

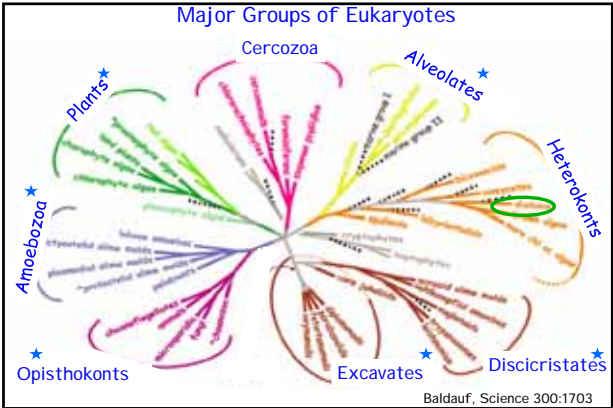
### Summary of the Talk

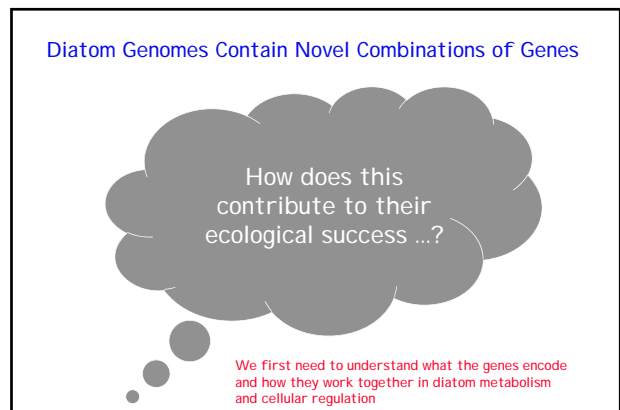
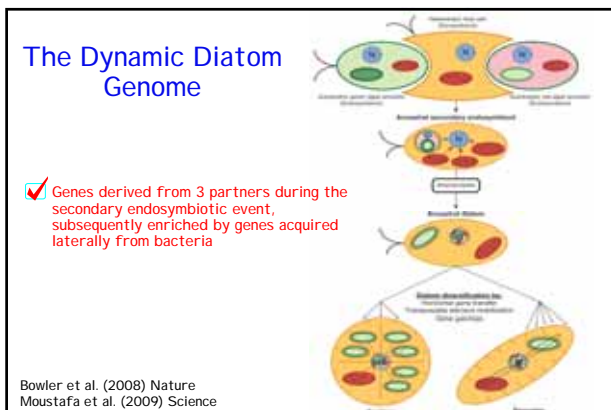
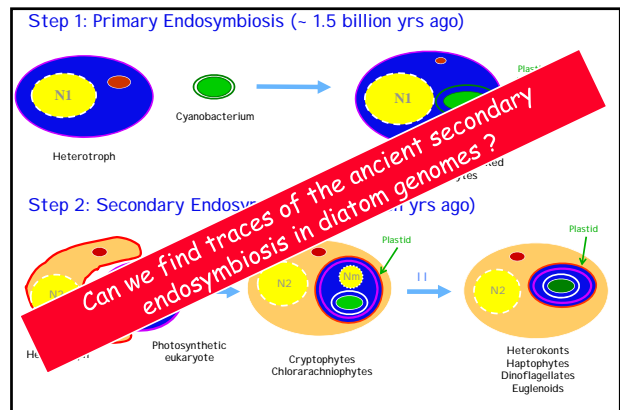
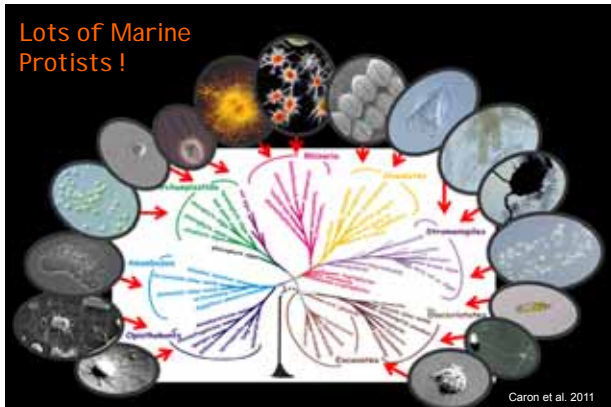
- ✓ Insights into diatom evolution from diatom genomes
- ✓ Diatom adaptation to chronically low iron bioavailability

