Pan-transcriptomic analysis identifies coordinated and orthologous functional modules in the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*

Justin Ashworth¹, Serdar Turkarslan¹, Micheleen Harris¹, Mónica V. Orellana^{1,2}, Nitin S. Baliga^{1,3}

¹Institute for Systems Biology, Seattle, WA USA

²Polar Science Center, University of Washington, Seattle, WA USA

³Department of Microbiology, University of Washington, Seattle, WA USA

Abstract

Diatoms are important primary producers in the ocean that thrive in diverse and dynamic environments. Their survival and success over changing conditions depend on the complex coordination of gene regulatory processes. Here we present an integrated analysis of all publicly available microarray data for the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*. This resource includes shared expression patterns, gene functions, and *cis*-regulatory DNA sequence motifs in each species that are statistically coordinated over many experiments. These data illustrate the coordination of transcriptional responses in diatoms over changing environmental conditions. Responses to silicic acid depletion segregate into multiple distinctly regulated groups of genes, regulation by heat shock transcription factors (HSFs) is implicated in the response to nitrate stress, and distinctly coordinated carbon concentrating, CO₂ and pH-related responses are apparent. Fundamental features of diatom physiology are similarly coordinated between two distantly related diatom species, including the regulation of photosynthesis, cellular growth functions and lipid metabolism. These integrated data and analyses can be explored publicly (<http://networks.systemsbiology.net/diatom-portal/>).

Introduction

Diatoms are important primary producers in marine ecosystems (Armbrust, 2009; Tsuda et al., 2003), and their ability and capacity to physiologically adjust to changing ocean conditions is critical to their ecological success, both now and in the future. The heterokonts (including diatoms) are phylogenetically distinct from other clades, and represent relatively undiscovered genomic and physiological territory. Recent studies of diatom genomes (Armbrust et al., 2004; Bowler et al., 2008), transcriptomes and proteomes (Allen et al., 2008; Ashworth et al., 2013; Brembu et al., 2011; Carvalho et al., 2011; Chauton et al., 2013; Hook and Osborn, 2012; Kustka et al., 2014; Levitan et al., 2015; Mock et al., 2008; Nymark et al., 2013, 2009; Sapriel et al., 2009; Shrestha et al., 2012; Thamtrakoln et al., 2013, 2012; Valle et al., 2014) hint at complex molecular and regulatory programs that control diatom physiology and acclimation to a variety of different environmental conditions. However, the specific coordination and regulation of these molecular and adaptive processes in diatoms remains largely unknown for multiple reasons: i) the size and complexity of diatom genomes and their molecular responses to change, ii) their genetic uniqueness and lack of sufficient homology in comparison to other well-studied clades, iii) low-throughput experimental genetics approaches, and iv) insufficient data and analysis methods to identify concurrent yet distinct molecular and regulatory pathways operating simultaneously under various conditions.

The integration and analysis of data collected through many different transcriptomic experiments can be used to discover fundamentally coordinated, conditional, and distinct molecular responses that are reflective of gene regulatory processes and can not be discovered through individual experiments (Brooks et al., 2014; Danziger et al., 2014; Reiss et al., 2006).

Data-driven approaches for the discovery of molecular coordination may be particularly useful in the case of diatoms, given the size and novelty of their genomes, transcriptomes and proteomes. To systematically discover and identify modules of putatively coordinated and

functionally related diatom genes, we aggregated all available microarray expression data for the model diatoms *T. pseudonana* and *P. tricornutum*, and performed hierarchical clustering (Eisen et al., 1998) and motif-guided biclustering (Reiss et al., 2006) over many experimental conditions. Highlights of this analysis in the context of specific conditions and functions are discussed herein, and the complete results have been made available for further use and exploration in a web portal at the following url: <http://networks.systemsbiology.net/diatom-portal/>.

Data Sources and Methods

Integrated diatom transcriptomic dataset. Transcriptome-wide microarray expression data *T. pseudonana* used in this analysis included: silica, iron, and nitrogen limitation, low temperature and elevated pH (Mock et al., 2008), exposure to pollutant and mutagen benzo[a]pyrene (Carvalho et al., 2011), iron starvation (Thamatrakoln et al., 2012), silica re-supplementation (Shrestha et al., 2012), diel growth from exponential to stationary phase (Ashworth et al., 2013), and growth at moderate and elevated CO₂ levels under moderate and elevated light (Gene Expression Omnibus (GEO) accession #GSE57737). Transcriptome-wide microarray expression data for *P. tricornutum* used in this analysis included: silica limitation (Sapriel et al., 2009), acclimation to high light (Nymark et al., 2009), exposure to cadmium (Brembu et al., 2011), acclimation to light and dark cycles (Chauton et al., 2013), exposure to a panel of pollutants (Hook and Osborn, 2012), darkness and re-illumination (Nymark et al., 2013), exposure to red, blue and green light (Valle et al., 2014). All public microarray data were downloaded from GEO, converted to log₂ expression ratios vs. within-experiment control samples, and aggregated by common transcript identifiers for each species. The normalization performed within these independent experimental datasets was preserved in order to reflect previous findings; further approaches to normalize these data were explored in conjunction with various distance metrics and hierarchical clustering methods, as described below in the section: Co-Expression Clustering.

