

Development of Renewable Biofuels Technology by Transcriptomic Analysis and Metabolic Engineering of Diatoms

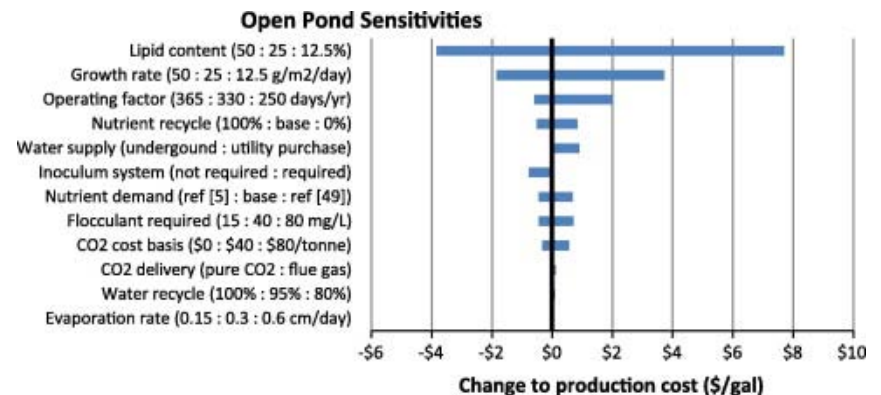
Award DE-EE0001222

May 21, 2013
Algae Review

Mark Hildebrand
Scripps Institution of Oceanography, UCSD

Goal Statement

- The goals of this project are to develop metabolic engineering approaches for diatoms to enable induction of lipid accumulation by controllable manipulation of intracellular processes rather than from external environmental conditions, and to manipulate carbon partitioning within the cell between lipid and carbohydrate synthesis to enable both abundant biomass and lipid accumulation.



Davis et al. 2011. Appl. Energy 88:3524-3531

- Achievement of the goals will result in increased overall lipid productivity and the development of diatoms as biofuels production organisms, which will facilitate the production of algal-based biofuels nationwide.

Quad Chart Overview

Timeline

- Project start date 09/01/09 (actual 02/01/10)
- Project end date: 08/31/13 (one year NCE)
- Percent complete: 90%

Budget

- Total project funding: \$300,000
 - DOE share: \$224,686
 - Contractor share: \$75,314
- Funding received in FY12: \$100,000
- Funding for FY13: \$0

Barriers

- Understanding fundamental processes of carbon flux and partitioning in diatoms.
- **Developing a versatile set of genetic manipulation tools.**
- **Developing the ability to manipulate carbon partitioning for abundant lipid accumulation coupled with high biomass accumulation.**

Partners

- J. Craig Venter Institute (Dr. Andrew Allen)
- UCLA (Dr. Matteo Pellegrini)

Project Overview

- Diatoms were identified as excellent candidate biofuel production microalgae in the Aquatic Species Program (ASP).
- Like most other algae, accumulation of triacylglycerol (TAG) requires cessation of growth to shift carbon flux from carbohydrate production to TAG accumulation.
- The fundamental aspects of carbon flux and partitioning and their regulation are unknown.
- By investigating mRNA-level changes during TAG accumulation under different limitation conditions and with different diatom species, carbon flux and its regulation can be elucidated.
- Further development of genetic manipulation tools, coupled with identification of key carbon partitioning regulatory genes, could enable metabolic engineering to facilitate high lipid productivity.

1 - Approach

- Objective 1: To perform comparative transcriptomic analysis in *Thalassiosira pseudonana* and *Cyclotella cryptica* of TAG accumulation resulting from silicon and nitrogen limitation, to identify common and key regulatory steps involved in controlling TAG accumulation and carbon partitioning.
- Objective 2: To metabolically engineer the cell to alter carbon partitioning to either improve the rate of TAG accumulation or to enable TAG accumulation along with high biomass accumulation.
- Metrics
 - Obj. 1: Develop a map of carbon flux pathways and identify key regulatory genes.
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 - Combined Obj.: Demonstrate improved TAG accumulation characteristics in engineered strains.

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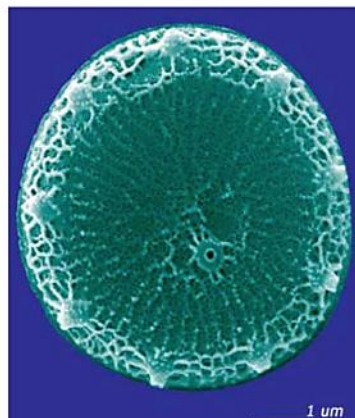
Comparative analysis of diatom genomes reveals substantial differences in the organization of carbon partitioning pathways

Sarah R. Smith, Raffaella M. Abbriano, Mark Hildebrand *

Marine Biology Research Division, Scripps Institution of Oceanography, UCSD, La Jolla, CA, United States

A comparative analysis of the genes involved in carbon partitioning metabolism from three available diatom genome sequences, from *Thalassiosira pseudonana*, *Phaeodactylum tricornutum*, and *Fragilariopsis cylindrus*

Centric

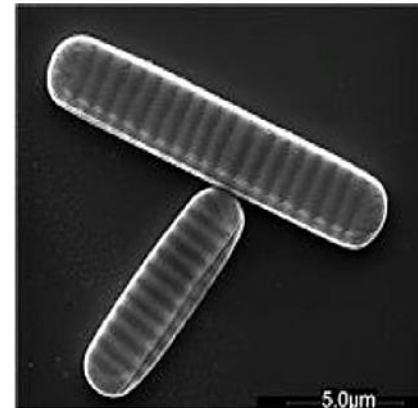


*Thalassiosira
pseudonana*

Pennates



*Phaeodactylum
tricornutum*



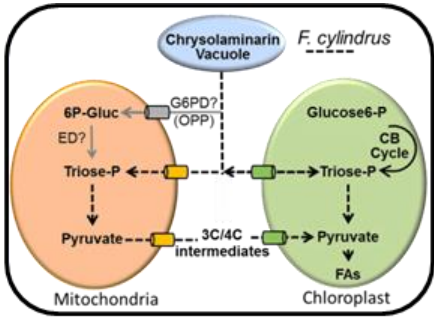
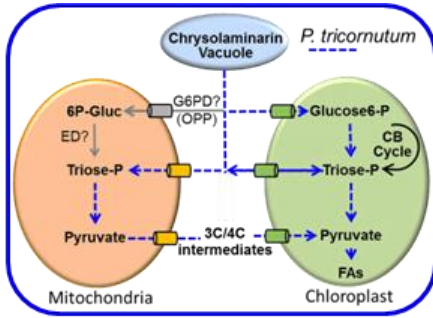
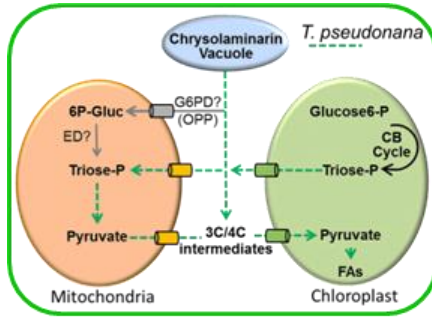
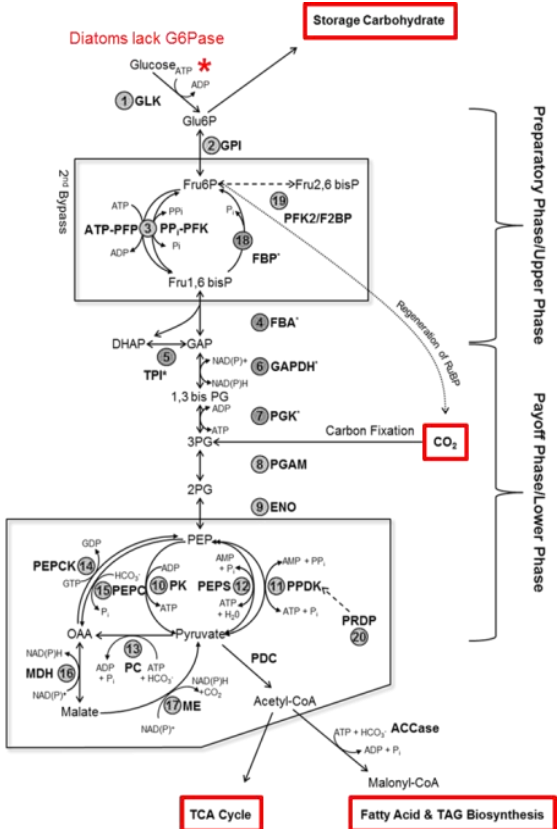
*Fragilariopsis
cylindrus*

Comparison of the Predicted Intracellular Location of Core Carbon Flux Isozymes Indicate Substantial Differences in Pathways

Diatoms

	<i>T. pseudonana</i>	<i>P. tricornutum</i>	<i>F. cylindrus</i>	<i>C. reinhardtii</i>
GLK	1	1	1	1
GPI	3 1	3 1	3 1	1
PFK	2	3 1	2	?? 1 ??
FBPase	1 2	1 2	2 5	1
ALDO	2 2	2 3	2 4	2 1 1
TPI	1 ^a 2 2	1 ^a 1 2	1 ^a 1+? ^b 3+? ^b	1
GAPDH	1 2 1	1+1 ^a 3 1	1+1 ^a 2 2	1 1 2
PGK	1 4 1	1 1 1	1 2 1	1 1
PGAM	4 2+? 2+?	5 2 3	1 4+?? 1+??	1
ENO	1 1	1	2	1
PK	2 2 1	2 4 1	2 1 2	4 1

Mitochondria
 Cytosol
 Chloroplast
 No enzyme



Key Findings:

1. The fundamental process of glycolysis is not conserved in different algal species.
2. Intercompartmental transport of metabolites will be an important regulatory process.
3. Diatoms are actively sampling carbon flux genes from different evolutionary sources.

Substantial Differences in Metabolic Organization and Processes Comparing Evolutionarily-diverse Algae

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Current Opinion in
Chemical Biology

Metabolic and cellular organization in evolutionarily diverse microalgae as related to biofuels production

Mark Hildebrand¹, Raffaella M Abbriano¹, Juergen EW Polle³,
Jesse C Traller¹, Emily M Trentacoste²,
Sarah R Smith¹ and Aubrey K Davis¹

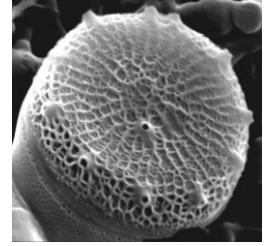
Key Findings:

1. Fundamental photosynthetic and metabolic processes substantially differ in different algal classes, particularly comparing primary with secondary endosymbionts. Limited data indicates that the efficiency of processes differ in different classes of microalgae.
2. Nature has “experimented” with different arrangements of carbon fixation and carbohydrate storage throughout evolutionary history. We hypothesize that this maximized overall carbon processing efficiency under prevalent environmental conditions.
3. Generalizations of metabolic or photosynthetic capabilities of “algae” should not be made.
4. We propose that comparative analysis of sub-steps in carbon flux across evolutionarily-divergent microalgae will provide invaluable insight into the relative efficiency of processes, leading to the development of optimized arrangements in organisms and synthetic systems.

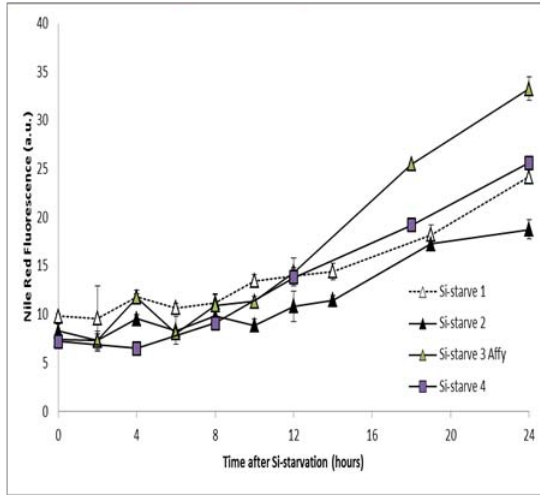
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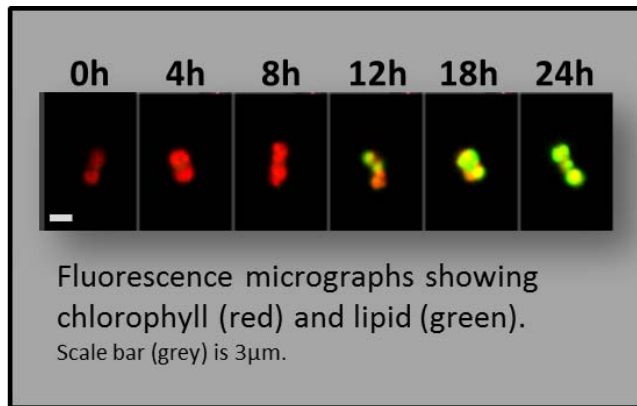
Fundamental Analysis of Short-term Lipid Accumulation Resulting from Silicon Limitation in *T. pseudonana*



