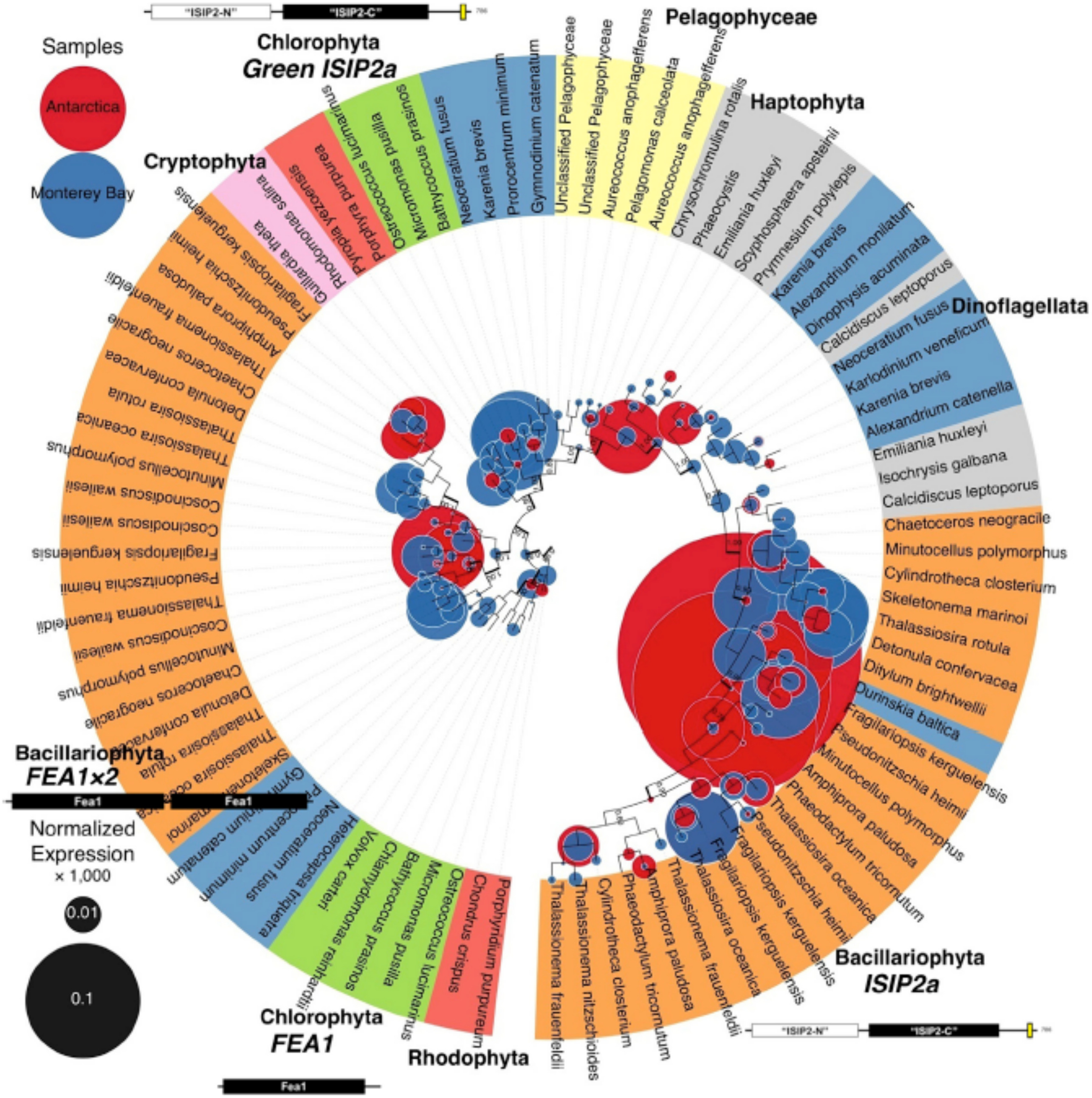
Morrissey et al., 2015, Current Biology *25*, 364–371
February 2, 2015 ©2015 Elsevier Ltd All rights reserved
http://dx.doi.org/10.1016/j.cub.2014.12.004