Genome information, protein annotations, functional enrichment analysis and

predictions of orthology. The genomes, gene, transcript and protein models, and functional

annotations for each diatom species (*T. pseudonana*:version 3; *P. tricornutum*:version 2) were

downloaded from the Joint Genome Institute (JGI) Genome Portal (Grigoriev et al., 2011).

Existing protein functional annotations were supplemented with significant matches to the

Conserved Domain Database (Marchler-Bauer et al., 2015). The enrichment of GO terms

(biological process) across all co-expression clusters was computed from existing JGI GO

annotations using a hypergeometric test and multiple hypothesis correction by the Benjamini-

Hochberg method (Benjamini and Hochberg, 1995). Putative orthologous proteins between

diatom species were inferred using a pairwise reciprocal best BLASTp(Altschul et al., 1990)

approach with an E-value cutoff of 1×10^{-10} . Significantly orthologous co-expression clusters

between species were identified by computing the hypergeometric probability of drawing

intersections of orthologous genes (Kalinka, 2013) and multiple hypothesis correction

(Benjamini and Hochberg, 1995).

Co-expression clustering. The co-expression of transcripts across all included experiments

was assessed using a Pearson correlation distance metric over all corresponding $\log_2$

expression ratios for each pair of transcripts. Fast agglomerative hierarchical clustering (Müllner,

2013) was used to generate a hierarchical tree of correlated transcripts, and ten thousand-fold

multi-scale bootstrap resampling (Suzuki and Shimodaira, 2006) was used to estimate the

significance and robustness of hierarchical subclusters to simulated noise and variation in the

data. For efficiency on large transcriptomes, all pairwise distance calculations, hierarchical

clustering using the fastcluster++ library (Müllner, 2013), and performance-critical functions of

the pvclust R package (Suzuki and Shimodaira, 2006) were computed using compiled c++ code

that is available for download. The false discovery rate of cluster-level changes in expression

over categorical conditions was computed based on empirical distributions ($n \geq 10,000$) of

clusters composed of randomly-selected transcripts for each cluster size over each condition,

with multiple hypothesis correction to account for total number of clusters. Significantly shared DNA sequence motifs in the upstream promoter regions of genes co-occurring in hierarchical co-expression clusters were detected using MEME (Bailey and Elkan, 1994) and compared to databases of known motifs using TOMTOM (Gupta et al., 2007), both of which are part of the MEME software suite (Bailey et al., 2009).

DNA motif-guided biclustering. The simultaneous clustering of genes based on i) co-expression over subsets of experimental conditions, ii) shared upstream non-coding DNA sequence patterns (possible *cis*-regulatory motifs), and iii) known gene and protein associations (Szkarczyk et al., 2011) was performed using version 4.9.21 of the R package cMonkey (Reiss et al., 2006). For biclustering using the cMonkey algorithm, the data were row-normalized as described in (Reiss et al., 2006). The bottom twenty-five percent least variant transcripts over all conditions were excluded from this analysis for computational and algorithmic efficiency. DNA sequences up to four hundred base pairs upstream of gene model start sites were iteratively searched using MEME (Bailey et al., 2009) for significantly occurring DNA sequence motifs in the putative promoters of co-expressed genes. The cMonkey algorithm was run for two thousand iterations to generate biclusters with a mean of thirty genes per cluster.

Results

Pan-transcriptomic clustering identifies separately coordinated groups of genes within singular condition-specific responses. Through integrative, whole-genome multi-experiment clustering (**Fig. 1**), numerous distinctly and significantly co-expressed sets of functionally-related transcripts were discovered in *T. pseudonana* and *P. tricornutum* (**Table 1, Table S1**). In addition, lists of transcripts that were significantly affected in individual previous experiments were segregated into separate and distinctly coordinated groups of genes. For example, transcripts whose expression increased in silica-limited *T. pseudonana* diatoms (Mock et al., 2008) represent at least three distinct transcriptional and physiological response

mechanisms (**Fig. S1**). Three distinct clusters of co-expressed genes contain the top five transcripts (protein ids 268895, 9619, 21665, 3250, 10363) whose expression increased exclusively under silica limitation in previous experiments, in addition to several other silica-sensitive transcripts (Mock et al., 2008). These functions appear to be divided into silica and membrane transport (**Fig. S1B**), cell membrane regulation (**Fig. S1C**), and vesicle-mediated transport (**Fig. S1D**).

Figure 1. Pan-transcriptomic discovery of co-expressed functional modules in diatoms.

Table 1. Clustering statistics.