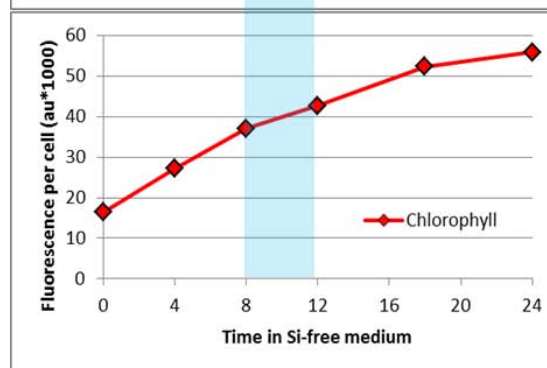
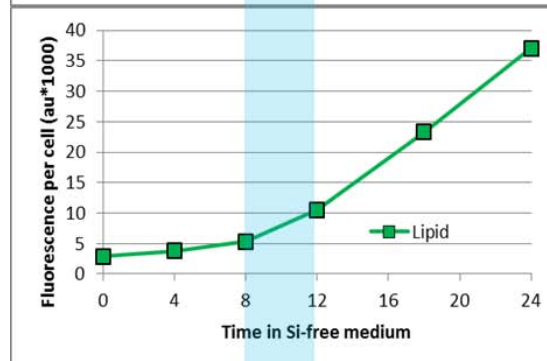
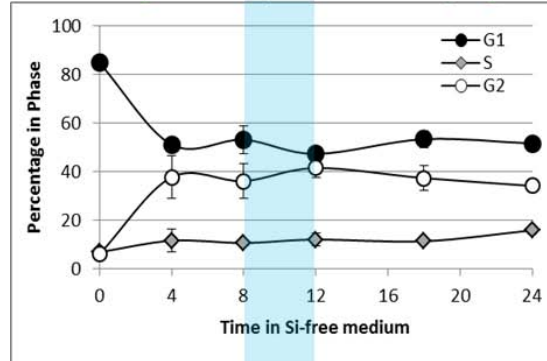
Reproducibility of Lipid Accumulation



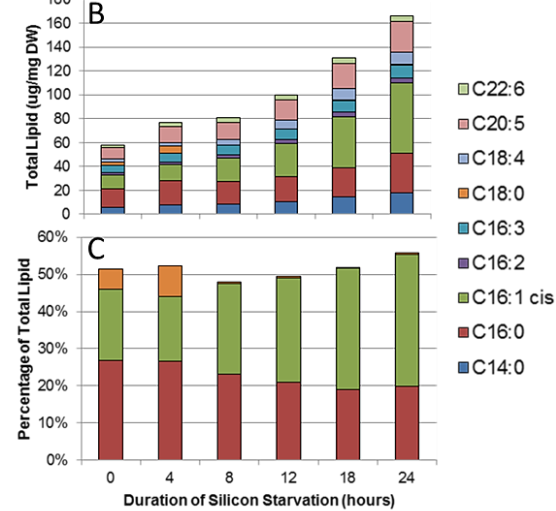
Microscopy



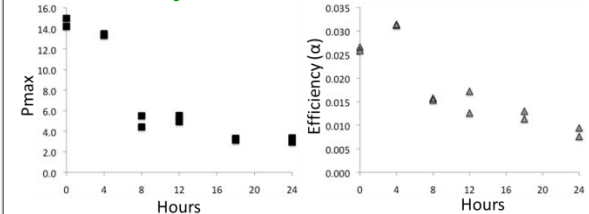
Cell Cycle, Lipid, Chlorophyll



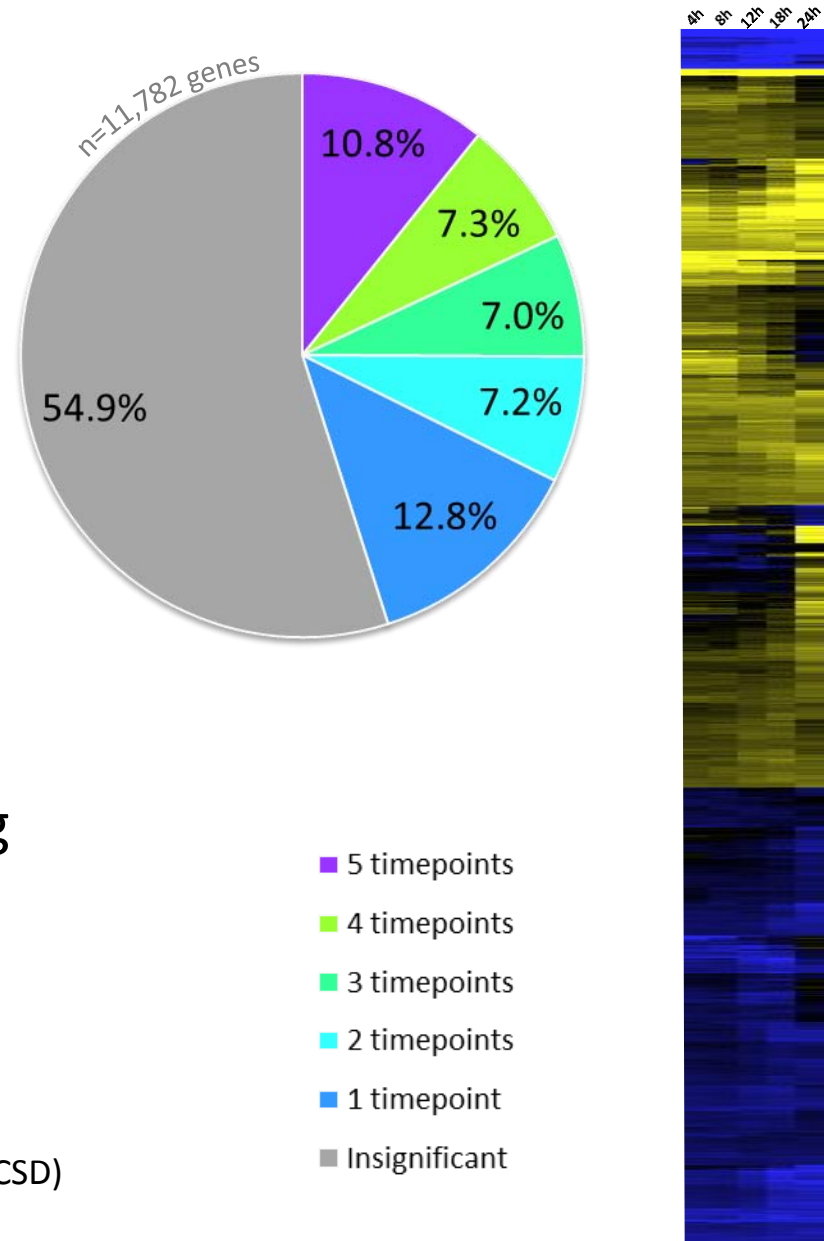
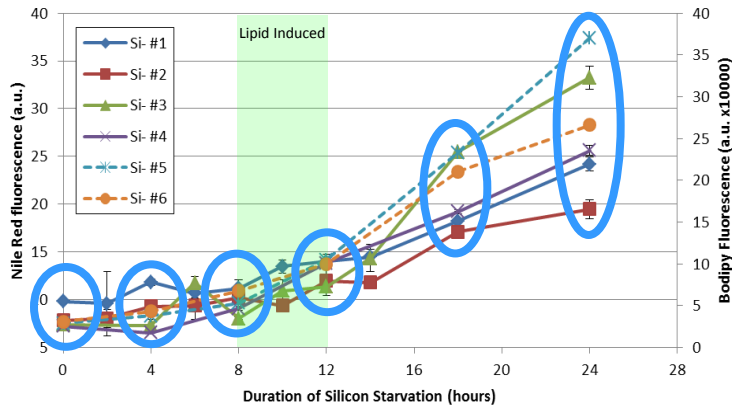
Fatty Acids



Photosynthesis

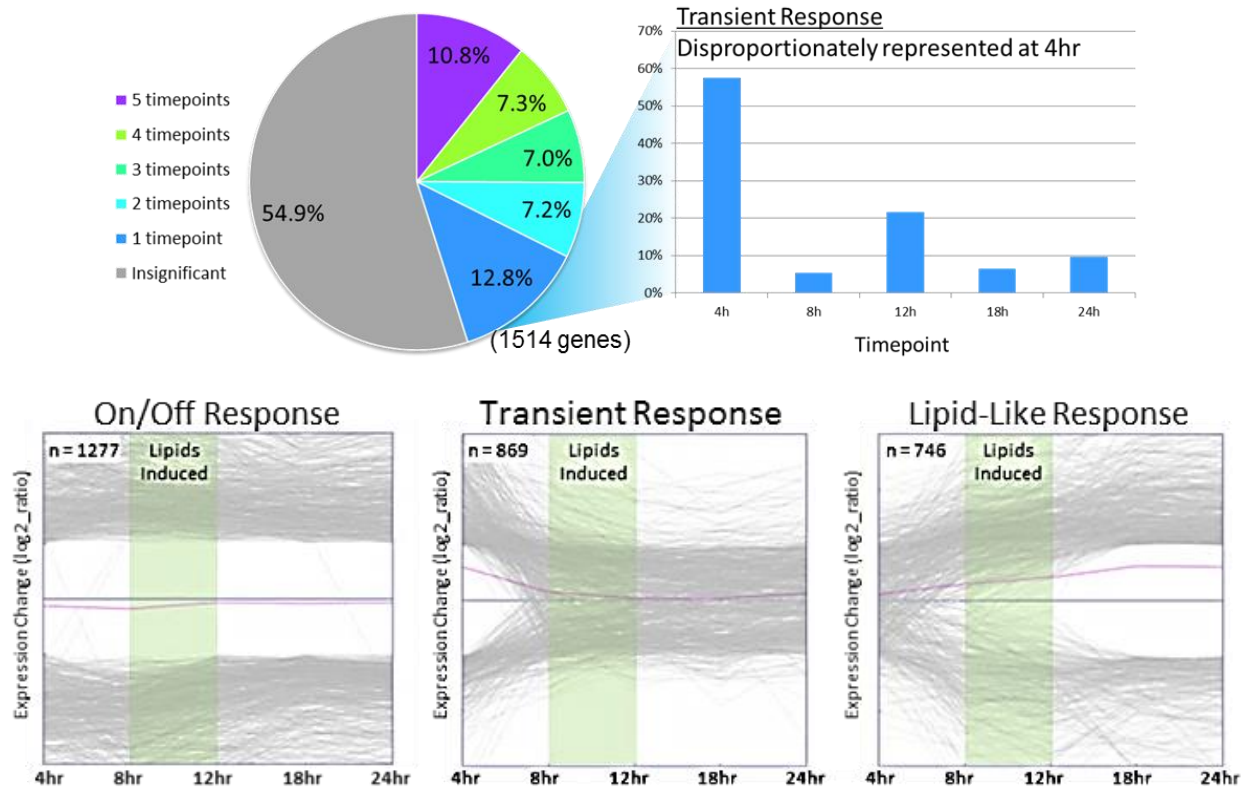


Whole-Genome mRNA Expression Using Microarrays



- RNA isolated from 6 timepoints (t=0h, t=4h, t=8h, t=12h, t=18h, t=24h)
- Affymetrix whole-genome tiling microarray in duplicate (Andy Allen, JCVI)
- Bioinformatics (11,782 genes) (Trina Norden-Krichmar, JCVI & Steve Federowicz, UCSD)

Transient Responses and Coordinate Transcriptional Regulation are Prevalent

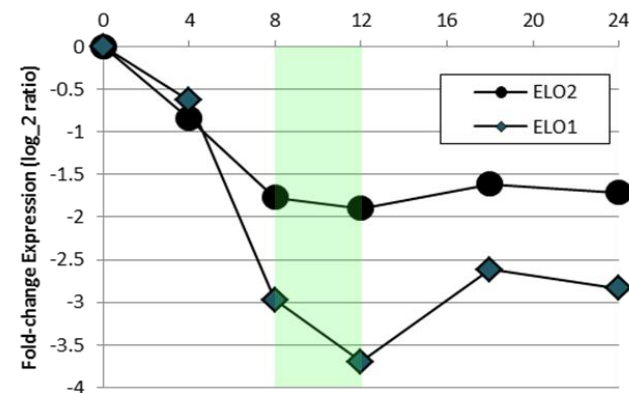
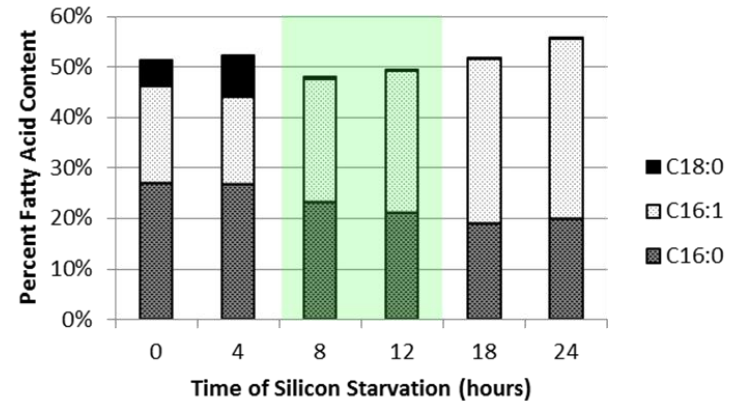


Key Findings:

1. 45% of the transcriptome was significantly up- or down-regulated during the process.
2. Transient responses were predominant at 4 hours.
3. Global alteration in transcript levels was common, suggesting “master” regulators.
4. Transcript level changes correlated with metabolic changes, indicating that the former is an accurate proxy for monitoring changes in cellular metabolic processes.

