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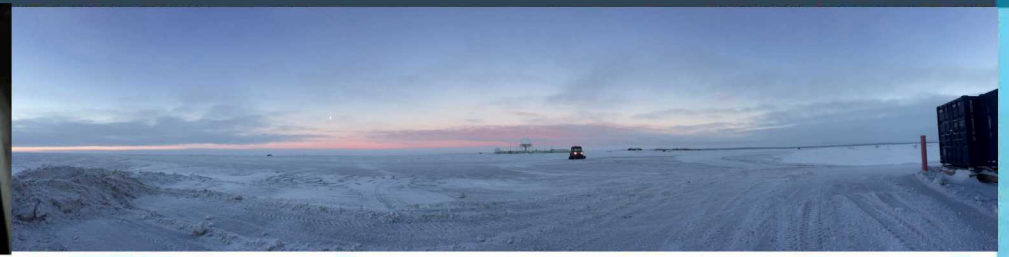
Sandia National Laboratories

U.S. DEPARTMENT OF ENERGY

Office of Science

Engineering of Diatoms for Novel Materials



PRESENTED BY

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Distinguished Member of Technical Staff, Systems Biology Dept.
October 22, 2019

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Pattern formation in diatom silica is highly conserved

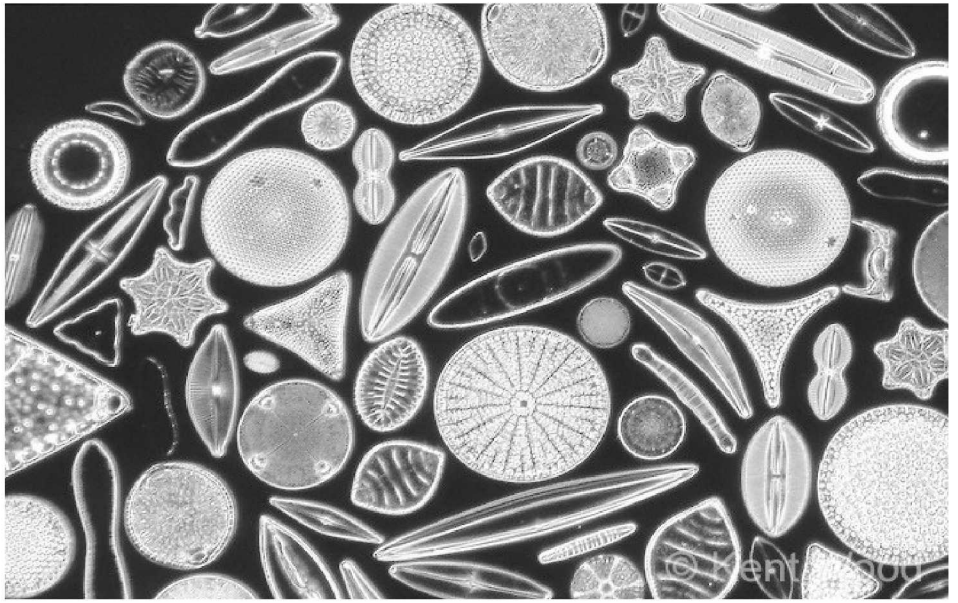
Silica is a major nutrient required for diatom growth: Silica starvation halts the cell cycle.