- A putative role for ISIP2a in non-Cu dependent uptake of Fe(III) was determined by combining Fe uptake studies, genetic manipulation, and physiological experiments

Morrissey et al., 2015, Current Biology 25, 364–371
February 2, 2015 ©2015 Elsevier Ltd All rights reserved
http://dx.doi.org/10.1016/j.cub.2014.12.004

- ISIP2a transcripts are abundant in Antarctica (red) and Monterey Bay (blue) metatranscriptomes
- ISIP2a expression illustrates Its ecological significance in the marine environment across different eukaryotic lineages



-omic/meta-omic identification of genetic capacity

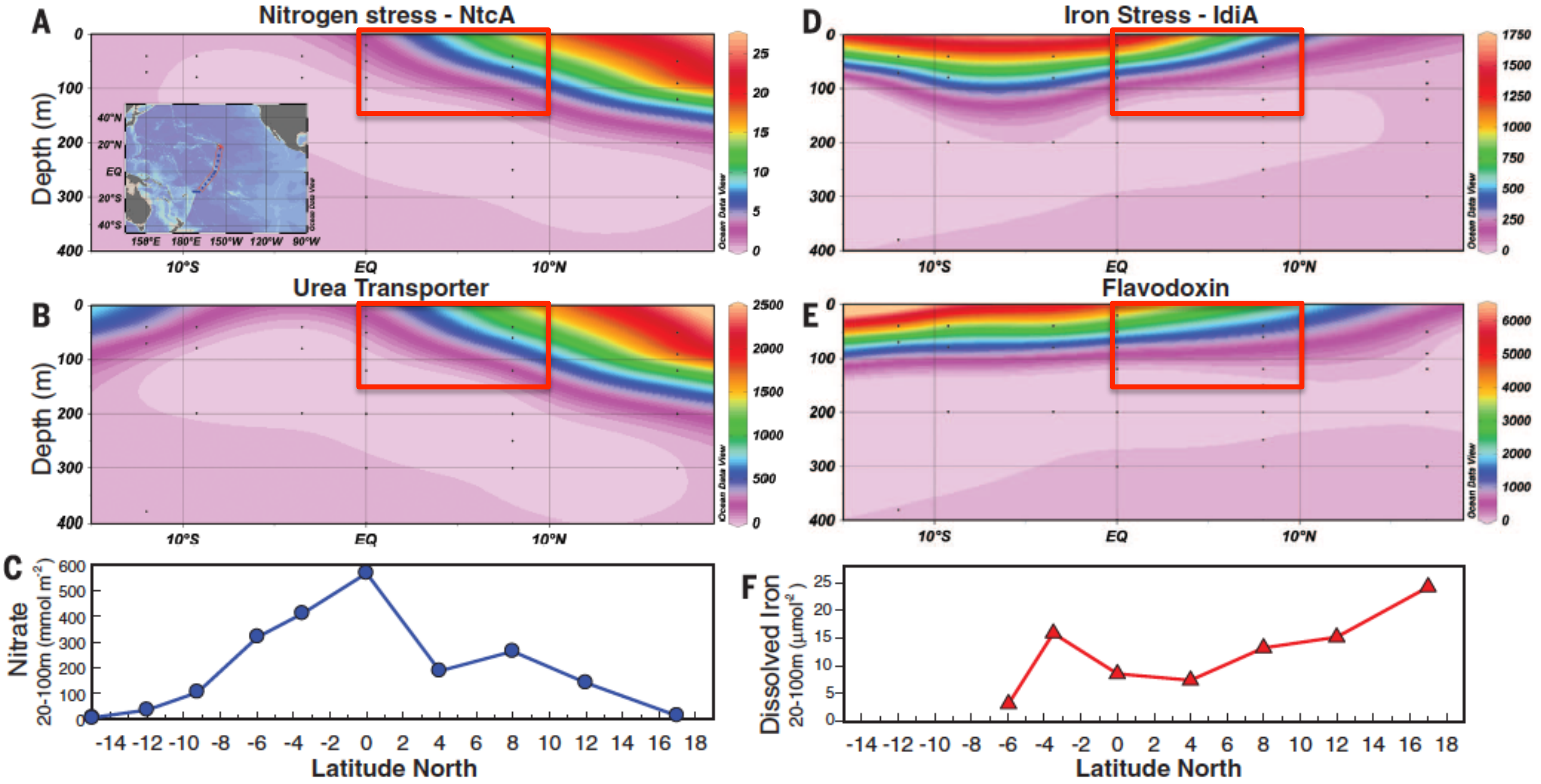
- Has proven to be a powerful tool to evaluate the potential for uptake of different forms of Fe and other metals
  - Especially when combined with genetic/ physiological characterization including Fe uptake experimental work
  - These efforts have also helped identify putative Fe stress markers that have been used to track Fe limitation in the field