Changes in Gene Expression and Physiology Can Be Correlated

- Shift in the chain length of fatty acids prior to the onset of lipid droplets
 - C18:0 (4hr: 8.3% → 24hr: 0.3%)
 - C16:1 (4hr: 17.4% → 24hr: 35.5%)
- Shift is coincident with a decrease in the expression of elongases

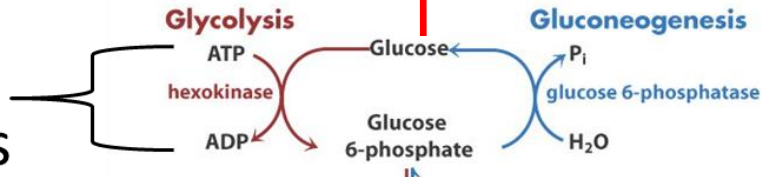


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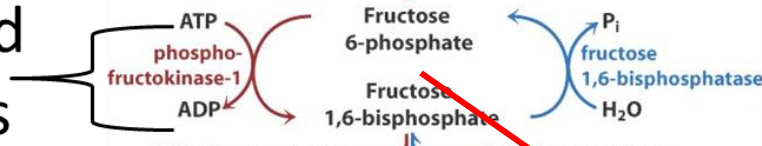
1. Gene expression data correlates with physiological changes.

Elucidation of Carbon Flux Processes: Transient Induction of Gluconeogenesis Genes

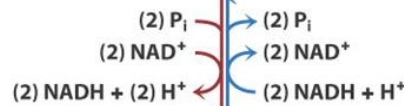
Third Bypass



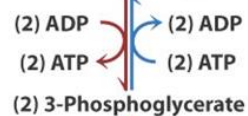
Second Bypass



(2) Glyceraldehyde 3-phosphate

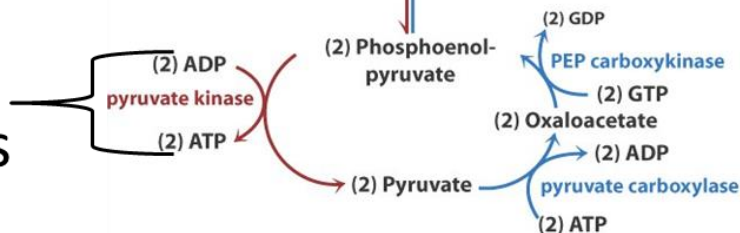


(2) 1,3-Bisphosphoglycerate

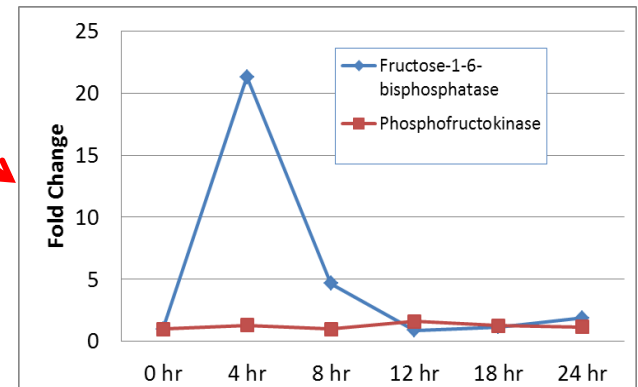
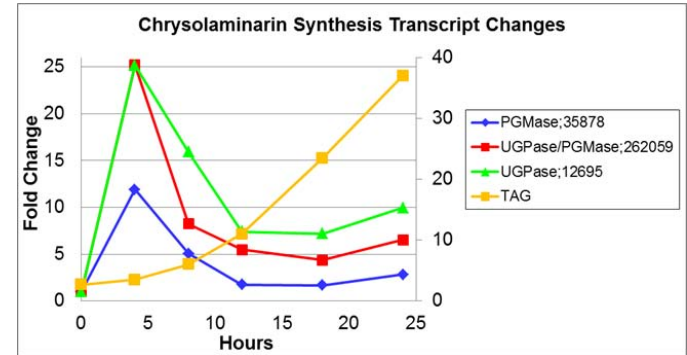


(2) 2-Phosphoglycerate

First Bypass



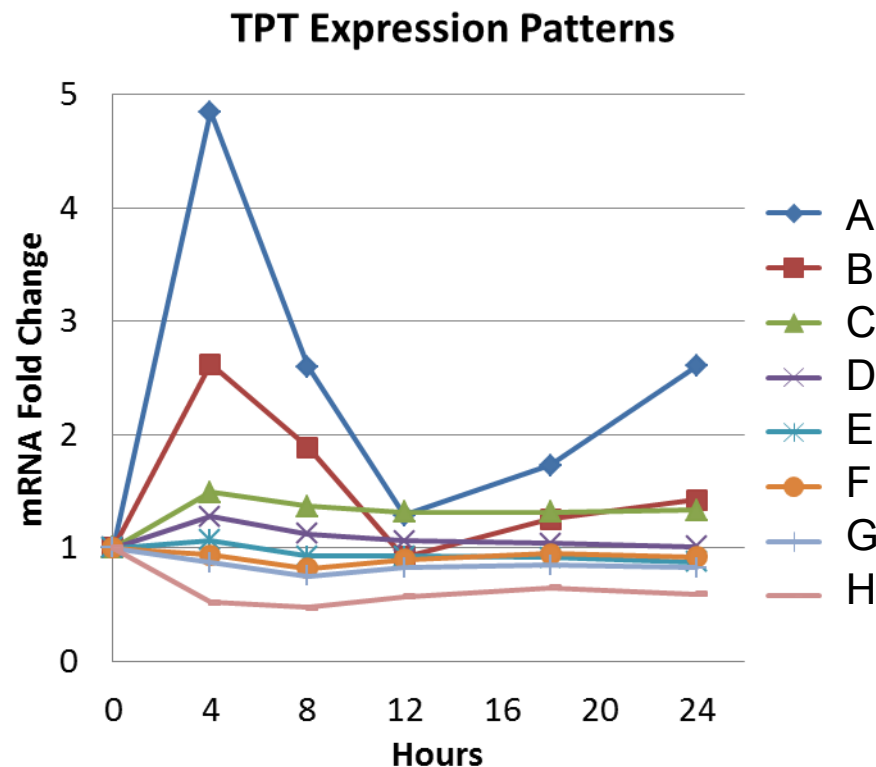
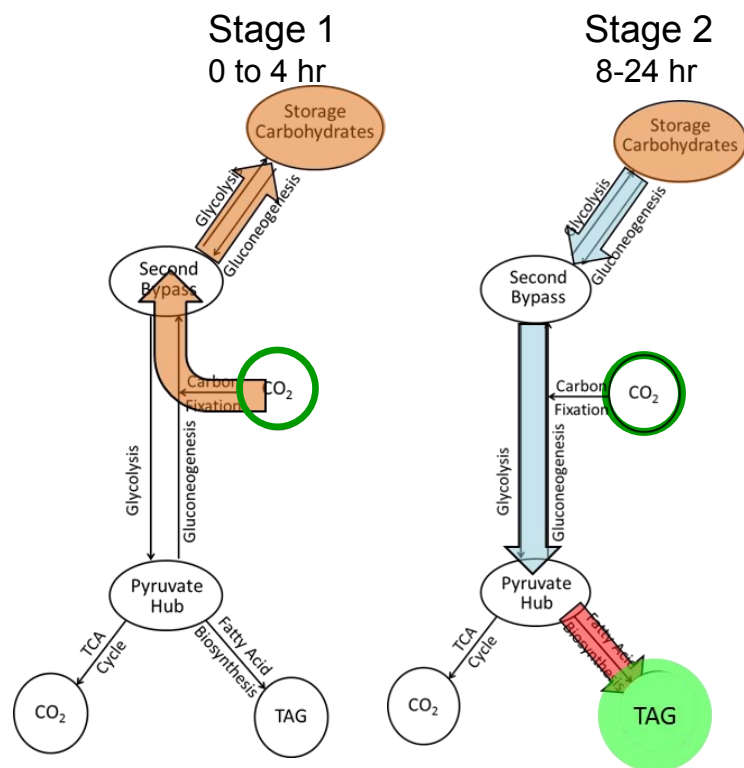
Storage Carbohydrate Genes



Key Finding:

1. The initial response to Si starvation is induction of gluconeogenesis and carbohydrate storage, followed later by TAG accumulation.

Expression Patterns Can Be Used to Identify Key Proteins



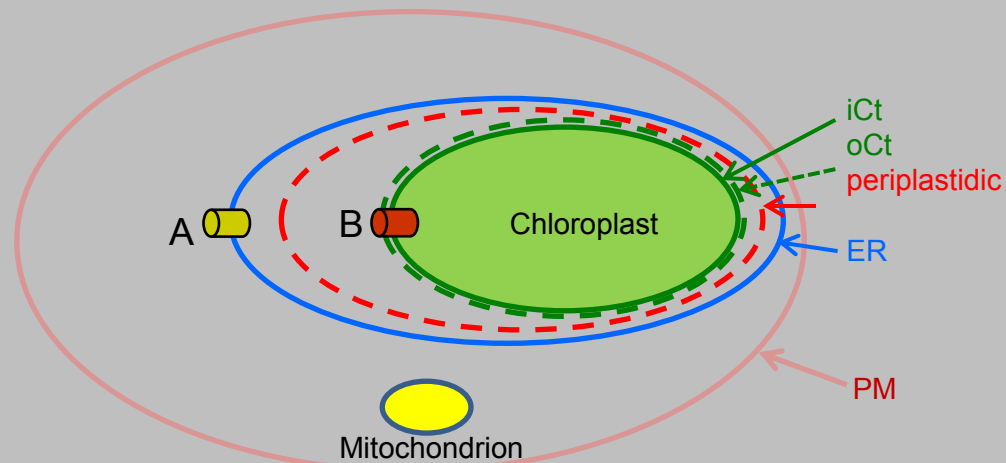
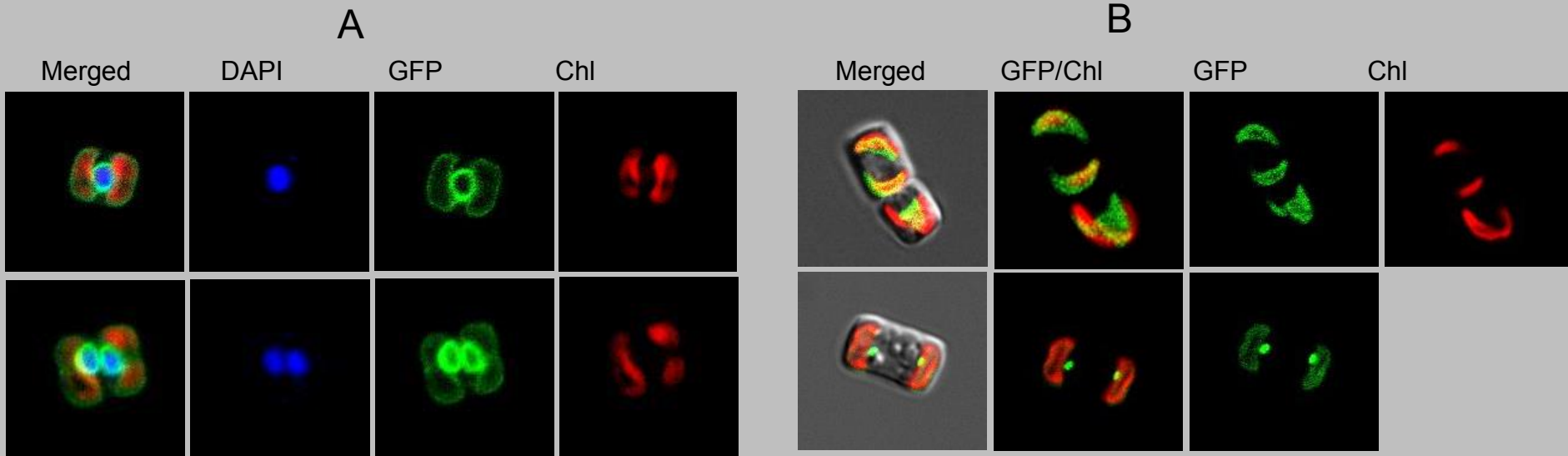
Triosephosphate translocators (TPTs) are responsible for carbon efflux from the chloroplast.

There are 8 TPT homologs in *T. pseudonana*.