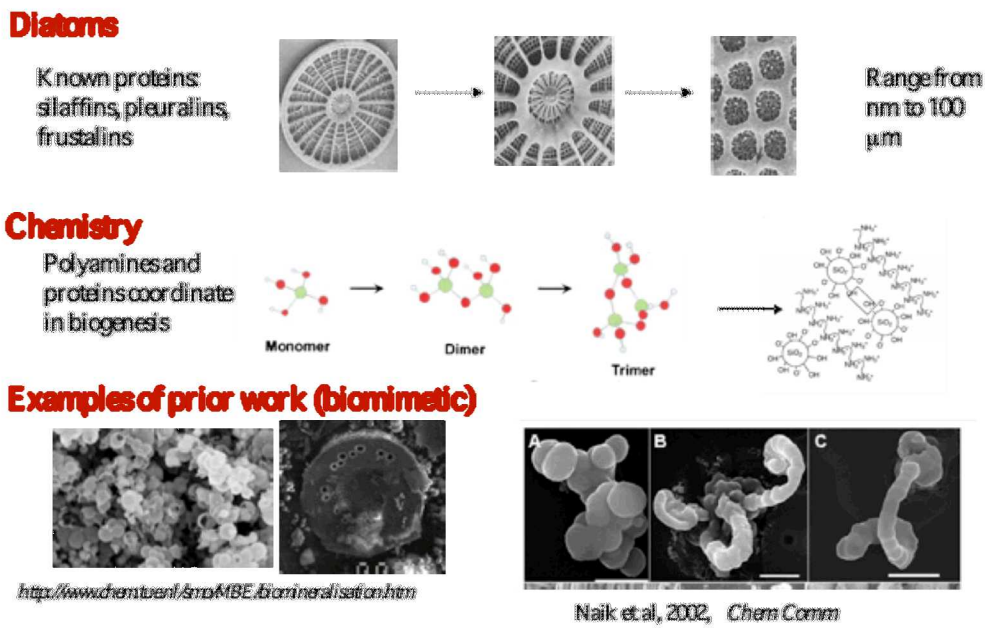
Pattern formation during frustule formation is highly conserved and likely to be genetically encoded.

A number of frustule associated proteins have been identified by proteomic analyses.

Objectives:

- Utilize a comparative transcriptomics & genomics approach to understand frustule morphogenesis
  - leverage both differences and similarities between basic physiology among different algal families and diatom species.
- Genetically control frustule morphogenesis
- Utilize this control of the finely patterned silica structure to develop functional materials

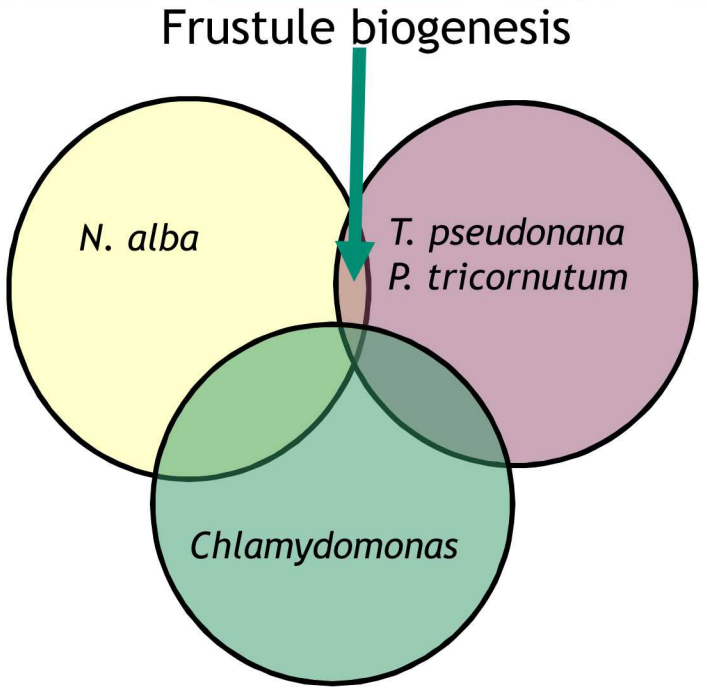


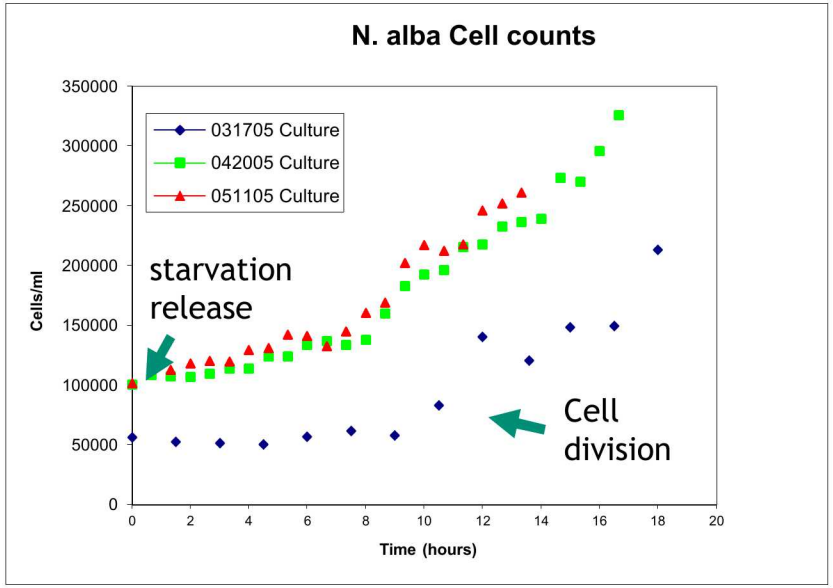


DOE-BER Workshop: Genome Engineering for Material Synthesis (GEMS) October 9-11, 2018, Rockville, MD