### Comparative Analysis of Diatom Genomes

- ✓ ~ 60% of genes are shared
- ✓ Little synteny and no segmental duplications
- ✓ Diatom-specific transposable elements may be important drivers of diversity
- ✓ Divergence equivalent to fish:human
- ✓ Novel combinations of genes encoding novel metabolisms
- ✓ Diatom-specific genes are evolving fast

Bowler et al. Nature 2008



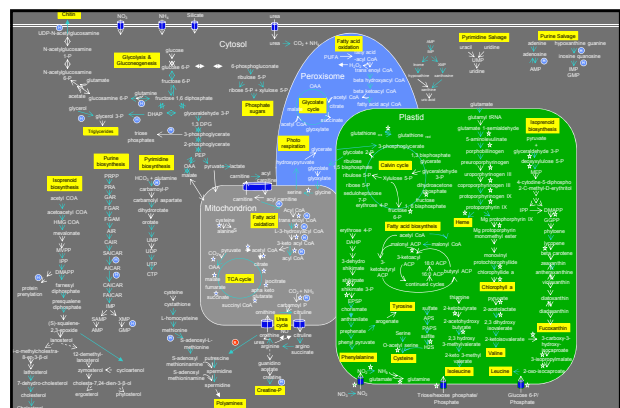


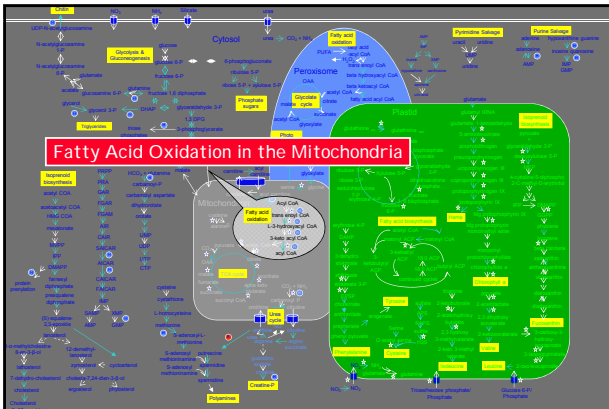
Genome-Enabled Resources for Diatoms

✓ The Diatom Digital Gene Expression Database:  
- <http://www.biologie.ens.fr/diatomics/est3>

✓ Genetic transformation

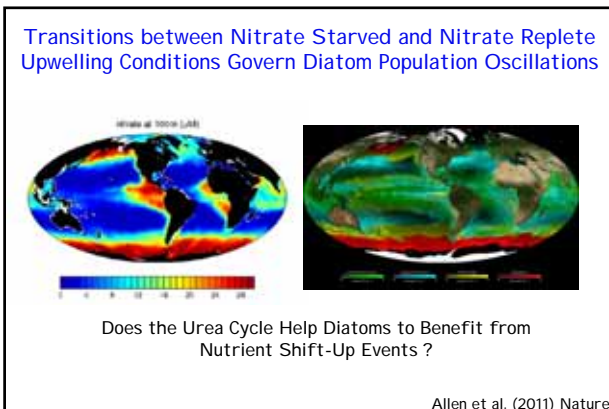
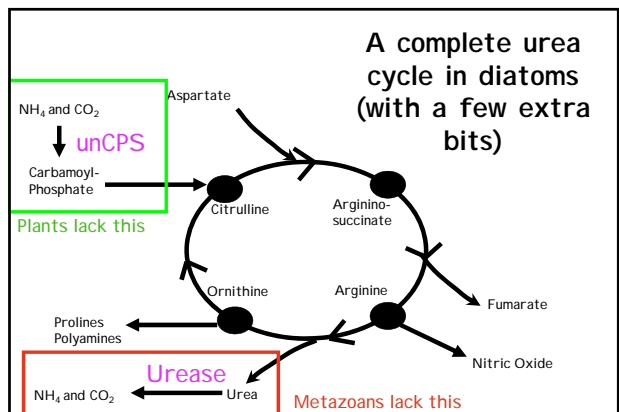
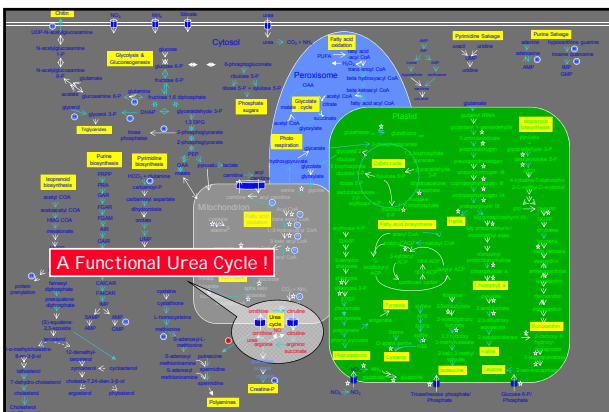
- Overexpression
- Subcellular localisation (GFP)
- Knockout (RNAi)