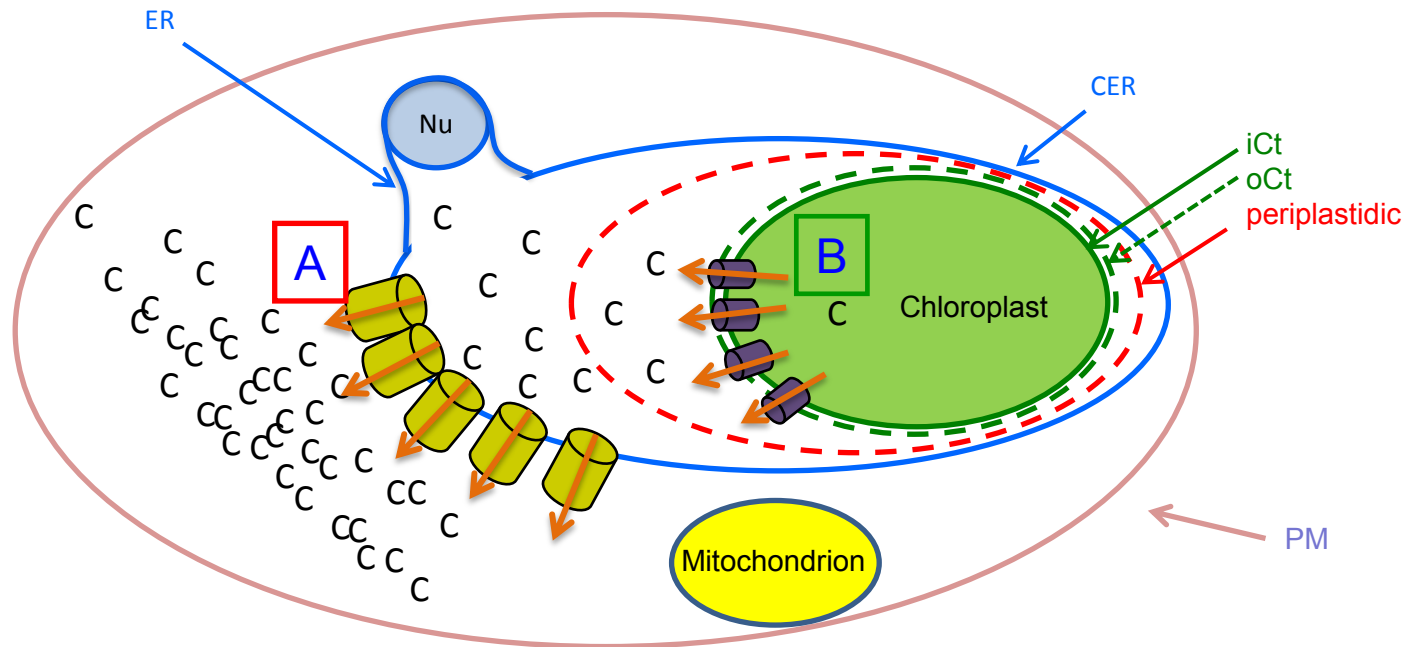
Substrate specificity cannot be determined by sequence comparisons.

Which are involved in export of carbon from the chloroplast? The two most highly upregulated at 4hr?

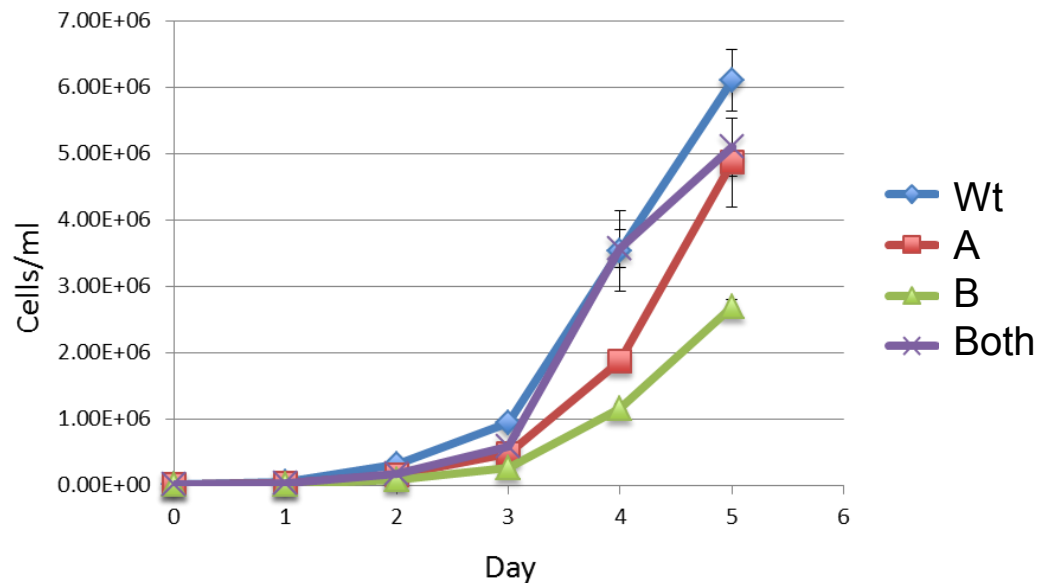
Intracellular Localization of Two Diatom Triosephosphate Translocators



Overexpression of TPTs in Transgenic *T. pseudonana* Affects Growth



Growth Comparisons (n=3 each)



Key Findings:

1. Transcript data can be used to identify key proteins involved in carbon flux.
2. Excellent candidates for translocators that export carbon from the chloroplast have been identified.
3. Manipulations of the translocators result in a phenotypic effect on growth.

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C. cryptica: A Model Lipid Accumulating Species

(collaboration with Pellegrini and Merchant labs, UCLA)

The top diatom species from the Aquatic Species Program, candidate production strain (grown outdoors).

Genome

- Assembly finished (150 Mbp), finalizing gene models

Transcriptome

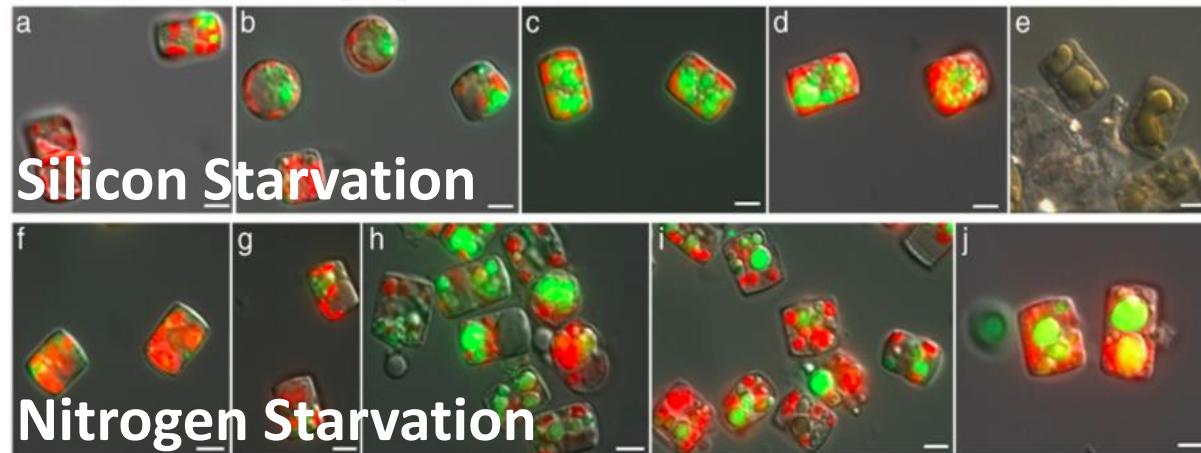
 -13 different libraries

- Nitrogen Starvation
- Silicon Starvation

Proteome - significant increases in key carbon metabolism enzymes

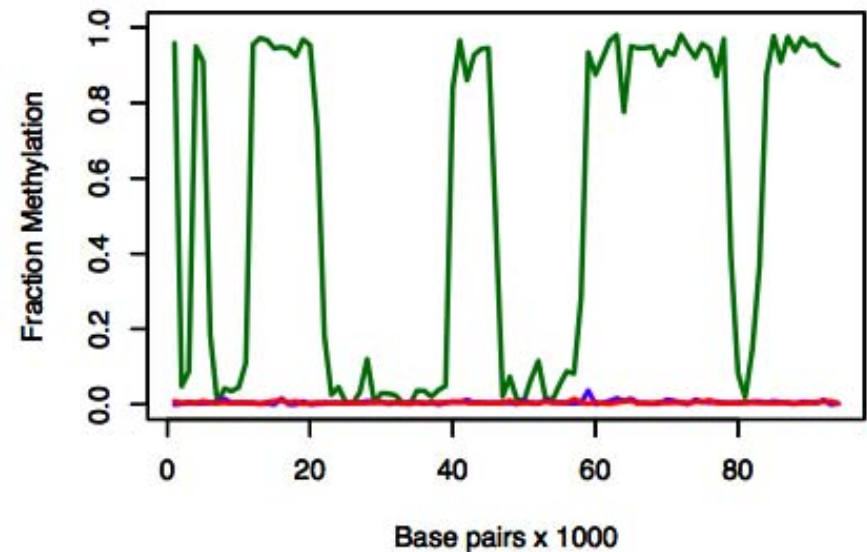
- ACCase (plastid localized)
- Pyruvate kinase (cytosolic)
- Enolase (plastid)

Methylome - ~53% of genome is methylated



Traller and Hildebrand, Algal Res. in press

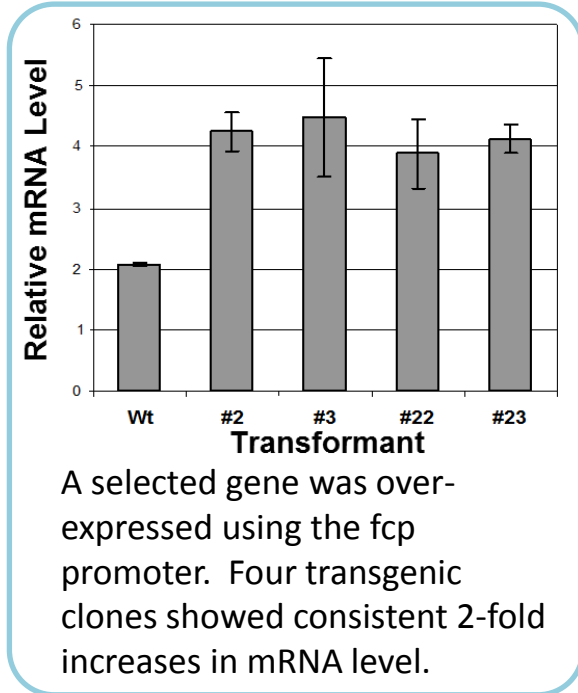
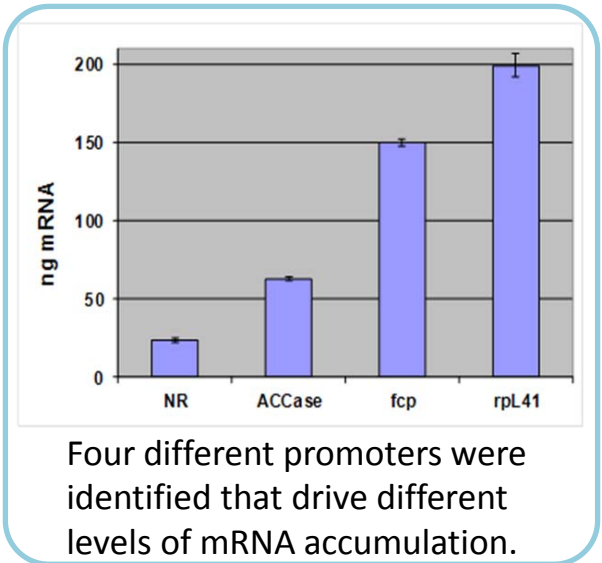
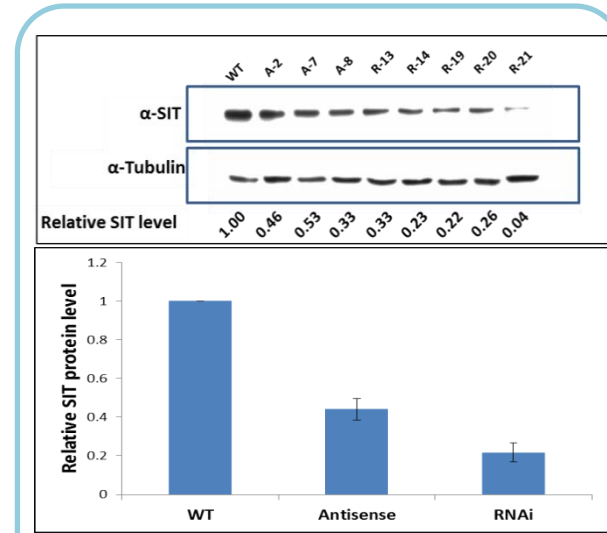
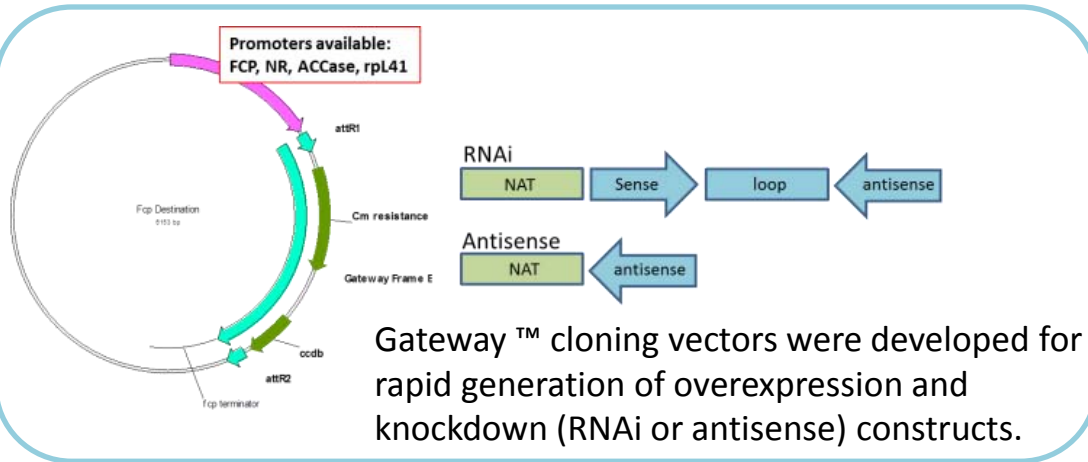
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1 - Approach