Proof of principle:

- Compared sequences obtained from a heterotrophic diatom (*N. alba*) to the genome of two photoautotrophic diatoms (*T. pseudonana and P. tricornutum*) and a chlorophytic algae (*Chlamydomonas*)
- Identified the genes that are held in common in the frustule forming diatoms and not found in *Chlamydomonas*.
- Allowed us to ignore or avoid genes that are not peculiar to silica frustule formation processes.
- Released *N.alba* from silica starvation
- Carried out transciptomic analysis to identify genes
- active during silica deposition and frustule formation
- Used comparative genomics to identify transcripts that were most likely to be associated with silica deposition versus other metabolic processes up regulated after release from from cell division block



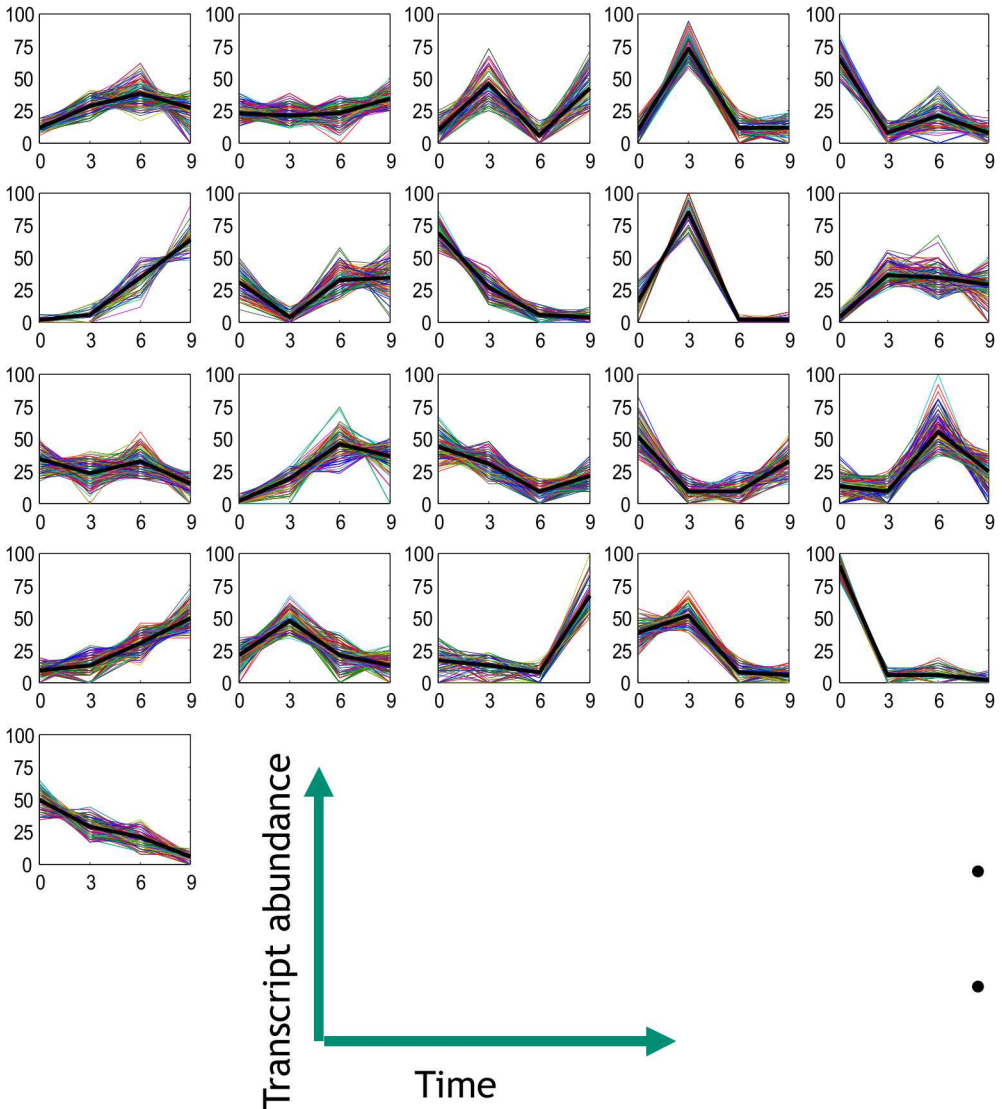


LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Identification of putative silica biogenesis genes

Optimal clustering in terms of defining time series patterns in the data resulted in a set 21 patterns representing changes in expression over 4 time points.