	<i>T. pseudonana</i>	<i>P. tricornutum</i>
Transcript models included in analysis	12,114	10,352
Protein models with GO annotations (biological process) ¹	3,818	3,195
Microarray series (# of distinct samples)	8 (56)	7 (123)
Hierarchical clusters (height-based selection)	500	500
Significantly enriched upstream DNA motifs in clusters ²	593	143
Bootstrap-supported hierarchical subclusters (pvclust) ³	899	1,006
Transcript models included in biclustering (cMonkey) ⁴	8,771	7,708
Biclusters (cMonkey)	292	257
Significantly enriched upstream DNA motifs in biclusters ²	87	43

¹JGI version 3 models and annotations for *T. pseudonana*; JGI version 2 for *P. tricornutum*.

²MEME *E*-value $\leq 1 \times 10^{-3}$.

³Approximately unbiased bootstrap *P*-value $\geq 90\%$, $n \geq 3$ transcripts.

⁴The top 75% most variant transcripts were included in biclustering.

Pan-transcriptomic clustering also identified clusters of tightly co-expressed genes for which evidence of direct co-regulation is present in upstream promoter regions. For example, among thousands of transcripts that significantly changed in expression between exponential and stationary/nutrient limited cultures in a previous microarray experiment (Ashworth et al.,

Figure 2. A putatively co-regulated cluster of genes responsible for nitrate assimilation and stress response in *T. pseudonana*. A tightly co-expressed cluster of transcripts (**A**) related to nitrate assimilation and potential stress-related mechanisms is significantly increased in expression during stationary, nutrient-limited conditions, high light, and iron and nitrate limitation (**B**). A shared putative *cis*-regulatory DNA motif (**C**) found in the upstream regions of most of the genes in this cluster is similar to the known binding site of a heat shock transcription factor found in *A. thaliana*. The presence of the discovered motif in individual gene promoters is indicated by asterisks in (**A**).

Newly discovered condition-specific clusters. Multi-experiment clustering resulted in the identification of new putative modules of co-expressed and co-regulated genes with shared functions and condition-specific transcriptional responses. For example, a cluster of *P. tricornutum* transcripts encoding several putative enzymes was tightly co-expressed over all conditions (bootstrap *P*-value = 91%), significantly differentially expressed over a range of conditions, and significantly increased in expression upon exposure to oil (Hook and Osborn, 2012) (**Fig. S3A**). A cluster of *T. pseudonana* transcripts encoding a silaffin protein, extracellular domains, peptidases and signaling proteins was tightly co-expressed over a range of conditions; The mean expression of these transcripts was significantly decreased at high pH and low silica, and significantly increased under elevated CO₂/low pH and during exponential growth (**Fig.S3B**).

Diatom species share orthologous clusters of co-expressed transcripts. Comparison of co-expression clusters between *T. pseudonana* and *P. tricornutum* revealed seventeensignificantly homologous clusters of transcripts between these two distantly related diatom species. Despite the different kinds of experiments from which transcriptome measurements were derived for the two species, similarly coordinated expression for groups of orthologous gene functions indicates fundamental processes whose transcriptional regulation is conserved between these diatoms. Among these conserved functions and processes are fatty acid metabolism (**Fig 3**),light harvesting complexes (**Fig. S4A**), photosynthesis (**Fig. S4B**), protein biosynthesis (ribosomal

proteins), RNA processing and nucleolar proteins, proteasomal proteins, cytokinesis, cell cycle,
cell division and frustule maintenance, chromatin regulation, and glycolysis (Table 2).

Figure 3. Conserved enzymes involved in fatty acid metabolism in both *T. pseudonana* and *P. tricornutum* are tightly co-expressed over large numbers of experimental conditions.

A) Hierarchical clusters (dendrograms) from both species containing transcripts for orthologous enzymes in the fatty acid elongation and degradation (β -oxidation) cycle (**D**). Arrows indicate orthologous proteins and selected branches are labeled with transcriptome-wide bootstrap *P*-values. **B)** Raw expression profile for these gene clusters over many conditions. **C)** Mean changes in expression over many conditions. Asterisks and shading indicate the significance (false discovery rate) for the magnitude of these changes. **D)** The fatty acid elongation and degradation (β -oxidation) cycle in *T. pseudonana* and *P. tricornutum*. Enzyme Commission (E.C.) numbers, enzyme names, and the orthologous enzymes in each species are listed. Red numbers indicate transcripts present in orthologous co-expression clusters (**A**).

Table 2. Orthologous hierarchical clusters shared between species. Hierarchical co-expression clusters from both species were compared to detect significantly orthologous clusters of protein-coding transcripts. The hypergeometric intersection method of (Kalinka, 2013) was used to assess significance, with Benjamini-Hochberg correction for multiple hypotheses.