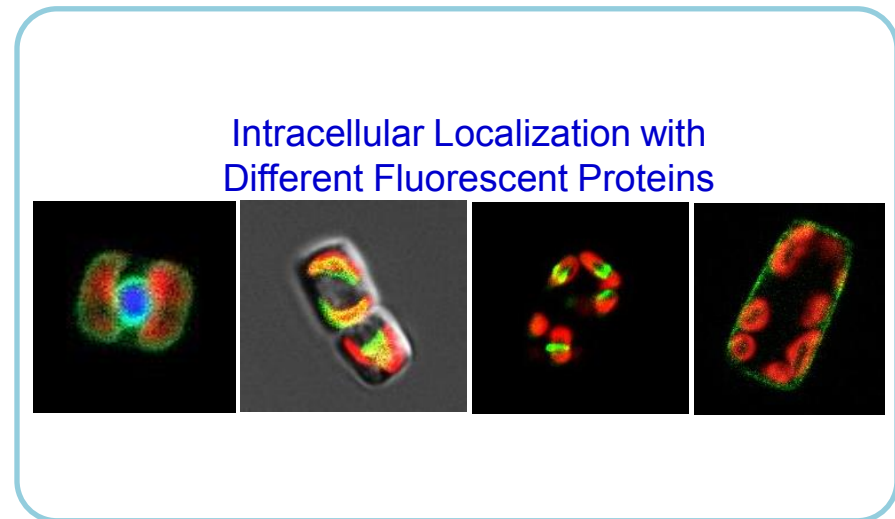
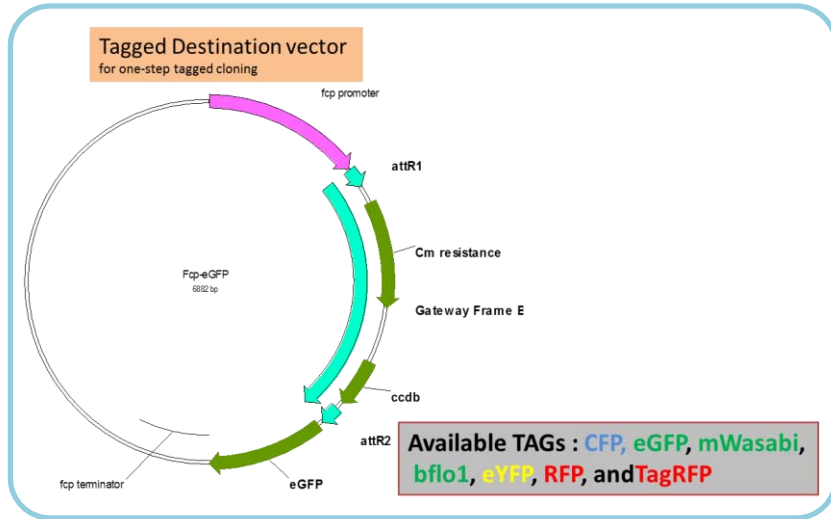
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Development of Diatom Genetic Manipulation Tools: Overexpression and Knockdowns



Silicon transporters (SIT) were knocked down using antisense or RNAi. On average RNAi worked better, and consistent levels of 25% expression compared to wild type were achieved, with an exceptional knockdown achieving 4%. Phenotypic effects consistent with knockdown have been observed.

Development of Diatom Genetic Manipulation Tools: Fluorescent Tagging and New Selectable Markers



Localization to the cytoplasm, chloroplast, chloroplast membrane, periplastid membrane, ER, plasma membrane, pyrenoid, mitochondria, cell wall, and possibly peroxisome have been demonstrated.

Previous to this project only one selectable marker (nourseothricin resistance) was available for *T. pseudonana*. We have developed two additional markers, for zeocin resistance (generated by codon optimization), and glyphosate resistance (interaction with the Weeks lab at UNL).

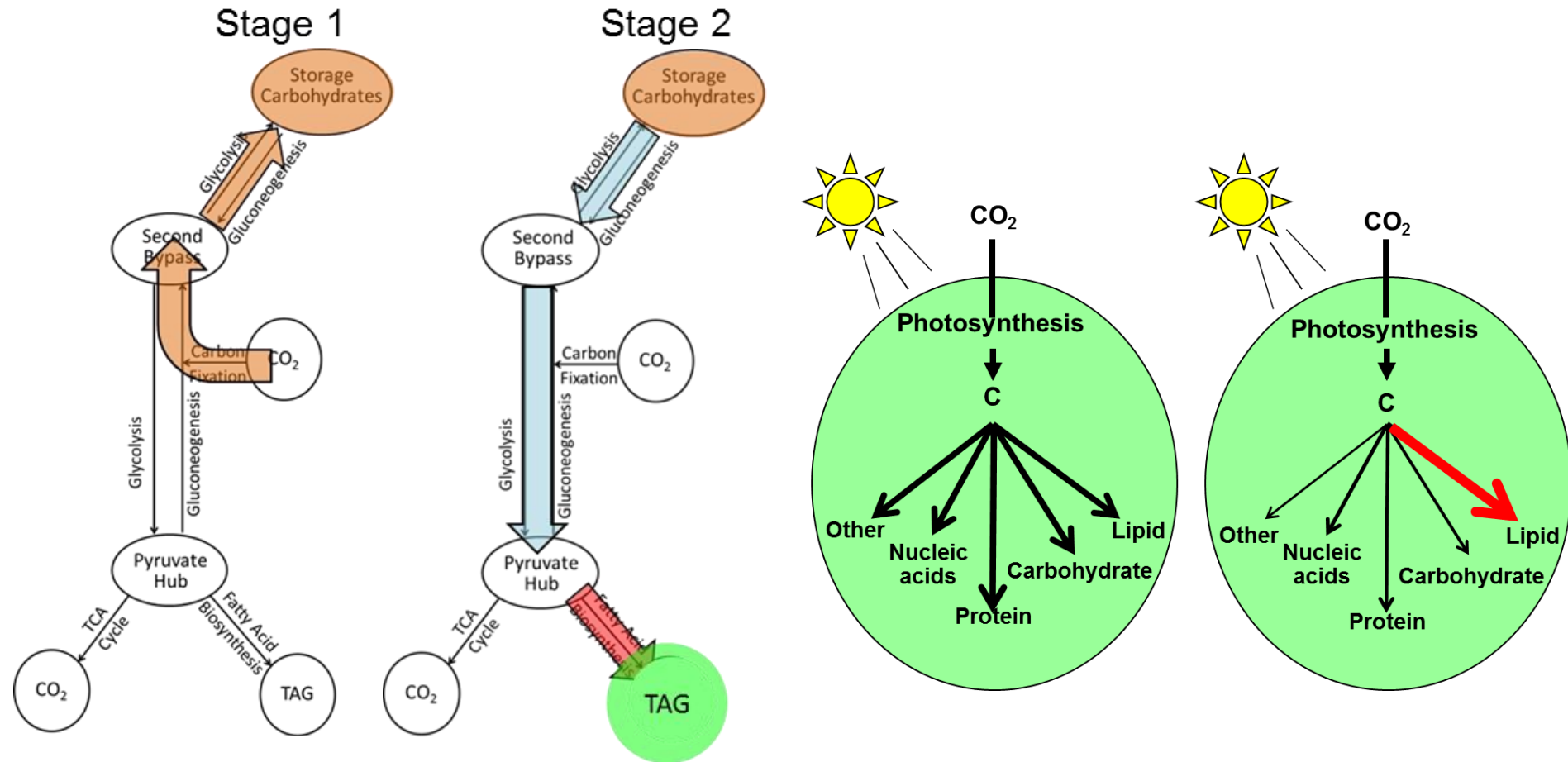
Key Advances:

1. A versatile set of genetic manipulation tools has been developed, bringing the technology for diatoms on a par with other model organisms.
2. We are defining intracellular membrane systems in detail for the first time in a secondary endosymbiont, which enables understanding of cellular compartmentation as related to carbon and other fluxes.
3. The technology has changed our fundamental approach from observation to direct testing of hypotheses via manipulation.

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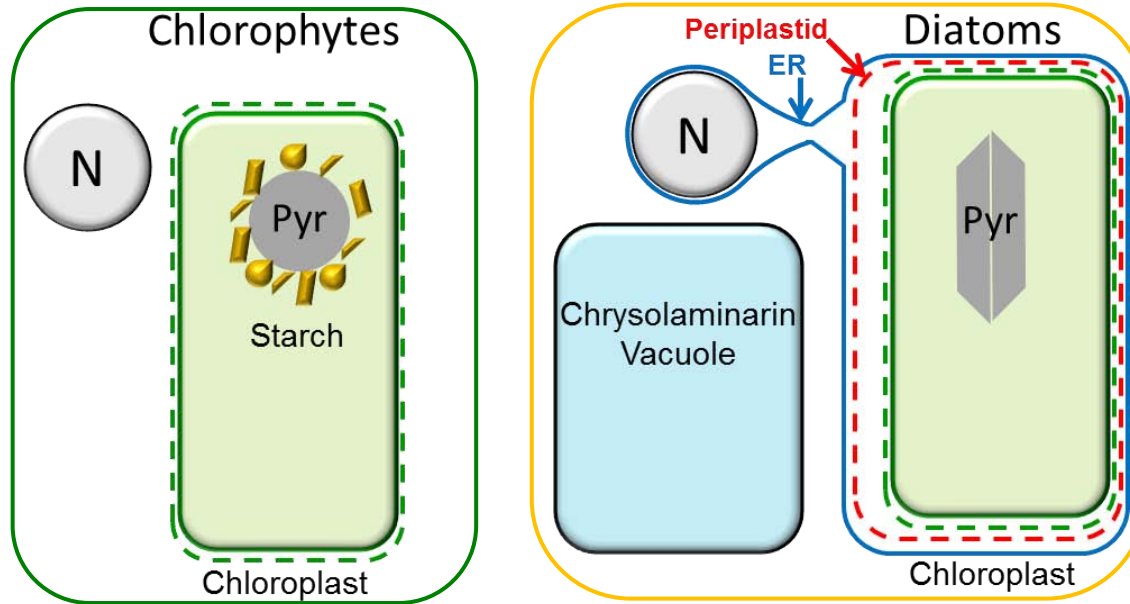
Rationale for Carbon Flux Manipulation



Previous data (Aquatic Species Program) indicate that the partitioning of carbon flux is a primary determinant of lipid accumulation.

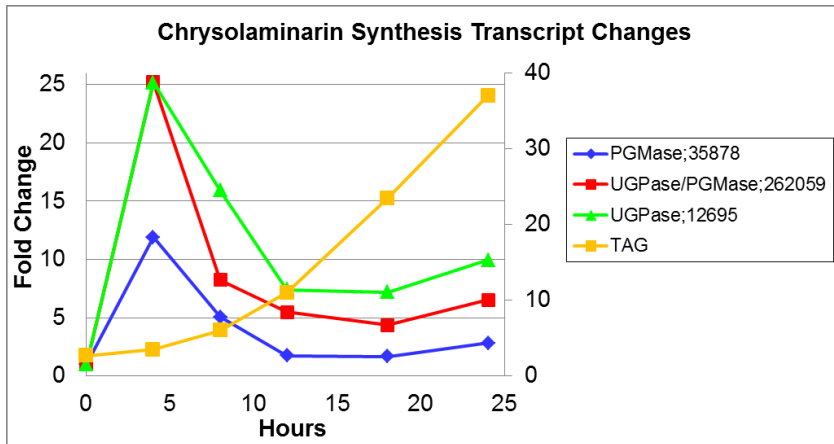
Hypothesis: Reducing the flux to competing pathways should improve lipid accumulation.