In Times of Need ...

Organism	Primary Storage Compound
Plants	Carbohydrates
Animals	Fats
Diatoms	Carbohydrates (short-term) Fats (long-term)



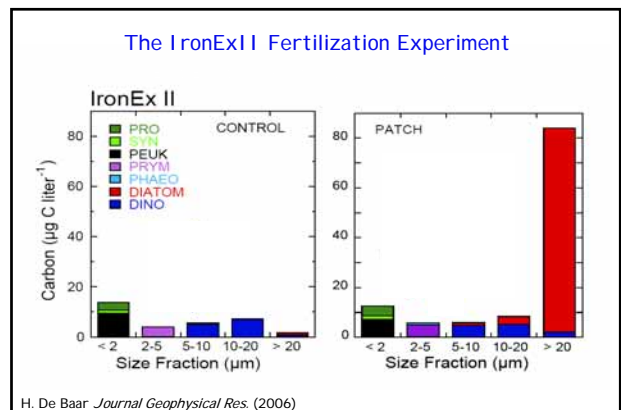
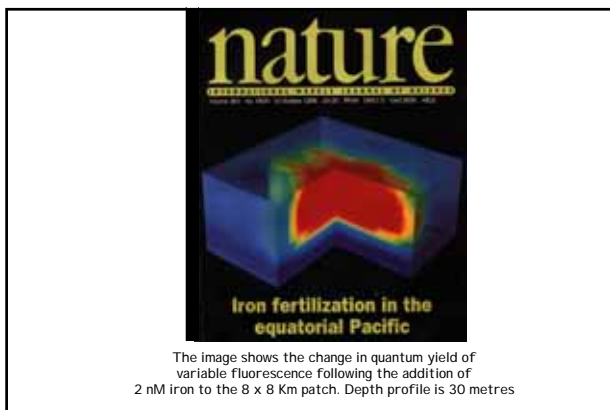
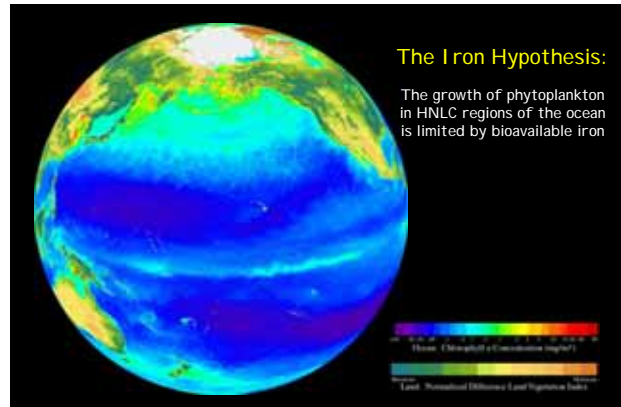
**Evolution and metabolic significance of the urea cycle in photosynthetic diatoms**

Andrew L. Allen<sup>1,2</sup>, Christopher L. Dugoni<sup>1</sup>, Miraluz Obornik<sup>1</sup>, Alek Itonik<sup>1</sup>, Adriano Nunes-Neto<sup>1</sup>, John P. McCrow<sup>1</sup>, Yong Zhang<sup>1</sup>, Daniel A. Johnson<sup>2</sup>, Hanhua He<sup>1</sup>, Alisdair R. Fernie<sup>3</sup> & Chris Bowler<sup>1</sup>

- ✓ RNAi knockdown of mitochondrial CPS111 impairs recovery from nutrient stress
- ✓ The urea cycle represents a hub for anaerobic carbon fixation into N compounds
- ✓ Derived compounds used for production of metabolites needed for diatom growth
- ✓ Genes acquired laterally from bacteria have significantly expanded the functionality of the urea cycle
- ✓ Metabolic coupling of bacterial and exosymbiont-derived processes appears to be fundamental to diatom physiology
- ✓ The urea cycle significantly contributes to the shift-up metabolic response of diatoms to episodic nutrient availability

## Summary of the Talk

- ✓ Insights into diatom evolution from diatom genomes
- ✓ Diatom adaptation to chronically low iron bioavailability



How do diatoms cope with feast-and-famine regimes imposed by iron bioavailability ?