<i>P. tricornutum</i>		<i>T. pseudonana</i>					
Cluster	Size	Cluster	Size	Orthologs	<i>p</i> -value	FDR	OrthologicalFunction
151	46	47	46	29	1.06E-54	2.66E-49	ribosomal proteins
111	51	22	66	20	8.69E-43	2.17E-37	light harvesting complexes
201	60	220	85	22	1.53E-29	3.82E-24	RNA processing and nucleolar proteins
183	26	199	42	14	2.15E-26	5.37E-21	proteasomal proteins
146	37	47	46	16	1.04E-25	2.60E-20	ribosomal proteins
146	37	46	25	12	1.25E-21	3.13E-16	ribosomal proteins
97	108	82	94	14	1.70E-20	4.25E-15	cytokinesis
151	46	46	25	11	4.36E-18	1.09E-12	ribosomal proteins
177	31	24	73	9	9.41E-14	2.35E-08	porphyrin biosynthesis
176	19	24	73	8	2.60E-13	6.50E-08	porphyrin biosynthesis
100	70	92	93	6	5.43E-12	1.36E-06	cell cycle, cell division, frustule
200	35	220	85	9	1.52E-10	3.80E-05	RNA processing and nucleolar proteins
108	34	440	14	5	8.03E-10	2.01E-04	fatty acid metabolism
96	103	82	94	9	1.25E-09	3.13E-04	chromatin regulation
134	21	41	53	6	2.33E-09	5.82E-04	ribosomal proteins and tRNAs
243	38	220	85	7	7.93E-08	1.98E-02	chromatin regulation
175	28	15	67	5	1.58E-07	3.94E-02	glycolysis

Putative co-regulatory modules and *cis*-regulatory signals: DNA motif-guided

biclustering. For several prokaryotic and some eukaryotic species it is possible to discover and refine groups of genes whose co-expression and regulation is explained by the presence of shared *cis*-regulatory DNA sequence motifs (Brooks et al., 2014; Danziger et al., 2014; Peterson et al., 2014; Reiss et al., 2006). Applying the cMonkey motif-guided biclustering methodology (Reiss et al., 2006) to combined genomic and transcriptomic data for *T. pseudonana* and *P. tricornutum* resulted in biclusters of functionally related co-expressed genes in which shared and potentially meaningful *cis*-regulatory DNA motif patterns were found in

putative upstream promoter regions (**Table 1 and Table S2**). Two examples are a bicluster of *T. pseudonana* genes putatively involved in tRNA and protein synthesis and a bicluster of *P. tricornutum* genes putatively involved in protein processing and amino acid metabolism (**Fig.S5**). Significantly enriched DNA sequence motifs found in these biclusters resemble the measured DNA binding sequences for Myb and PIF transcription factors in *Arabidopsis thaliana* (**Fig. S5C and S5D**, respectively) (Franco-Zorrilla et al., 2014). The significantly enriched *cis*-regulatory sequences in the upstream regions of the genes in these clusters may be key to the transcriptional regulation of co-expressed diatom genes. However, these inferences must be experimentally investigated, and additional data will be required to accurately describe and model diatom gene regulatory networks, including true transcriptional start sites, known transcription factor binding site and other *cis*-regulatory sequences, and genome-wide transcriptional factor-DNA binding measurements. Additionally, pre- and post-transcriptional and post-translational regulatory mechanisms such as microRNAs, protein signaling, nuclear translocation and metabolite levels may also factor significantly into the regulation of physiology in diatoms.

Exploration of data and comprehensive results: The Diatom Portal. The aggregated data and clustering described and discussed here have been incorporated into a public web portal (The Diatom Portal) for further exploration and analysis. This web interface includes gene, transcript and protein level information for fully sequenced and transcriptionally profiled diatoms (currently *T. pseudonana* and *P. tricornutum*), a lightweight genome context browser, and the results of hierarchical expression clustering and motif-directed biclustering over many aggregated datasets and conditions (**Fig. 4**). The contents of this portal are organized into gene transcripts, clusters of co-expressed genes, and detected potential DNA *cis*-regulatory sequence motifs. These can be explored by using a variety of filters, or searched using a variety of search terms including JGI protein identification numbers, annotation keywords, and GO

terms. There are two primary record types represented in the portal: Genes and Clusters. Pages for individual genes contain a genome browser (**Fig. 4A**), gene and transcript model information, synonymous external identifiers, other most highly correlated transcripts (**Fig. 4B**), conserved domains (**Fig. 4C**), predicted orthologous genes detected in other diatoms and plant species (**Fig. 4D**), previews of co-expression clusters in which the transcript occurs (**Fig. 4E**), KEGG mapping and gene ontology (GO) terms. Pages for individual co-expression clusters contain groups of gene transcripts whose expression was correlated over many experiments, changes in expression over categorical conditions, GO term enrichments, and potential *cis*-regulatory DNA motifs detected in the upstream promoter regions of the genes occurring in the cluster. The details and position weight matrices (PWMs) for these motifs can also be downloaded in MEME format for comparison against databases of known motifs (e.g. TOMTOM (Gupta et al., 2007)). The aggregate data set used for expression clustering can also be downloaded for additional or independent analysis. The goal of this Diatom Portal is to facilitate discoveries, hypothesis generation, collaboration and continued data integration within the diatom research community in order to accelerate the advancement of the science and understanding of diatom biology and gene regulation. For example, the incorporation of additional transcriptomic data (including mRNA-sequencing data and the MMETSP (Keeling et al., 2014)) as it becomes available will increase the resolution and conditionality of gene expression clustering, and continued cross-species analysis will expand the set of conserved, related and unique regulatory features and coordination that can be detected and inferred within the diatom clade.