Diatom Carbohydrate Storage

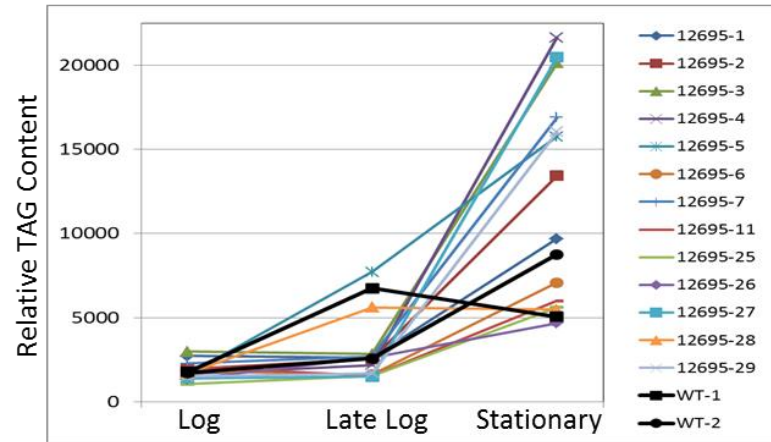


Diatoms store carbohydrates as a soluble β -1,3-linked glucan called chrysolaminarin outside of the chloroplast in the chrysolaminarin vacuole.

Knockdown of a Chrysolaminarin Synthase Gene Improves TAG Accumulation



Three genes encoding steps in chrysolaminarin synthesis are transiently upregulated during silicon starvation

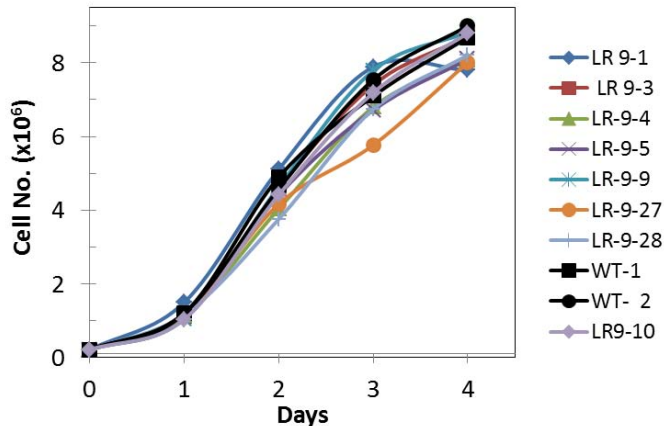


Several 12695 knockdown strains exhibit improved TAG accumulation

The best knockdowns resulted in 1.5-3 fold improvement in TAG levels over wild-type.

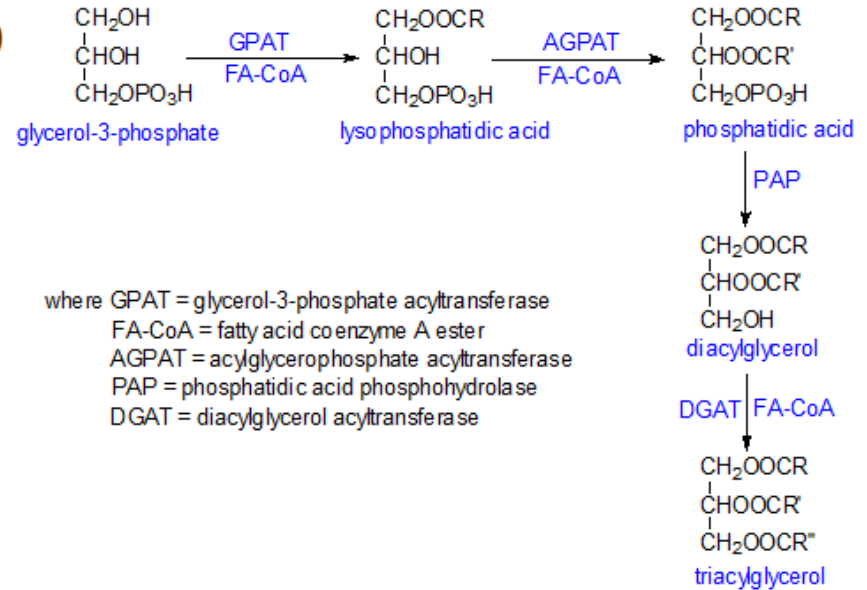
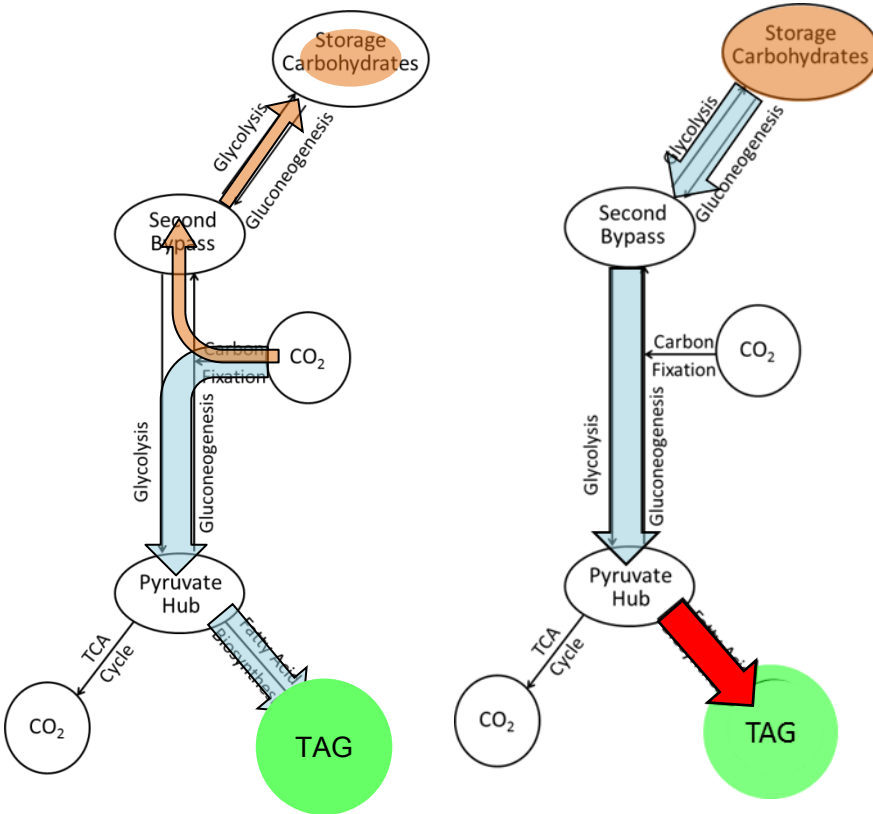
We are characterizing knockdowns in the two other genes and generating simultaneous knockdowns in multiple genes.

Key Finding:
Inhibition of carbon flux to a competing pathway (carbohydrate storage) improves TAG accumulation, in some cases without detriment to growth.



Most strains grow as well as wild-type

Overexpression of a DGAT Improves TAG Accumulation

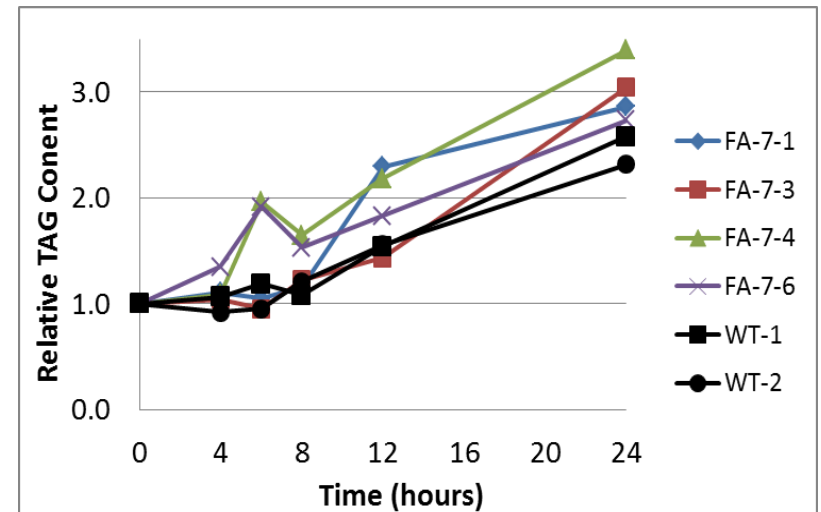


Overexpression of DGAT resulted in 1.2-1.5 fold improvement in TAG levels over wild-type.

We are characterizing other enzymes involved in TAG synthesis and have generated overexpression strains.

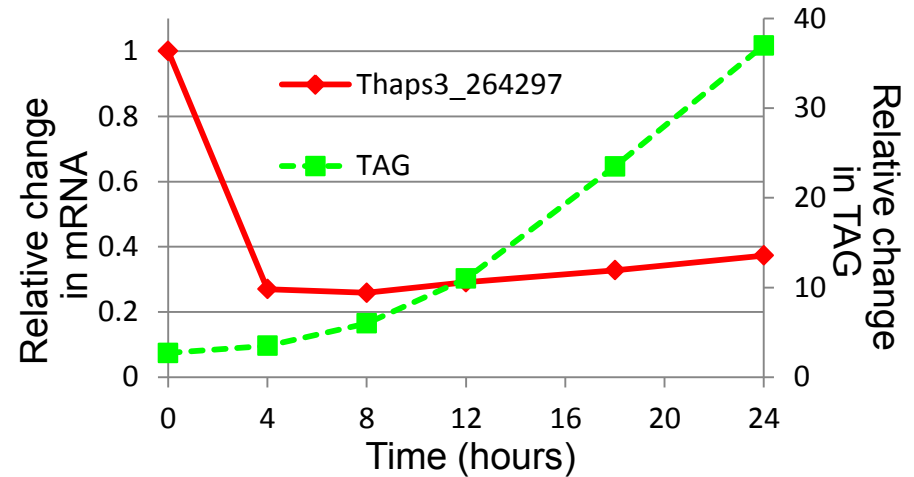
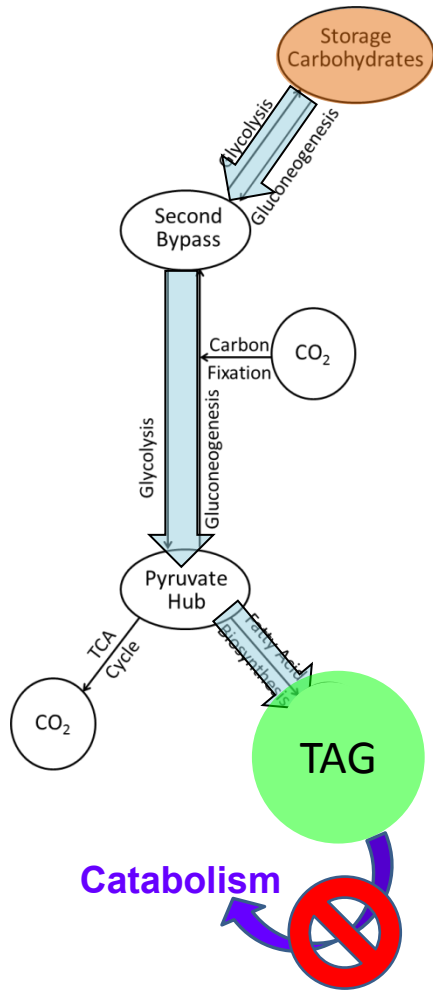
Key Finding:
 Increased TAG accumulation results from overexpression of a TAG synthesis enzyme.

DGAT overexpression strains



Characterization of CGI-58 Lipase Homologs

Emily Trentacoste, Jennifer Hull, Bill Gerwick



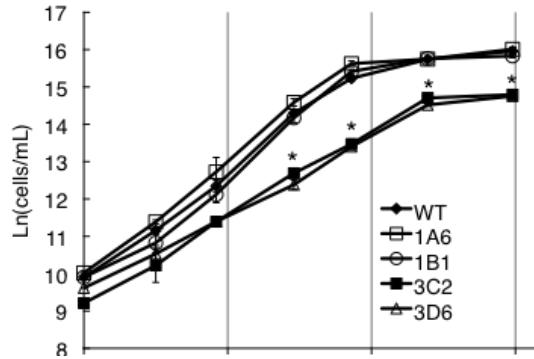
CGI-58	TPLVLLHGFGGGGLGLWALNFGDLCNRP--VYAFDLLGFGRSSRPFDS-----	122
ABHD4	TPLVVHGHGGVGLWILNMDLSARRT--LHTFDLLGFGRSSRPAPFR-----	115
At4g24160	PTLVVHGHGASQGFRRNF DALASRFR--VIAIDQLGWGSSRPFDCR-----	168
Thaps3_264297	APLVLLHGFIANGSLYFYRNLMGLSHFHFSGSIYALDMLGWGLSSRPTFDLQLLGDNDG--	158
Ict1p	TPTVLLHGFIYAASSMAFYRTFENLSDNIK--DLYAIDL PANGASEAPALQVNKTKKIKSLRF	129
CGI-58	-----DAEEVENQFVESIEENRCALGLDKMILIGHNLGQFLA	159
ABHD4	-----DPEGADEFVTSIETWRETMGIPSMILIGHSLGQFLA	152
At4g24160	-----STEETEAWFIDSFEEWRKAQNLNSFILLGHSGFGYVA	205
Thaps3_264297	-----TNDDKRSNEHKQVASAEHFFVESLESWRKQHDLPKITLAGHSMGGYLS	206
Ict1p	KHIEDDVVIPVIEKRPPAEDIKSHLEQYESYFVDRIEQWRKDNKLRKINVVGHSGFYIS	189
CGI-58	-----RPHSYVKTIAILGACHYVYAD-----QPEEFNQK	340
ABHD4	-----RPDSYVRDMEIKGASHHVYAD-----QPHIFNAV	333
At4g24160	-----SMKVPCEIIRVPQGHFVFD-----NPIGFHSA	392
Thaps3_264297	KRTEWEKQNNNESPPPKVFLHGVRNACHLLMLDYYEEFN SALI IAAGGEDRLPSNFRP	433
Ict1p	-----MLKNKAKASYVEVPDACHNLFID-----NPQHFASS	387