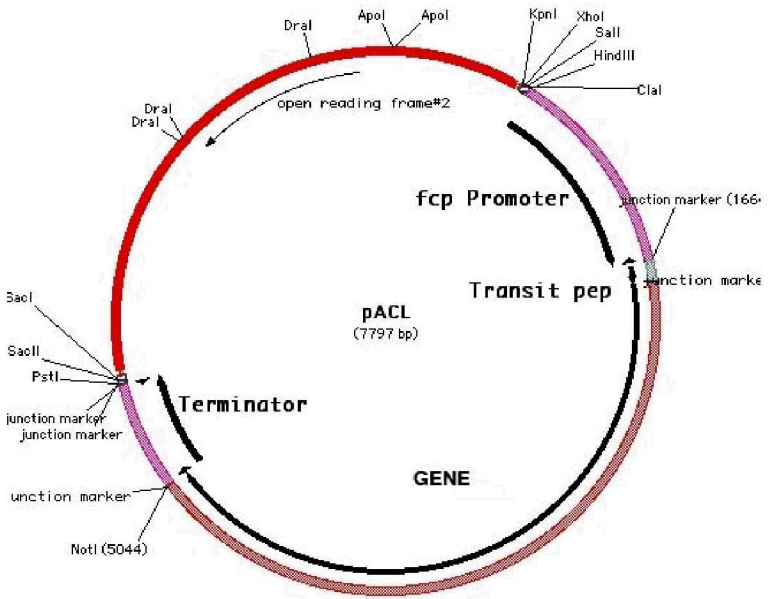
Identified several diatom specific genes that may play a role in frustule formation