Diatom Portal v1

Enter your search term, e.g. "268895", "lipase", "GO:0003755" etc.
Put quotes around phrases to match all the words: "lipase superfamily". You can require or exclude terms using + and -

268895 (SIT1) Silic_transp superfamily

Thalassiosira pseudonara

View Edit Manage display Track Log

B Most Highly Correlated Transcripts

9619	41392	bd415	4108	bd1787	bd1959
hypothetical protein(SIT2) Silic_transp superfamily(bd415) arom_aa_hydroxylase superfamilyhypothetical protein(bd1787) GLTP(bd1959) NA					
0.8705	0.8593	0.859	0.8515	0.8497	0.8447
bd1941	21968	bd1808	bd721		
(bd1941) NA	hypothetical protein	(bd1808) NA	(bd721) NA		
0.8377	0.8313	0.8268	0.825		

C Conserved Domains

Name	CD Accession	Definition	Superfamily	Bitscore	E-Value	From - To	Hit Type	PSSMID
Silic_transp superfamily	cd04286	Silicon transporter; Silicon transporter.	-	780.964	0	1-461	superfamily	146463

D Homologs in Other Species

T. pseudonara	P. tricornutum	P. tricornutum DiatomCyc	F. cylindrus	Pseudo-nitzschia multiseriis	E. huxleyi	C. reinhardtii	A. thaliana	P. sojae
Not available	PHATRDRAFT_23423	PHATRDRAFT_23423	Not available	261458	Not available	Not available	Not available	Not available

E Hierarchical Clustering

Figure 4. The Diatom Portal. This figure illustrates the Diatom Portal web interface, using search results for the SIT1 silicic acid transporter (protein id: 268895) in *T. pseudonana* as an example. These results include A) a lightweight genome context browser, B) highly correlated transcripts, C) detected conserved protein domains, D) putative orthologs in other related species, and E) expression clustering with de novo detection of potential *cis*-regulatory DNA sequence motifs shared in the upstream promoter regions of co-expressed genes.

Conclusion

In this work, an integrated analysis of transcriptomic data for two model diatom species (*T. pseudonana* and *P. tricornutum*) over many independent experiments resulted in the comprehensive inference of coordinated and conditional transcriptional responses that are indicative of distinct functional and gene regulatory processes operating robustly in the cell. The clustering and analysis of combined datasets performed here allowed the partitioning of the large transcriptomes of these diatom species into distinct groups of putatively coordinated and functionally related genes that are enriched in diverse essential functions, and were not previously evident in individual transcriptome experiments. The continued integration and meta-analysis of genomic and transcriptomic data from diatom species will be essential to accelerate the discovery of molecular coordination in these complex and novel biological systems, and help to inform ongoing and future studies of these and other related diatoms and microalgae.

Acknowledgements

This work was supported by the National Science Foundation (OCB-0928561 and MCB-1316206 to M.V.O. and N.S.B.)