MARINE MICROBES

Multiple nutrient stresses at intersecting Pacific Ocean biomes detected by protein biomarkers

Mak A. Saito,[1]* Matthew R. McIlvin,[1] Dawn M. Moran,[1] Tyler J. Goepfert,[1] Giacomo R. DiTullio,[2] Anton F. Post,[3] Carl H. Lamborg[1]

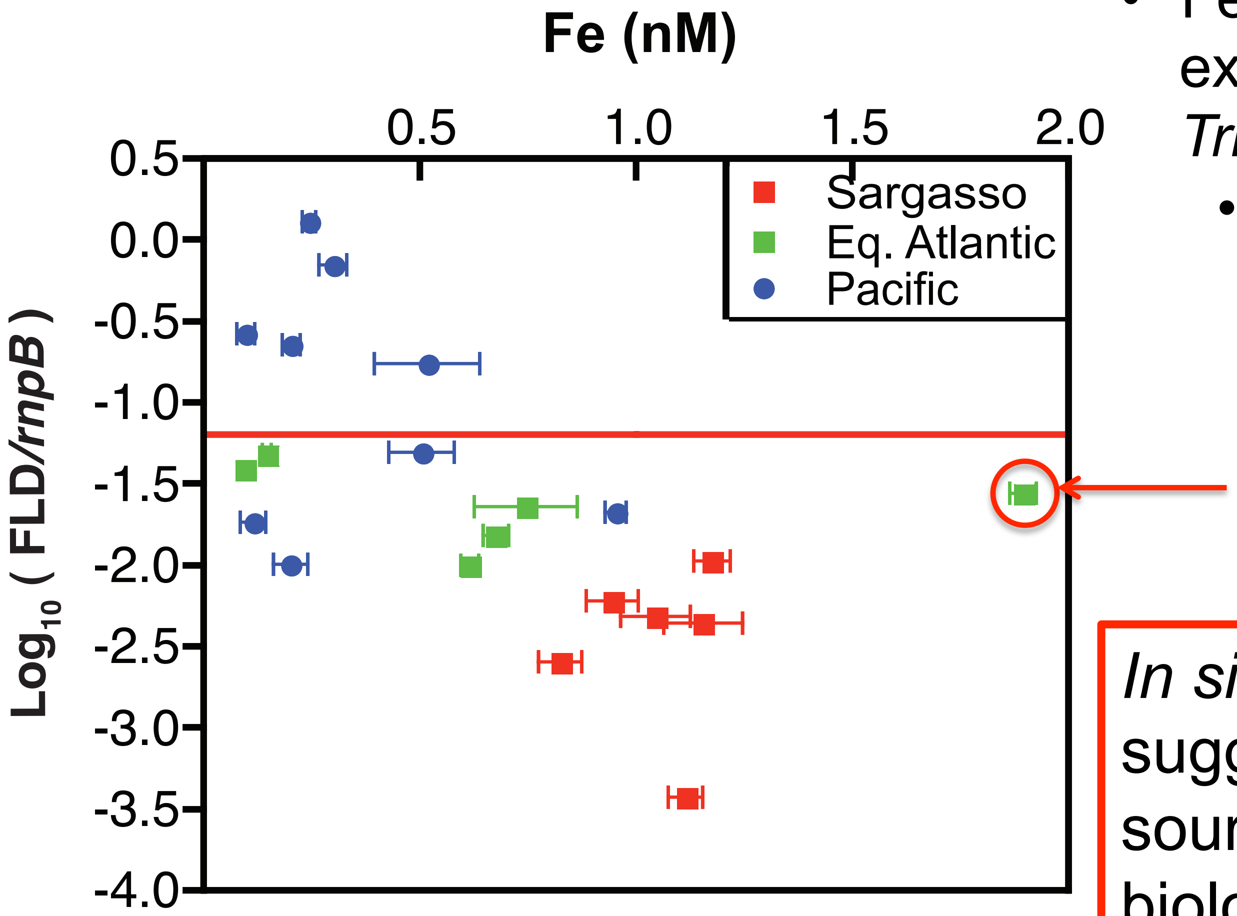
- N and Fe stress markers in *Prochlorococcus*:
    - Successfully track biome shifts (N lim, Fe lim)
    - Highlight areas of possible co-limitation