### The Global Response of Phaeodactylum to Iron Limitation

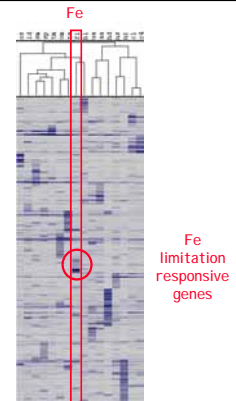
- ✓ **Analysis of gene expression**
  - ESTs from iron-limited libraries
  - Microarray hybridisations
  - qRT-PCR
- ✓ **Analysis of metabolome**
  - Non-targeted GC-MS profiling
- ✓ **Characterization of novel gene functions**
  - Reverse genetics

### Genes Expressed in Different Conditions in *Phaeodactylum*

Library	Condition / Medium	Number of Sequences
Pt - "Original"	f/2	12,136
Silica Plus	350 $\mu$ M metasilicate in artificial seawater	8,012
Silica Minus	Artificial seawater	8,157
Nitrate Replete	1.12 mM in chemostat culture	3,722
Nitrate Depleted	50 $\mu$ M for 3 days in chemostat culture	9,657
Ammonium	75 $\mu$ M	9,466
Urea	50 $\mu$ M	9,040
Low decadienal	0.5 $\mu$ g/ml 2E,4E-decadienal for 6 h	9,610
High decadienal	5 $\mu$ g/ml 2E,4E-decadienal for 6 h	3,541
Iron limited	5 nM Fe	8,669
Oval morphotype	English strain PT3 grown at low salinity	4,661
Blue light	48 hours dark adapted cells exposed to 1 h blue light	10,834
Triradiate morphotype	Canadian strain PT8	11,005
Tropical strain	Pt 9 grown at 15 $^{\circ}$ C	4,942
CO2 High - 1 day	3.2 mM DIC (level equivalent to 2300)	10,307
CO2 High - 4 day	3.2 mM DIC (level equivalent to 2300)	10,283

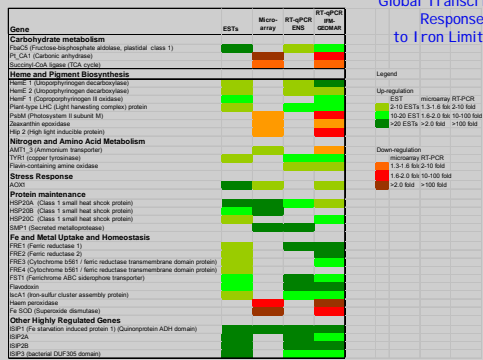
Allen et al. (2008) PNAS; Maheswari et al (2010) Genome Biol

### Hierarchical Clustering of 13,000 Genes in 16 Different Growth Conditions



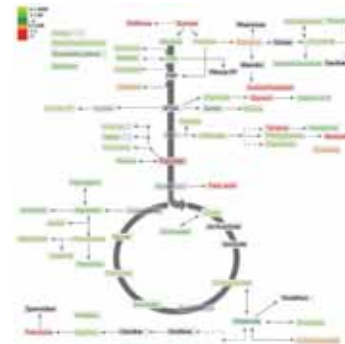
Allen et al. (2008) PNAS; Maheswari et al (2010) Genome Biol

### Global Transcriptional Responses to Iron Limitation



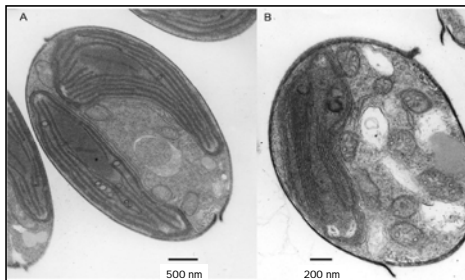
Allen et al. (2008) PNAS; Maheswari et al (2010) Genome Biol