References

- Allen, A.E., Laroche, J., Maheswari, U., Lommer, M., Schauer, N., Lopez, P.J., Finazzi, G., Fernie, A.R., Bowler, C., 2008. Whole-cell response of the pennate diatom *Phaeodactylum tricornutum* to iron starvation. *Proc. Natl. Acad. Sci. U. S. A.* 105, 10438–10443. doi:10.1073/pnas.0711370105
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. doi:10.1016/S0022-2836(05)80360-2
- Armbrust, E.V., 2009. The life of diatoms in the world's oceans. *Nature* 459, 185–192. doi:10.1038/nature08057
- Armbrust, E.V., Berges, J.A., Bowler, C., Green, B.R., Martinez, D., Putnam, N.H., Zhou, S., Allen, A.E., Apt, K.E., Bechner, M., Brzezinski, M.A., Chaal, B.K., Chiovitti, A., Davis, A.K., Demarest, M.S., Detter, J.C., Glavina, T., Goodstein, D., Hadi, M.Z., Hellsten, U., Hildebrand, M., Jenkins, B.D., Jurka, J., Kapitonov, V.V., Kröger, N., Lau, W.W.Y., Lane, T.W., Larimer, F.W., Lippmeier, J.C., Lucas, S., Medina, M., Montsant, A., Obornik, M., Parker, M.S., Palenik, B., Pazour, G.J., Richardson, P.M., Rynearson, T.A., Saito, M.A., Schwartz, D.C., Thamatrakoln, K., Valentin, K., Vardi, A., Wilkerson, F.P., Rokhsar, D.S., 2004. The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. *Science* 306, 79–86. doi:10.1126/science.1101156
- Ashworth, J., Coesel, S., Lee, A., Armbrust, E.V., Orellana, M.V., Baliga, N.S., 2013. Genome-wide diel growth state transitions in the diatom *Thalassiosira pseudonana*. *Proc. Natl. Acad. Sci. U. S. A.* 110, 7518–7523. doi:10.1073/pnas.1300962110
- Bailey, T.L., Boden, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., Ren, J., Li, W.W., Noble, W.S., 2009. MEME Suite: tools for motif discovery and searching. *Nucleic Acids Res.* 37, W202–W208. doi:10.1093/nar/gkp335
- Bailey, T.L., Elkan, C., 1994. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proc. Int. Conf. Intell. Syst. Mol. Biol. ISMB Int. Conf. Intell. Syst. Mol. Biol.* 2, 28–36.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300.
- Bowler, C., Allen, A.E., Badger, J.H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otiillar, R.P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., Verret, F., Berges, J.A., Brownlee, C., Cadoret, J.-P., Chiovitti, A., Choi, C.J., Coesel, S., De Martino, A., Detter, J.C., Durkin, C., Falciatore, A., Fournet, J., Haruta, M., Huysman, M.J.J., Jenkins, B.D., Jiroutova, K., Jorgensen, R.E., Joubert, Y., Kaplan, A., Kröger, N., Kroth, P.G., La Roche, J., Lindquist, E., Lommer, M., Martin-Jézéquel, V., Lopez, P.J., Lucas, S., Mangogna, M., McGinnis, K., Medlin, L.K., Montsant, A., Secq, M.-P.O., Napoli, C., Obornik, M., Parker, M.S., Petit, J.-L., Porcel, B.M., Poulsen, N., Robison, M., Rychlewski, L., Rynearson, T.A., Schmutz, J., Shapiro, H., Siaut, M., Stanley, M., Sussman, M.R., Taylor, A.R., Vardi, A., von Dassow, P., Vyverman, W., Willis, A., Wyrwicz, L.S., Rokhsar, D.S., Weissenbach, J., Armbrust, E.V., Green, B.R., Van de Peer, Y., Grigoriev, I.V., 2008. The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* 456, 239–244. doi:10.1038/nature07410
- Brembu, T., Jørstad, M., Winge, P., Valle, K.C., Bones, A.M., 2011. Genome-Wide Profiling of Responses to Cadmium in the Diatom *Phaeodactylum tricornutum*. *Environ. Sci. Technol.* 45, 7640–7647. doi:10.1021/es2002259
- Brooks, A.N., Reiss, D.J., Allard, A., Wu, W.-J., Salvanha, D.M., Plaisier, C.L., Chandrasekaran, S., Pan, M., Kaur, A., Baliga, N.S., 2014. A system-level model for the microbial regulatory genome. *Mol. Syst. Biol.* 10, 740.