Molecular evidence of iron limitation and availability in the global diazotroph *Trichodesmium*

Phoebe Dreux Chappell[1,2], James W Moffett[3], Annette M Hynes[1,4] and Eric A Webb[3]

The ISME Journal (2012) 6, 1728–1739



- Fe stress gene expression markers in *Trichodesmium*:
  - Mostly show good correlation with dFe

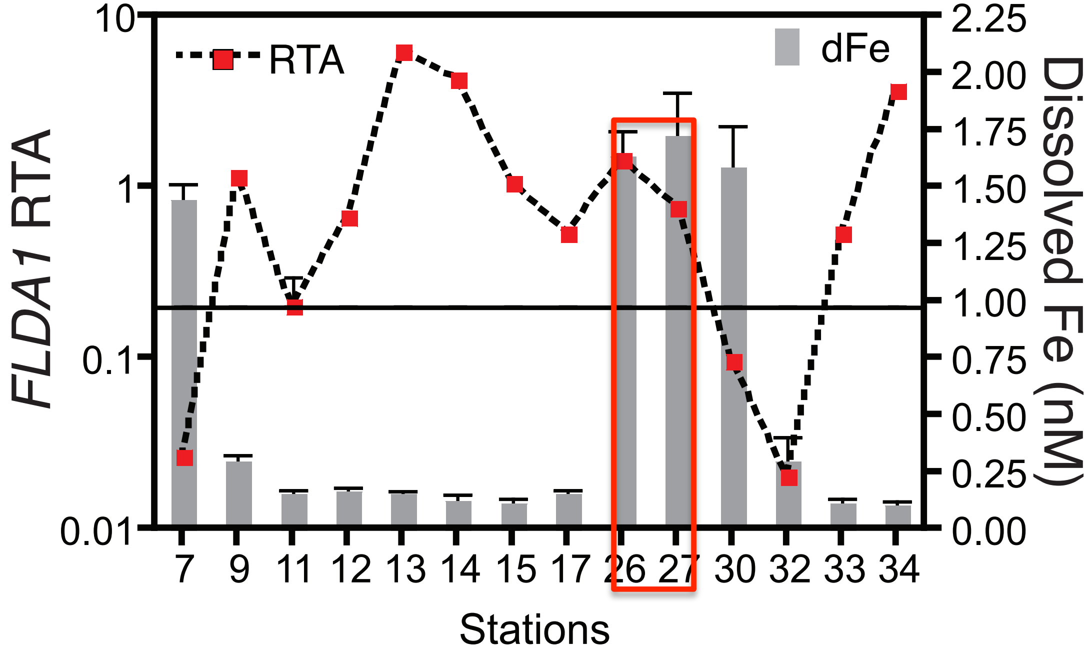
Sample from the Amazon River Plume

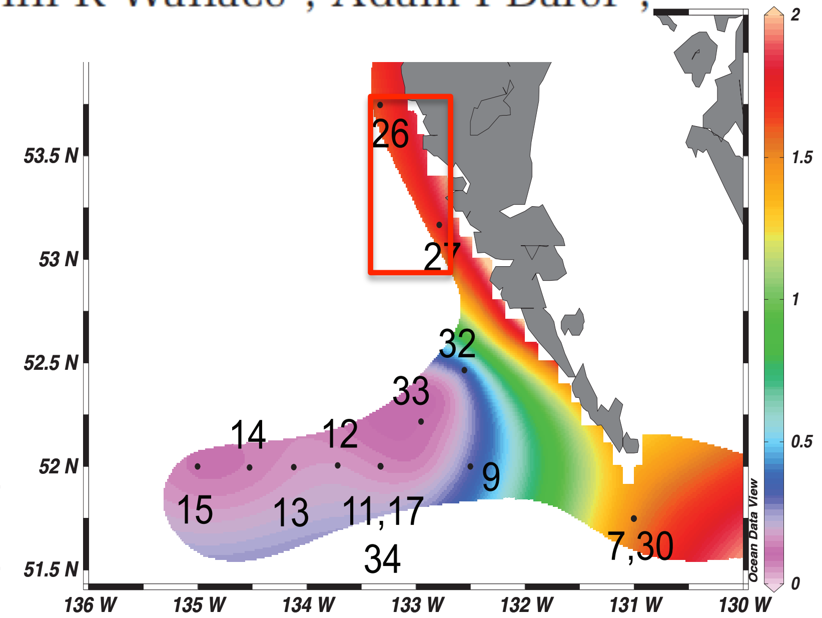
In situ expression suggests some dFe sources may not be biologically available

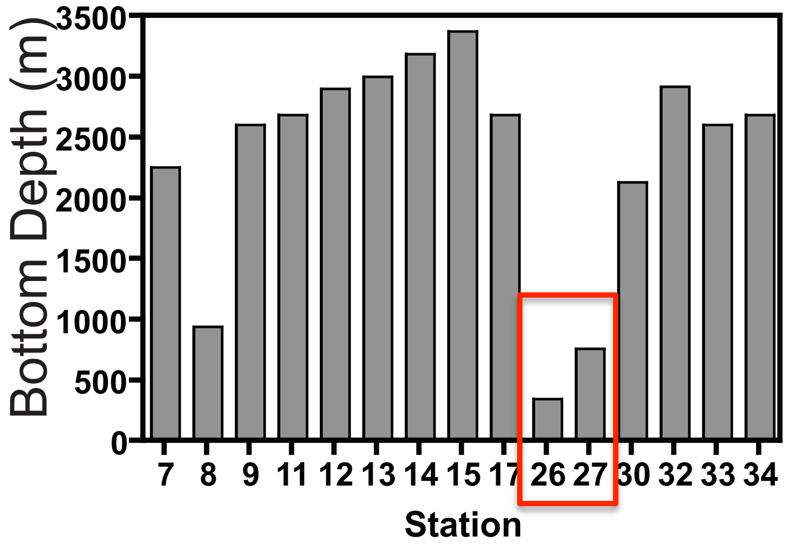
Genetic indicators of iron limitation in wild populations of *Thalassiosira oceanica* from the northeast Pacific Ocean

ISME J 2015 **9:** 592-602.

P Dreux Chappell[1,4,6], LeAnn P Whitney[1,5,6], Joselynn R Wallace[1], Adam I Darer[2], Samua Jean-Charles[1] and Bethany D Jenkins[1,3]







Again, *in situ* expression suggests some dFe sources may not be biologically available

Targeted genomics/proteomics

- Can be used with trace element measurements (preferably a more complete suite) to evaluate limitation in the field
- Has helped identify potential areas of co-limitation (multiple stressors) and some surprising limitation results
- Can we use these tools to answer questions:
  - About Fe bioavailability? Was it terrestrial Fe? Was it a colloidal or particulate form that was unavailable? Was it humics? Another unavailable ligand?
  - About what drives the ecological success of phytoplankton from different habitats? Is there a terrestrial iron source that's unavailable to oceanic phytoplankton? Can coastal species access it?

Conclusions/Outlook

- Omics methods provide valuable information about phytoplankton uptake pathways
  - Especially useful when combined with uptake analyses, physiology, and genetic manipulation
  - Could possibly identify universal markers for limitation?
- Combining surveys of trace elements with transcriptomic/proteomic analysis in both surveys and process studies can identify:
  - Areas of limitation/co-limitation (where would it be useful to do more process studies?)
  - Which form(s) of trace elements are bioavailable to different phytoplankton groups