CGI-58 knockdowns in *Arabidopsis* and mutations in humans resulted in high TAG accumulation

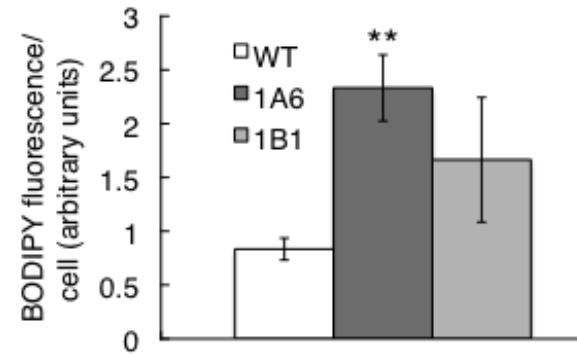
Characterization of CGI-58 Lipase Homolog Knockdowns

Emily Trentacoste, Jennifer Hull, Bill Gerwick

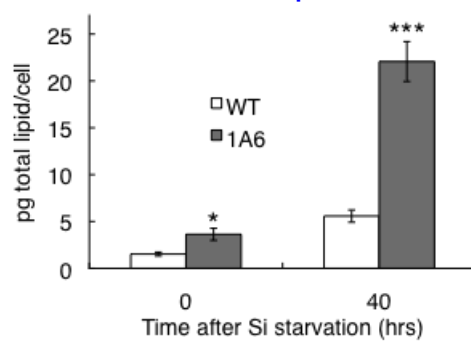
Growth Curves



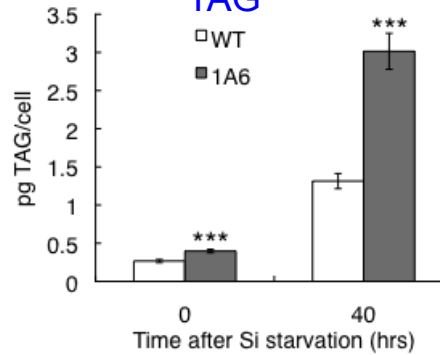
BODIPY



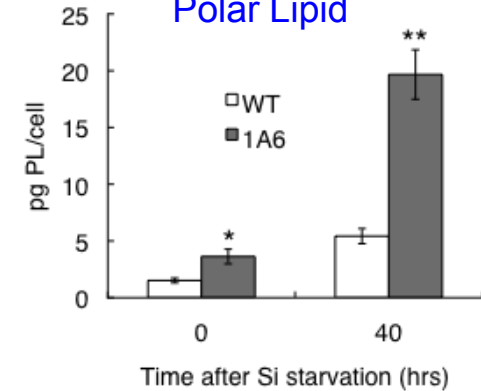
Total Lipid



TAG



Polar Lipid



Key Finding: CGI-58 homolog knockdowns improve both TAG and polar lipid content with (in antisense cases) no detrimental affect on growth.

TAG was increased 1.9 - 4 fold

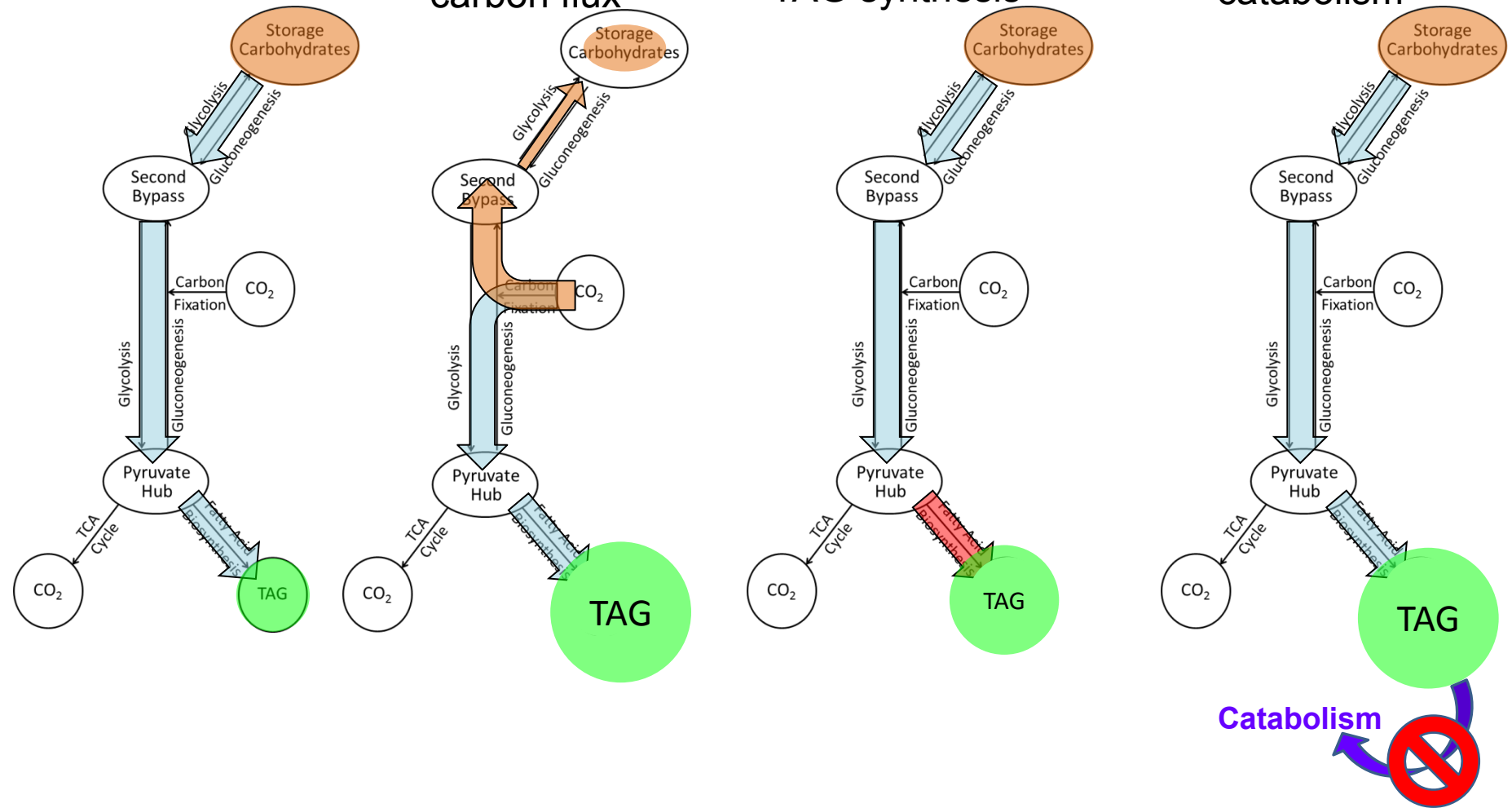
"1" = antisense
"3" = RNAi

Key Finding: A Variety of Manipulations Lead to Increased TAG

Redirecting
carbon flux

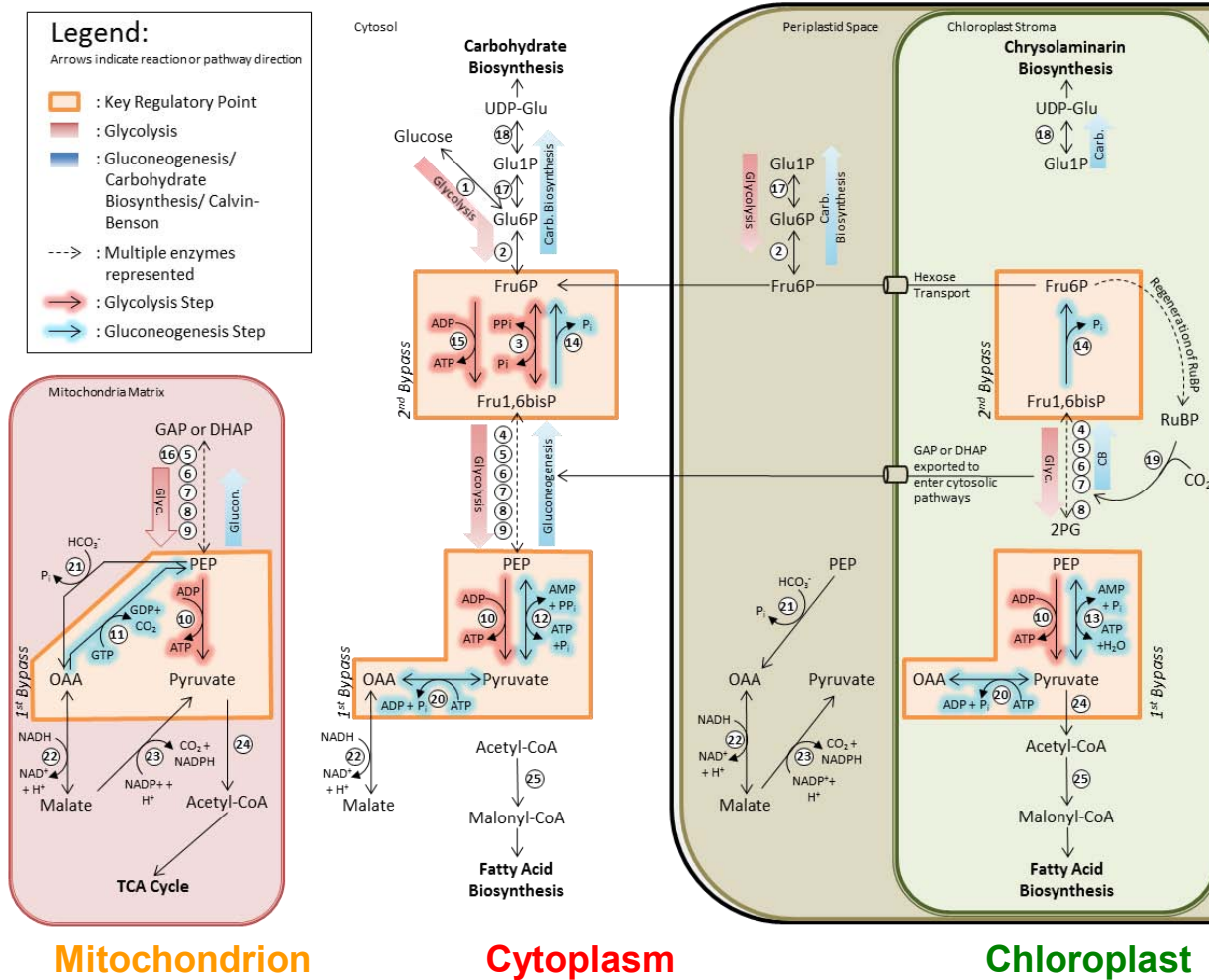
Increasing
TAG synthesis

Inhibiting lipid
catabolism



Flexibility in choosing gene targets could minimize detrimental effects and maintain fast growth and high biomass accumulation

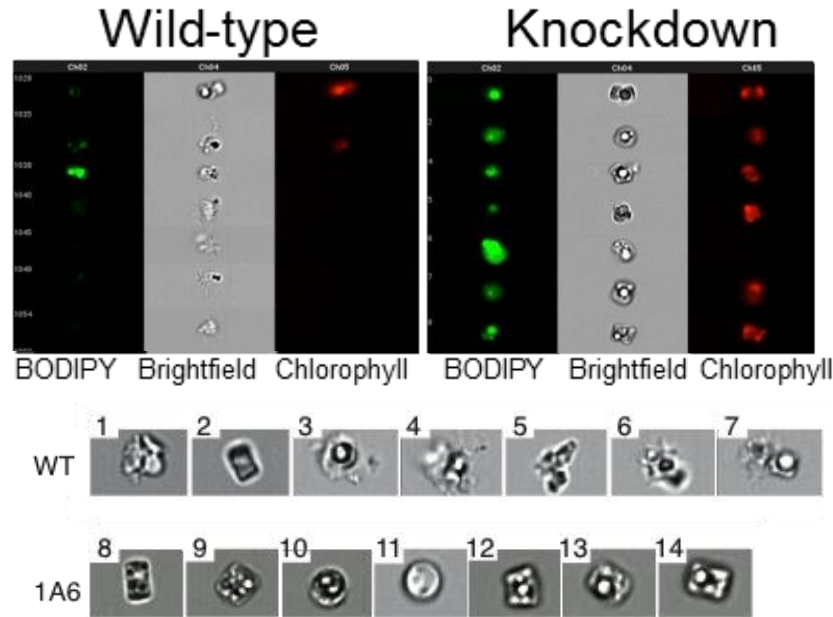
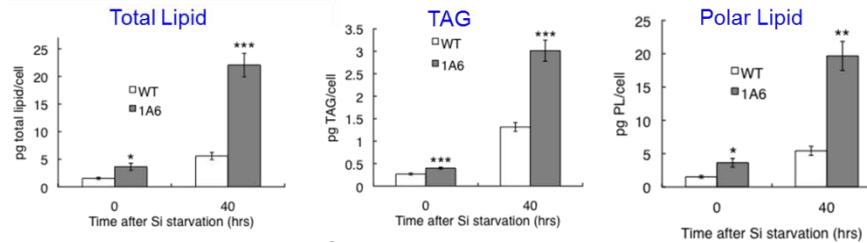
There are Many More Gene Targets to Consider