- Carvalho, R.N., Bopp, S.K., Lettieri, T., 2011. Transcriptomics Responses in Marine Diatom *Thalassiosira pseudonana* Exposed to the Polycyclic Aromatic Hydrocarbon Benzo[a]pyrene. *PLoS ONE* 6, e26985. doi:10.1371/journal.pone.0026985
- Chauton, M.S., Winge, P., Brembu, T., Vadstein, O., Bones, A.M., 2013. Gene Regulation of Carbon Fixation, Storage, and Utilization in the Diatom *Phaeodactylum tricornutum* Acclimated to Light/Dark Cycles. *Plant Physiol.* 161, 1034–1048. doi:10.1104/pp.112.206177
- Danziger, S.A., Ratushny, A.V., Smith, J.J., Saleem, R.A., Wan, Y., Arens, C.E., Armstrong, A.M., Sitko, K., Chen, W.-M., Chiang, J.-H., Reiss, D.J., Baliga, N.S., Aitchison, J.D., 2014. Molecular mechanisms of system responses to novel stimuli are predictable from public data. *Nucleic Acids Res.* 42, 1442–1460. doi:10.1093/nar/gkt938
- Eisen, M.B., Spellman, P.T., Brown, P.O., Botstein, D., 1998. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci.* 95, 14863–14868.
- Franco-Zorrilla, J.M., López-Vidriero, I., Carrasco, J.L., Godoy, M., Vera, P., Solano, R., 2014. DNA-binding specificities of plant transcription factors and their potential to define target genes. *Proc. Natl. Acad. Sci.* 111, 2367–2372. doi:10.1073/pnas.1316278111
- Grigoriev, I.V., Nordberg, H., Shabalov, I., Aerts, A., Cantor, M., Goodstein, D., Kuo, A., Minovitsky, S., Nikitin, R., Ohm, R.A., Otilar, R., Poliakov, A., Ratnere, I., Riley, R., Smirnova, T., Rokhsar, D., Dubchak, I., 2011. The Genome Portal of the Department of Energy Joint Genome Institute. *Nucleic Acids Res.* gkr947. doi:10.1093/nar/gkr947
- Gupta, S., Stamatoyannopoulos, J.A., Bailey, T.L., Noble, W.S., 2007. Quantifying similarity between motifs. *Genome Biol.* 8, R24. doi:10.1186/gb-2007-8-2-r24
- Hennon, G.M.M., Ashworth, J., Groussman, R.D., Berthiaume, C., Morales, R.L., Baliga, N.S., Orellana, M.V., Armbrust, E.V., 2015. Diatom acclimation to elevated CO₂ via cAMP signalling and coordinated gene expression. *Nat. Clim. Change* 5, 761–765. doi:10.1038/nclimate2683
- Hook, S.E., Osborn, H.L., 2012. Comparison of toxicity and transcriptomic profiles in a diatom exposed to oil, dispersants, dispersed oil. *Aquat. Toxicol. Amst. Neth.* 124-125, 139–151. doi:10.1016/j.aquatox.2012.08.005
- Kalinka, A.T., 2013. The probability of drawing intersections: extending the hypergeometric distribution. *ArXiv13050717 Math.*
- Keeling, P.J., Burki, F., Wilcox, H.M., Allam, B., Allen, E.E., Amaral-Zettler, L.A., Armbrust, E.V., Archibald, J.M., Bharti, A.K., Bell, C.J., Beszteri, B., Bidle, K.D., Cameron, C.T., Campbell, L., Caron, D.A., Cattolico, R.A., Collier, J.L., Coyne, K., Davy, S.K., Deschamps, P., Dyhrman, S.T., Edvardsen, B., Gates, R.D., Gobler, C.J., Greenwood, S.J., Guida, S.M., Jacobi, J.L., Jakobsen, K.S., James, E.R., Jenkins, B., John, U., Johnson, M.D., Juhl, A.R., Kamp, A., Katz, L.A., Kiene, R., Kudryavtsev, A., Leander, B.S., Lin, S., Lovejoy, C., Lynn, D., Marchetti, A., McManus, G., Nedelcu, A.M., Menden-Deuer, S., Miceli, C., Mock, T., Montresor, M., Moran, M.A., Murray, S., Nadathur, G., Nagai, S., Ngam, P.B., Palenik, B., Pawlowski, J., Petroni, G., Piganeau, G., Posewitz, M.C., Rengefors, K., Romano, G., Rumpho, M.E., Ryneerson, T., Schilling, K.B., Schroeder, D.C., Simpson, A.G.B., Slamovits, C.H., Smith, D.R., Smith, G.J., Smith, S.R., Sosik, H.M., Stief, P., Theriot, E., Twary, S.N., Umale, P.E., Vulot, D., Wawrik, B., Wheeler, G.L., Wilson, W.H., Xu, Y., Zingone, A., Worden, A.Z., 2014. The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. *PLoS Biol.* 12, e1001889. doi:10.1371/journal.pbio.1001889
- Kustka, A.B., Milligan, A.J., Zheng, H., New, A.M., Gates, C., Bidle, K.D., Reinfelder, J.R., 2014. Low CO₂ results in a rearrangement of carbon metabolism to support C₄ photosynthetic carbon assimilation in *Thalassiosira pseudonana*. *New Phytol.* 204, 507–520. doi:10.1111/nph.12926