### Global Metabolite Responses to Iron Limitation



Allen et al. (2008) PNAS; Wagner et al (2012) TIPS

### Plastid Ultrastructure in Fe-Replete and Fe-limited Conditions



Fe-replete

Fe-limited

Allen et al. (2008) PNAS

### The Global Response of *Phaeodactylum* to Iron Limitation

Parameter	Fe-limited	Fe-replete	Fe-limited/Fe replete
Chl/cell (pg/cell)	0.22	0.5	0.44
Cell diameter ( $\mu$ m)	2.5	3.5	0.71
Cell volume ( $\mu$ m <sup>3</sup> )	60	100	0.60
Chl/volume (fg/ $\mu$ m <sup>3</sup> )	3.67	5.0	0.73
Growth rate	0.18 $\pm$ 0.05	0.88 $\pm$ 0.01	0.2 $\pm$ 0.06
Fv/Fm	0.18 $\pm$ 0.1	0.5 $\pm$ 0.05	0.36 $\pm$ 0.2
NPQ	4.8 $\pm$ 0.8	3.1 $\pm$ 0.51	1.5 $\pm$ 0.4
AOX activity	0.6 $\pm$ 0.15	0.25 $\pm$ 0.18	2.4 $\pm$ 1.83
PSII	1.2 $\pm$ 0.2	1	1.2 $\pm$ 0.14
PSI	0.6 $\pm$ 0.1	1.4 $\pm$ 0.2	0.43 $\pm$ 0.10
Cytochrome <i>b<sub>6</sub>f</i>	0.6 $\pm$ 0.1	0.8 $\pm$ 0.1	0.75 $\pm$ 0.13
Cytochrome <i>c<sub>2</sub></i>	1.4 $\pm$ 0.1	2 $\pm$ 0.2	0.70 $\pm$ 0.05
Carbon fixation @ 150 $\mu$ E (pmol C/cell*hour)	0.010 $\pm$ 0.006	0.141 $\pm$ 0.022	0.07 $\pm$ 0.04
Ferric reductase assay (A.U./cell)	0.717 $\pm$ 0.03	0.020 $\pm$ 0.004	35.9 $\pm$ 7.3
Ferric reductase assay (A.U./ $\mu$ m <sup>2</sup> )	0.01	0.0002	50

### The Global Response of *Phaeodactylum* to Iron Limitation

Parameter	Fe-limited	Fe-replete	Fe-limited/Fe replete
Chl/cell (pg/cell)	0.22	0.5	0.44
Cell diameter (µm)	2.5	3.5	0.71
Cell volume (µm <sup>3</sup> )	60	100	0.60
Chl/volume (fg/µm <sup>3</sup> )	3.67	5.0	0.73
Growth rate	0.18 ± 0.05	0.88 ± 0.01	0.2 ± 0.06
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Ferric reductase assay (A.U./µm <sup>3</sup> )	0.01	0.0002	50

### Alternative Oxidase is the 5<sup>th</sup> most highly expressed gene under Fe-Limitation

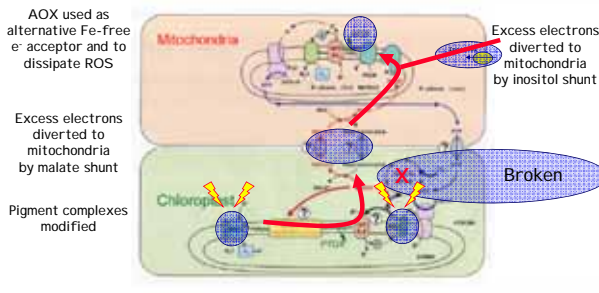
#### Top Fe-regulated genes

Gene ID	Gene Name	BLASTX description
156	SSIP1	SSIP1
120	mp6	mp6 metalloprotease smp1
115	AUTO TRANSPORT BARREL/ton-b?	AUTO TRANSPORT BARREL/ton-b?
114	SSIP2A	SSIP2A
55	AOX	AOX
44	SSIP2B	SSIP2B
43	SSIP3	SSIP3

Allen et al, PNAS 2008; Maheswari et al, Genome Biol 2010

- ✓ AOX is an alternative terminal electron acceptor in the respiratory electron transport chain in plants

### The Cellular Response to Iron Limitation, as Revealed by Coupled Transcriptome/Metabolome Profiling



### The Diatom Photosynthesis and Respiratory Pathways Work Together

Diatoms are obligate photomixotrophs !!