Not only specific metabolic steps, but compartmentation needs to be considered

Characterization of CGI-58 Lipase Homologs: Improved Lipid Content Plus a Non GMO Selection Approach

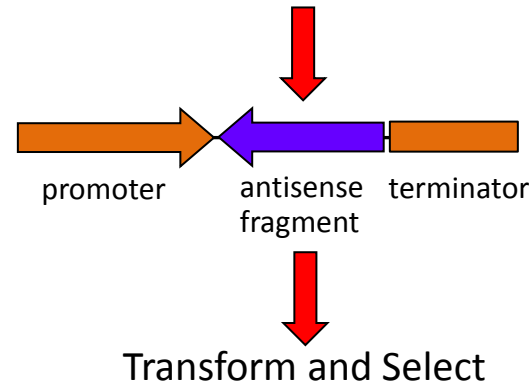
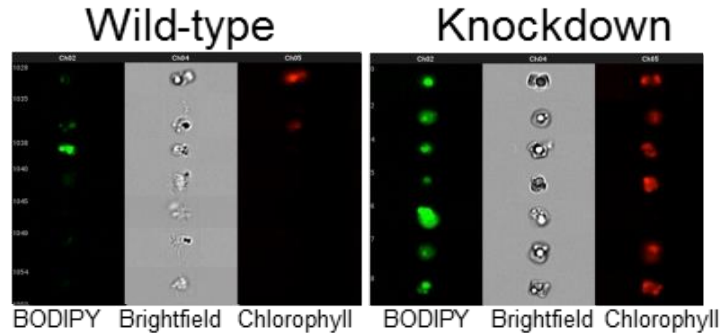
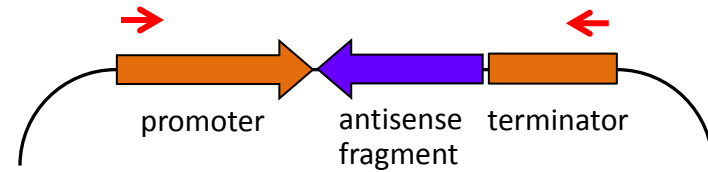
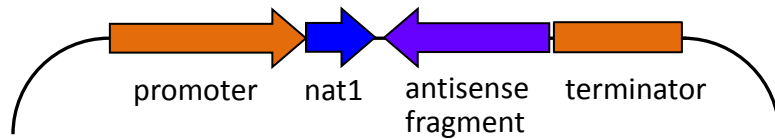
Emily Trentacoste, Jennifer Hull, Bill Gerwick



Knockdowns exhibited a phenotype of increased TAG plus increased cell viability under Si-limitation conditions.

Increased viability plus increased fluorescence (BODIPY and Chlorophyll) are selectable phenotypes.

Native Sequence, Non-GMO Selection Approach



Organisms are classified as GMOs only if DNA from another genus is introduced. Theoretically, through recombination and cross-over such an arrangement could be generated by the native host.

Transformants generated this way will not be classified as GMOs, can be selected, and may have improved viability under stress than wild-type.

Additional genes can be manipulated by co-transformation.

Key Finding: Application of genetic manipulation approaches has led to the possibility of native sequence-based metabolic engineering for field applications with no regulatory issues.

3 - Relevance

- ***The mission of the Biomass Program (the Program) is to:
Develop and transform our renewable biomass resources into cost-competitive, high-performance biofuels, bioproducts, and biopower through **targeted research**, development, demonstration, and deployment supported through public and private partnerships.***
- ***This project (Award DE-EE0001222) relates to Conversion R&D to develop technologies for converting feedstocks into cost-competitive liquid transportation fuels.***

4 - Critical Success Factors

- **Critical success factor**

Successful deployment of metabolically-engineered strains in production systems with maintenance of productivity.

- **Challenges:**

1. *Adapt the technology to production strains (being done, *C. cryptica* has been deployed in outdoor systems).*

2. *Continue research to identify manipulations that provide the highest lipid yield with no negative impact on growth and biomass accumulation.*

- **Demonstrate that success of the project will advance the state of technology and positively impact the commercial viability of biomass and /or biofuels**

Technoeconomic analyses indicate that improvement of lipid content is the single most important factor in reducing the cost of the biological component of biofuels production. (Davis et al. 2011. Appl. Energy 88:3524-3531).

5. Future Work

- *Project end date will come prior to a complete investigation of gene targets for improved performance. Additional funding is needed!*
- *We plan to continue manipulating genes in different aspects of carbon metabolism to provide an overview of critical metabolic processes required for high lipid accumulation coupled with a detailed look at specific aspects within a given metabolic process.*
- *Upcoming milestone – demonstrate that multiple manipulations result in “trait stacking” for improved performance.*
- *Decisions forward will be made based on the data obtained, manipulations that show the most improvement will be pursued in more detail.*

Summary

Three major goals of the project were to:

1. Understand fundamental processes of carbon flux and partitioning in diatoms.
2. Develop a versatile set of genetic manipulation tools.
3. Develop the ability to manipulate carbon partitioning for abundant lipid accumulation coupled with high biomass accumulation.

All three goals were achieved, but more can be done.

In addition, novel insights were gained into:

1. Evolution-based differences in fundamental metabolic processes in microalgae.
2. The contribution of compartmentation to intracellular carbon flux and processing.
3. The relation between transcriptomic data and changes in cellular metabolic processes.
4. The intracellular membrane organization of a secondary endosymbiont.
5. The importance of lipid catabolism on overall cellular lipid status.

A major technical advance is the development of an approach to use native sequences to metabolically engineer the cell, avoiding a GMO classification.

Targets identified at the 2011 Program Review were to demonstrate that metabolic engineering could improve TAG accumulation. The goal was achieved by this review.

Additional Slides

Responses to Previous Reviewers' Comments

- The major reviewer's comment to be addressed in the 2011 review was the concept of how valid the approaches would be in a production scenario. Concerns were related to: 1) whether the technology would work, 2) the relatedness of lab-based improvements to production conditions, and 3) whether GMO organisms could be deployed.
- The response to the comments at that time were that 1) the fundamental work needed to be done in order to assess its feasibility, 2) unless improvements were generated in the lab, there would be no possibility for assessment in production systems, and 3) the decision to deploy GMOs was beyond the scope of the project, however based on precedence in crop plants, the prospect was considered positive.
- As a result of the work completed since the 2011 review, we have demonstrated that the approaches work and have generated an approach that could avoid GMO classification. Testing of lab-improved strains outdoors is outside the scope of the current project, but is desired.

Publications and Presentations

Publications

- Traller, JC, Hildebrand M. 2013. Application of high throughput imaging to the diatom *Cyclotella cryptica* demonstrates substantial intrapopulation heterogeneity in the rate and extent of triacylglycerol accumulation. *Algal Research*. Accepted, in press.
- Hildebrand, M, Abbriano, RM, Polle, JEW, Traller, JC, Trentacoste, EM, Smith, SR, Davis, AK. Metabolic and cellular organization in evolutionarily diverse microalgae as related to biofuels production. *Curr. Opin. Chem. Biol.* <http://dx.doi.org/10.1016/j.cbpa.2013.02.027>.
- Smith, S.R., Abbriano, R.M. & Hildebrand, M. 2012. Comparative analysis of diatom genomes reveals substantial differences in the organization of carbon partitioning pathways. *Algal Research*, 1:2-16. DOI: 10.1016/j.algal.2012.04.003. ISSN: 2211-9264
- Hildebrand M, Davis AK, Smith SR, Traller JC, Abbriano R. 2012. The place of diatoms in the biofuels industry. *Biofuels*, 3:221-240.

Presentations

- Seminar speaker, The Development of Diatoms for Nanotechnology And Biofuels Production, University of Nebraska-Lincoln Biotechnology/Life Sciences Seminar Series, Lincoln, NE, April, 28, 2010.
- Seminar Speaker, The Development of Diatoms for Nanotechnology And Biofuels Production, Danforth Plant Science Center, Washington University, St. Louis, MO, May 5, 2010.
- Invited Speaker, POSTECH Symposium, Pohang Institute of Science and Technology, “Development of Microalgal-based Biofuels Using Diatoms as a Model System”, Pohang Korea, Sept. 5-7, 2010.
- Invited Speaker, Fourth Annual Algae Biomass Summit 2010, “Development of Microalgal-based Biofuels Using Diatoms as a Model System”, Phoenix, AZ, Sept. 28-30, 2010.
- Speaker, SD-CAB Algal Biofuels Symposium 2011, “Development of Diatoms as Biofuels Production Organisms”. La Jolla, CA, April 30, 2011.
- Speaker and Session Chair, The Molecular Life of Diatoms Conference, “Development of Diatoms as Biofuels Production Organisms”, Atlanta GA, June 5-9, 2011.

Publications and Presentations

Presentations – cont.

- Invited Keynote Speaker and Session Chair, The First International Conference on Algal Biomass, Biofuels, and Bioproducts, “Development of Diatoms as Biofuels Production Organisms” St. Louis MO, July 17-20, 2011.
- M. Hildebrand: Development of Diatom Genetic Manipulation Tools; oral presentation at the Food and Fuel for the 21st Century, April 22-23, 2012, La Jolla, CA, USA
- Invited Keynote Speaker and Session Chair, The Second International Conference on Algal Biomass, Biofuels, and Bioproducts, “Evolutionary-based Differences in Microalgal Cellular Organization and Processes as Related to Biofuels Production” San Diego, CA, June 10-13, 2012.
- Invited Speaker, The 22nd Annual Western Photosynthesis Conference, “Differences in Carbon Flux Pathways and Photosynthetic Processes in Evolutionarily-distinct Microalgae” Asilomar, CA, January 3-6, 2013.
- Invited Speaker, M Hildebrand, AK Davis, K Manandhar-Shrestha, RM Abbriano, JE Polle, SR Smith, JC Traller, EM Trentacoste, R Roth, U Goodenough. The Third International Conference on Algal Biomass, Biofuels, and Bioproducts, “Metabolic and Cellular Organization in Evolutionarily Diverse Microalgae As Related to Biofuels Production” Toronto Canada, June 16-19, 2013.
- EM Trentacoste, R Shrestha, SR Smith, C Gle, A Hartmann, M Hildebrand & WH Gerwick. The Third International Conference on Algal Biomass, Biofuels, and Bioproducts, “Increased lipid accumulation without compromising growth: Metabolic engineering of lipid catabolism in *Thalassiosira pseudonana*” Toronto Canada, June 16-19, 2013.
- J Traller, S Cokus, D Lopez, M Pellegrini, M Hildebrand. The Third International Conference on Algal Biomass, Biofuels, and Bioproducts. “Genome and Methyloome Of A Candidate Biofuel Organism: How ‘Omic’ Data Can Inform Approaches To Improve Productivity”, Toronto Canada, June 16-19, 2013.
- R Abbriano, S Smith, M Hildebrand, The Third International Conference on Algal Biomass, Biofuels, and Bioproducts. “Manipulation of a Key Glycolytic Regulatory Step and Its Effect on Growth and Lipid Accumulation in the Diatom *Thalassiosira pseudonana*”, Toronto Canada, June 16-19, 2013.
- SR Smith, AE Allen, M Hildebrand. The Third International Conference on Algal Biomass, Biofuels, and Bioproducts. “A global regulatory mechanism integrates carbon and energy metabolism in the diatom *Thalassiosira pseudonana*.” Toronto Canada, June 16-19, 2013.

2 - Technical Accomplishments/Progress/Results

Project Management Plan Breakdown

A.	Transcriptomic and bioinformatic analysis of environmentally-triggered lipid accumulation.
A.1	Bioinformatic identification of genes involved in lipid accumulation and carbon partitioning.
A.1.1.	Analysis of Affymetrix microarray probing of <i>T. pseudonana</i> during short-term lipid accumulation resulting from silicon limitation.
A.1.1.1	Confirmation done by Illumina-based RNAseq
A.2.1.	Transcriptomes and genome done by Illumina-based RNAseq
A.2.1.1.	
A.2.1.2	Confirmation done by Illumina-based RNAseq
B.	Metabolic engineering of the cell to alter carbon partitioning for abundant lipid accumulation coupled with high biomass accumulation
B.1	Manipulation of selected genes
B.1.1	Manipulation of selected genes from genome sequences
B.1.2	Manipulation of selected genes from transcriptomics analyses