- Levitan, O., Dinamarca, J., Zelzion, E., Lun, D.S., Guerra, L.T., Kim, M.K., Kim, J., Van Mooy, B.A.S., Bhattacharya, D., Falkowski, P.G., 2015. Remodeling of intermediate metabolism in the diatom *Phaeodactylum tricornutum* under nitrogen stress. *Proc. Natl. Acad. Sci. U. S. A.* 112, 412–417. doi:10.1073/pnas.1419818112
- Marchler-Bauer, A., Derbyshire, M.K., Gonzales, N.R., Lu, S., Chitsaz, F., Geer, L.Y., Geer, R.C., He, J., Gwadz, M., Hurwitz, D.I., Lanczycki, C.J., Lu, F., Marchler, G.H., Song, J.S., Thanki, N., Wang, Z., Yamashita, R.A., Zhang, D., Zheng, C., Bryant, S.H., 2015. CDD: NCBI's conserved domain database. *Nucleic Acids Res.* 43, D222–226. doi:10.1093/nar/gku1221
- Mock, T., Samanta, M.P., Iverson, V., Berthiaume, C., Robison, M., Holtermann, K., Durkin, C., Bondurant, S.S., Richmond, K., Rodesch, M., Kallas, T., Huttlin, E.L., Cerrina, F., Sussman, M.R., Armbrust, E.V., 2008. Whole-genome expression profiling of the marine diatom *Thalassiosira pseudonana* identifies genes involved in silicon bioprocesses. *Proc. Natl. Acad. Sci. U. S. A.* 105, 1579–1584. doi:10.1073/pnas.0707946105
- Müllner, D., 2013. Fastcluster: Fast Hierarchical, Agglomerative Clustering Routines for R and Python. *J. Stat. Softw.* 53, 1–18.
- Nymark, M., Valle, K.C., Brembu, T., Hancke, K., Winge, P., Andresen, K., Johnsen, G., Bones, A.M., 2009. An Integrated Analysis of Molecular Acclimation to High Light in the Marine Diatom *Phaeodactylum tricornutum*. *PLoS ONE* 4, e7743. doi:10.1371/journal.pone.0007743
- Nymark, M., Valle, K.C., Hancke, K., Winge, P., Andresen, K., Johnsen, G., Bones, A.M., Brembu, T., 2013. Molecular and Photosynthetic Responses to Prolonged Darkness and Subsequent Acclimation to Re-Illumination in the Diatom *Phaeodactylum tricornutum*. *PLoS ONE* 8, e58722. doi:10.1371/journal.pone.0058722
- Pelham, H., 1985. Activation of heat-shock genes in eukaryotes. *Trends Genet.* 1, 31–35. doi:10.1016/0168-9525(85)90012-5
- Peterson, E.J.R., Reiss, D.J., Turkarslan, S., Minch, K.J., Rustad, T., Plaisier, C.L., Longabaugh, W.J.R., Sherman, D.R., Baliga, N.S., 2014. A high-resolution network model for global gene regulation in *Mycobacterium tuberculosis*. *Nucleic Acids Res.* 42, 11291–11303. doi:10.1093/nar/gku777
- Reiss, D.J., Baliga, N.S., Bonneau, R., 2006. Integrated biclustering of heterogeneous genome-wide datasets for the inference of global regulatory networks. *BMC Bioinformatics* 7, 280. doi:10.1186/1471-2105-7-280
- Sapriel, G., Quinet, M., Heijde, M., Jourden, L., Tanty, V., Luo, G., Le Crom, S., Lopez, P.J., 2009. Genome-Wide Transcriptome Analyses of Silicon Metabolism in *Phaeodactylum tricornutum* Reveal the Multilevel Regulation of Silicic Acid Transporters. *PLoS ONE* 4, e7458. doi:10.1371/journal.pone.0007458
- Shrestha, R.P., Tesson, B., Norden-Krichmar, T., Federowicz, S., Hildebrand, M., Allen, A.E., 2012. Whole transcriptome analysis of the silicon response of the diatom *Thalassiosira pseudonana*. *BMC Genomics* 13, 499. doi:10.1186/1471-2164-13-499
- Suzuki, R., Shimodaira, H., 2006. Pvcust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* 22, 1540–1542. doi:10.1093/bioinformatics/btl117
- Szklarczyk, D., Franceschini, A., Kuhn, M., Simonovic, M., Roth, A., Minguez, P., Doerks, T., Stark, M., Muller, J., Bork, P., Jensen, L.J., von Mering, C., 2011. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Res.* 39, D561–568. doi:10.1093/nar/gkq973
- Thamatrakoln, K., Bailleul, B., Brown, C.M., Gorbunov, M.Y., Kustka, A.B., Frada, M., Joliot, P.A., Falkowski, P.G., Bidle, K.D., 2013. Death-specific protein in a marine diatom regulates photosynthetic responses to iron and light availability. *Proc. Natl. Acad. Sci. U. S. A.* 110, 20123–20128. doi:10.1073/pnas.1304727110

- Thametrakoln, K., Korenovska, O., Niheu, A.K., Bidle, K.D., 2012. Whole-genome expression analysis reveals a role for death-related genes in stress acclimation of the diatom *Thalassiosira pseudonana*. *Environ. Microbiol.* 14, 67–81. doi:10.1111/j.1462-2920.2011.02468.x
- Troyanskaya, O., Cantor, M., Sherlock, G., Brown, P., Hastie, T., Tibshirani, R., Botstein, D., Altman, R.B., 2001. Missing value estimation methods for DNA microarrays. *Bioinforma. Oxf. Engl.* 17, 520–525.
- Tsuda, A., Takeda, S., Saito, H., Nishioka, J., Nojiri, Y., Kudo, I., Kiyosawa, H., Shiimoto, A., Imai, K., Ono, T., Shimamoto, A., Tsumune, D., Yoshimura, T., Aono, T., Hinuma, A., Kinugasa, M., Suzuki, K., Sohrin, Y., Noiri, Y., Tani, H., Deguchi, Y., Tsurushima, N., Ogawa, H., Fukami, K., Kuma, K., Saino, T., 2003. A Mesoscale Iron Enrichment in the Western Subarctic Pacific Induces a Large Centric Diatom Bloom. *Science* 300, 958–961. doi:10.1126/science.1082000
- Valle, K.C., Nymark, M., Aamot, I., Hancke, K., Winge, P., Andresen, K., Johnsen, G., Brembu, T., Bones, A.M., 2014. System Responses to Equal Doses of Photosynthetically Usable Radiation of Blue, Green, and Red Light in the Marine Diatom *Phaeodactylum tricornutum*. *PLoS ONE* 9, e114211. doi:10.1371/journal.pone.0114211