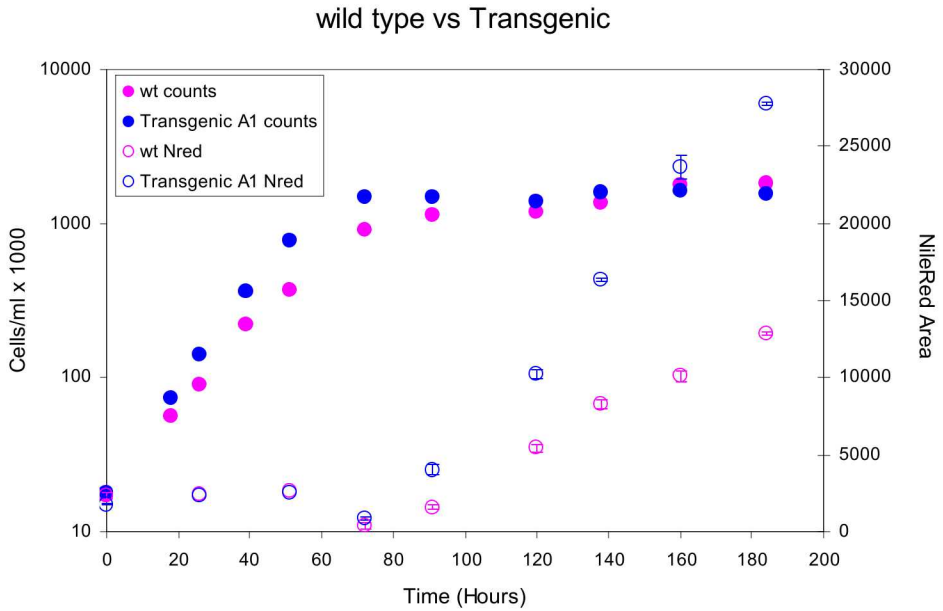


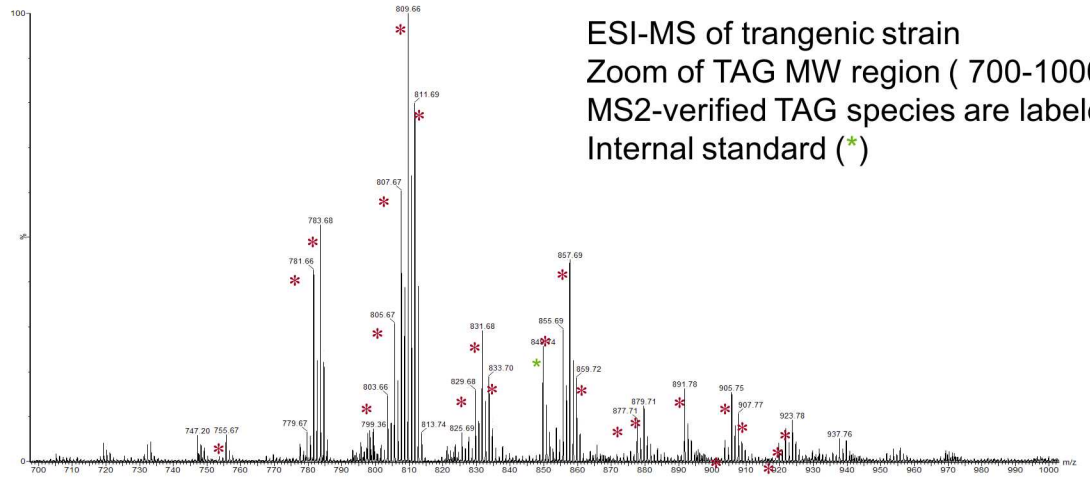
Clone	Description of best BLAST hit	Hit in P. tricornutum	Hit in T. pseudonana
1-10	Predicted protein,	GENE ID: 7194775 PHATRDRAFT_39015	
1-26	Predicted protein,	GENE ID: 7195890 PHATRDRAFT_40079	GENE ID: 7449863 THAPSDRAFT_23833
1-35	Prolyl 4-hydroxylase, alpha subunit	predicted protein	GENE ID: 7451246 THAPSDRAFT_264177 prolyl 4-hydroxylase alpha subunit
10-3	prolyl 4-hydroxylase alpha subunit-like protein	GENE ID: 7195168 PHATRDRAFT_48891	GENE ID: 7444578 THAPSDRAFT_6480
12-1	Hypothetical protein,	GENE ID: 7201119 PHATRDRAFT_45880	n.f
12-46	proline rich? hypothetical protein	GENE ID: 7204199 PHATR_18585	n.f.
12-57	hypothetical protein	GENE ID: 7195734 PHATRDRAFT_15968	n.f.
15-73	hypothetical protein	GENE ID: 7197686 PHATRDRAFT_33885	n.f.
16-3	Hypothetical protein	GENE ID: 7197835 PHATRDRAFT_44357	GENE ID: 7449684 THAPSDRAFT_22599
1-38	Sec4 Rab family GTPase Rab8	GENE ID: 7195643 Sec4	GENE ID: 7441917 TpSec4b
1-41	Rab family GTPase Rab8	GENE ID: 7202891 PHATRDRAFT_47709	n.f.

- Identified several diatom specific genes: putative silica biogenesis functions.
- Several were specific to the pennate rather than the centric diatom

Metabolic engineering of marine diatom *T. pseudonana*







ESI-MS of trangenic strain
Zoom of TAG MW region (700-1000 m/z)
MS2-verified TAG species are labeled (*)
Internal standard (*)

Gene fused to chloroplast transit peptide and expressed off of *fcp* promoter (constitutive).

Microalgae transfected by biolistic method.

Increase rate of accumulation and over TAG abundance in transgenic strain.

Increase in this example: ~35%

- WT 15.3% TAG w/w
- Transgenic 20.7% TAG w/w

We have created a number of additional transgenic algae and are completing characterization.

We have also created classical mutants that increase TAG production.

Future work

Understand, harness, and manipulate silica biomineralization in diatoms

- Extend transcriptomic analysis to the comparison of multiple heterotrophic and autotrophic diatoms
- Comparison of centric and pennate species: leverage morphological diversity
- Employ molecular genetics to manipulate structure and pattern formation in diatoms
- Control the creation of morphological variants in diatom frustules
- Control and manipulate the biochemistry at the organic inorganic interface

Potential materials applications: use the hierarchical structures present in diatoms to develop stable, active, and easily deployed photocatalysts for decontamination applications