This may contribute to their ecological success

### ISIP2a is the 4<sup>th</sup> Most Highly Expressed Gene in Fe-Limiting Conditions

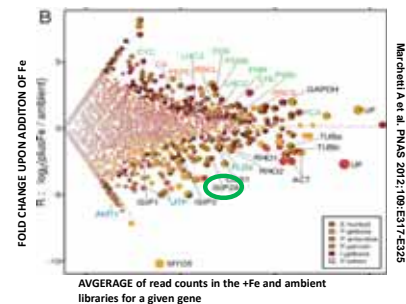
#### Top Fe-regulated genes

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120	mp6	mp6 metalloprotease smp1
115	AUTO TRANSPORT BARREL/ton-b?	AUTO TRANSPORT BARREL/ton-b?
114	SSIP2A	SSIP2A
55	AOX	AOX
44	SSIP2B	SSIP2B
43	SSIP3	SSIP3

Allen et al, PNAS 2008; Maheswari et al, Genome Biol 2010

- ✓ ISIP2A is predicted to be membrane localized
- ✓ ISIP2A has some similarity with known Fe transporters

### ISIP2a is Fe-Regulated in Ocean Metatranscriptome Datasets



## ISIP1 is the most numerous EST under -Fe

### Top Fe-regulated genes

Gene	120	115	114	58	48	45
ISIP1	11	11	11	11	11	11
ISIP2	11	11	11	11	11	11
ISIP3	11	11	11	11	11	11
ISIP4	11	11	11	11	11	11
ISIP5	11	11	11	11	11	11
ISIP6	11	11	11	11	11	11
ISIP7	11	11	11	11	11	11
ISIP8	11	11	11	11	11	11
ISIP9	11	11	11	11	11	11
ISIP10	11	11	11	11	11	11
ISIP11	11	11	11	11	11	11
ISIP12	11	11	11	11	11	11
ISIP13	11	11	11	11	11	11
ISIP14	11	11	11	11	11	11
ISIP15	11	11	11	11	11	11
ISIP16	11	11	11	11	11	11
ISIP17	11	11	11	11	11	11
ISIP18	11	11	11	11	11	11
ISIP19	11	11	11	11	11	11
ISIP20	11	11	11	11	11	11
ISIP21	11	11	11	11	11	11
ISIP22	11	11	11	11	11	11
ISIP23	11	11	11	11	11	11
ISIP24	11	11	11	11	11	11
ISIP25	11	11	11	11	11	11
ISIP26	11	11	11	11	11	11
ISIP27	11	11	11	11	11	11
ISIP28	11	11	11	11	11	11
ISIP29	11	11	11	11	11	11
ISIP30	11	11	11	11	11	11
ISIP31	11	11	11	11	11	11
ISIP32	11	11	11	11	11	11
ISIP33	11	11	11	11	11	11
ISIP34	11	11	11	11	11	11
ISIP35	11	11	11	11	11	11
ISIP36	11	11	11	11	11	11
ISIP37	11	11	11	11	11	11
ISIP38	11	11	11	11	11	11
ISIP39	11	11	11	11	11	11
ISIP40	11	11	11	11	11	11
ISIP41	11	11	11	11	11	11
ISIP42	11	11	11	11	11	11
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ISIP44	11	11	11	11	11	11
ISIP45	11	11	11	11	11	11
ISIP46	11	11	11	11	11	11
ISIP47	11	11	11	11	11	11
ISIP48	11	11	11	11	11	11
ISIP49	11	11	11	11	11	11
ISIP50	11	11	11	11	11	11
ISIP51	11	11	11	11	11	11
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ISIP55	11	11	11	11	11	11
ISIP56	11	11	11	11	11	11
ISIP57	11	11	11	11	11	11
ISIP58	11	11	11	11	11	11
ISIP59	11	11	11	11	11	11
ISIP60	11	11	11	11	11	11
ISIP61	11	11	11	11	11	11
ISIP62	11	11	11	11	11	11
ISIP63	11	11	11	11	11	11
ISIP64	11	11	11	11	11	11
ISIP65	11	11	11	11	11	11
ISIP66	11	11	11	11	11	11
ISIP67	11	11	11	11	11	11
ISIP68	11	11	11	11	11	11
ISIP69	11	11	11	11	11	11
ISIP70	11	11	11	11	11	11
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ISIP75	11	11	11	11	11	11
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ISIP77	11	11	11	11	11	11
ISIP78	11	11	11	11	11	11
ISIP79	11	11	11	11	11	11
ISIP80	11	11	11	11	11	11
ISIP81	11	11	11	11	11	11
ISIP82	11	11	11	11	11	11
ISIP83	11	11	11	11	11	11
ISIP84	11	11	11	11	11	11
ISIP85	11	11	11	11	11	11
ISIP86	11	11	11	11	11	11
ISIP87	11	11	11	11	11	11
ISIP88	11	11	11	11	11	11
ISIP89	11	11	11	11	11	11
ISIP90	11	11	11	11	11	11
ISIP91	11	11	11	11	11	11
ISIP92	11	11	11	11	11	11
ISIP93	11	11	11	11	11	11
ISIP94	11	11	11	11	11	11
ISIP95	11	11	11	11	11	11
ISIP96	11	11	11	11	11	11
ISIP97	11	11	11	11	11	11
ISIP98	11	11	11	11	11	11
ISIP99	11	11	11	11	11	11
ISIP100	11	11	11	11	11	11

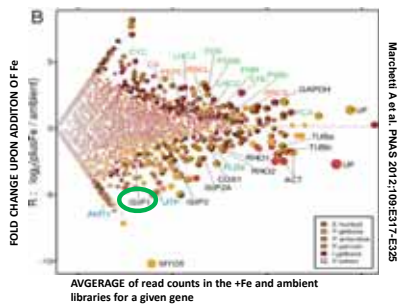
Allen et al, PNAS 2008; Maheswari et al, Genome Biol 2010

- ✓ ISIP1 is also predicted to be secreted to the cell surface and has one predicted transmembrane span.
- ✓ ISIP1 does not rescue Fe-uptake in *Δfet3 fet4*.

## ISIP Distributions in Phytoplankton

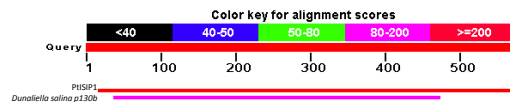
	ISIP1	ISIP2a	ISIP3
<i>P. tricornutum</i>	YES	YES	YES
<i>T. oceanica</i>	YES	YES	YES
<i>Fragilariopsis cylindrus</i>	YES	YES	YES
<i>Pseudo-nitzschia granii</i>	YES	YES	YES
<i>T. pseudonana</i>	NO	NO	YES
<i>O. lucimarinus</i>	NO	YES	NO
<i>O. RCC809</i>	NO	YES	NO
<i>O. tauri</i>	NO	NO	NO
<i>Aureococcus anophagefferens</i>	NO	YES	NO
<i>Ectocarpus siliculosus</i>	NO	YES	NO

## ISIP1 is Fe-regulated in ocean metatranscriptome sets



AVGERAGE of read counts in the +Fe and ambient libraries for a given gene

## ISIP1 and Fe-uptake?



The only non-diatom ortholog of ISIP1 is p130 in *D. salina*.

*D. salina* transferrin binds extracellular Fe and internalizes it.

p130b binds transferrin complex, but not Fe (Paz et al, 2007, JBC)

## However, diatom ISIP1 has a cysteine rich core absent in *D. salina* p130b



Cysteine motifs are associated with metal binding, and thus could served to concentrate iron.

Figure 6 from Lommer et al., 2012, Genome Biology

Lommer et al. (2012) Genome Biol.

## Study of Iron Limitation Responses in Phaeodactylum has :

- ✓ Led to a new understanding of the fundamentals of diatom cell physiology
- ✓ I identified a novel mechanism for Fe<sup>3+</sup> capture and transport
- ✓ These mechanisms are likely of significance in the oceans

## Life at low iron concentrations

- Chris Bowler, Uma Maheswari, Andy Allen (now at JCVI) and Joe Morrissey (IBENS-Paris)
- Julie La Roche and Markus Lommer (IFM-Geomar, Kiel)
- Alisdair Fernie and Nic Schauer (MPI-MP, Golm)
- Giovanni Finazzi (CEA, Grenoble)
- Emmanuel Lesuisse (IJM, Paris)

Allen et al. (2008) PNAS; Maheswari et al (2010) Genome Biol