



GENOME ANNOTATION AND TRANSCRIPTOMICS OF OIL-PRODUCING ALGAE

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Final Report

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14. ABSTRACT
Most algae accumulate triacylglycerols (TAGs) when they are starved for essential nutrients like N, S, P (or Si in the case of some diatoms). We had proposed to use whole transcriptome analyses to detail the changes in gene expression that occur during N-starvation induced TAG accumulation in Chlamydomonas. We used RNA-Seq on the Illumina platform for quantitative determination of the Chlamydomonas transcriptome. Deep coverage over multiple time points in nearly a dozen different experiments in at least 3 strains allowed us to distinguish early responses to the stress signal and to unequivocally identify relevant changes in RNA abundance. This led to the functional identification of 8 acyltransferases, several of which were documented to have a role in TAG accumulation in stressed algal cells, and candidate regulators of the N starvation response. We used our RNA-Seq data analysis pipeline for building and quantitating transcripts from Chlorella and Cyclotella in TAG-producing conditions and we assembled a draft genome for Cyclotella. During a period of project extension, we generated new strand-specific RNA-Seq data from ribosomal RNA-depleted preparations of RNA for the purpose of identifying regulatory lncRNAs.

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RNA-Seq, Chlamydomonas, biodiesel, triacylglycerol, Cyclotella, transcriptomics, regulation, stress

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Genome annotation and transcriptomics of oil-producing algae

FA9550-10-1-0095

Reporting period: 04/01/2010-12/31/2014

Abstract

Most algae accumulate triacylglycerols (TAGs) when they are starved for essential nutrients like N, S, P (or Si in the case of some diatoms). We had proposed to use whole transcriptome analyses to detail the changes in gene expression that occur during N-starvation induced TAG accumulation in *Chlamydomonas*. We used RNA-Seq on the Illumina platform for quantitative determination of the *Chlamydomonas* transcriptome. Deep coverage over multiple time points in nearly a dozen different experiments in at least 3 strains allowed us to distinguish early responses to the stress signal and to unequivocally identify relevant changes in RNA abundance. This led to the functional identification of 8 acyltransferases, several of which were documented to have a role in TAG accumulation in stressed algal cells, and candidate regulators of the N starvation response. We used our RNA-Seq data analysis pipeline for building and quantitating transcripts from *Chlorella* and *Cyclotella* in TAG-producing conditions and we assembled a draft genome for *Cyclotella*. During a period of project extension, we generated new strand-specific RNA-Seq data from ribosomal RNA-depleted preparations of RNA for the purpose of identifying regulatory lncRNAs. Some of these candidates, which map to intergenic regions, show sharp transient patterns of expression during the cell cycle consistent with a regulatory role.

Key Accomplishments

1. The analysis of the *Chlamydomonas* transcriptome in N-starved cells in 3 time course experiments is complete. We have curated enzymes involved in TAG metabolism. Besides the 5 previously described *DGTT1-DGTT5* genes (encoding type 2 diacylglycerol acyltransferases), we identified two additional genes, *DGAT1* and *DGAT3*, encoding distinct candidate enzymes. *DGAT1* had eluded prior discovery because of incomplete sequence coverage in that region of the genome. By using new sequence data and manual assembly, we have increased the sequence coverage of *DGAT1* from ~ 50% to ~ 90%. *DGTT1*, *DGAT1* and *DGAT3* are coordinately expressed in N-starved *Chlamydomonas* cells. The genes are also up-regulated in other stress situations that promote TAG accumulation, consistent with a causal connection. We developed an in vivo method for testing the function of algal genes in TAG synthesis. *DGAT3*, conserved in the plant lineage, does not support TAG synthesis in this assay, and we wonder whether it may have a regulatory role.
2. We have developed methodology for and now have considerable experience with the analysis of Illumina sequence data for transcriptome studies, and also for genome and transcript assembly. These methods have been applied to transcriptome data from *Chlorella* (sub-contract to Sayre) and *Cyclotella* (sub-contract to Hildebrand). In both cases the reads were used to assemble better transcript models and estimate transcript abundance under specific situations.
3. We also expanded the scope of the project (without increases in cost) to include genome sequencing of *Cyclotella cryptica* in collaboration with the Hildebrand group. We generated libraries from DNA and RNA and performed *de novo* assembly to generate a first draft of the genome and transcriptome of *Cyclotella cryptica*. The first draft of the genome has an N50 (i.e. typical fragment size) of 10kb and a total genome size of approximately 160Mb. The total genome estimate is in agreement with that generated previously based on calorimetric measurements. The transcript-based gene models include alternative forms generated by splicing or from different start sites. Gene models were constructed by using MAKER, which combines multiple gene prediction tool including Augustus and Fgenesh. We found that Augustus models that overlapped Maker models were the most reliable, and allowed us to generate a high confidence set of approximately 10,000 genes. We also generated bisulfite data to produce a map of cytosine methylation, and found that this mark is associated with repeat regions of the genome. The manuscript describing these results is in preparation.

4. We generated new strand-specific RNA-Seq data from rRNA-depleted samples with parallel CHIP-Seq data from 18 samples collected during the *Chlamydomonas* cell cycle. We used these reads to assemble nearly 900 new transcripts that appear to be long non-coding RNAs (lncRNAs). These lncRNAs map to intergenic regions and also to the opposite strand of protein-coding mRNAs. In individual cases that were manually curated, the RNAs do not appear to code for long proteins and their pattern of expression is transient, suggesting that they may be regulatory. Manual curation also indicates that the 5' ends have chromatin marks that are typical for PolIII transcripts, consistent with these being lncRNAs. We are very interested in continuing this work should funding permit.

Background

Most algae accumulate triacylglycerols (TAGs) when they are starved for essential nutrients like N, S, P (or Si in the case of some diatoms). In the absence of such essential nutrients they are unable to synthesize macromolecules that require these elements. Therefore, they cannot grow; rather, they divert carbon towards storage molecules – either starch or neutral lipids like TAGs. The TAGs are precursors for biodiesel because the fatty acid constituents of TAG can be transesterified to generate methyl esters.

We had proposed to use transcriptome approaches to detail the changes in gene expression that occur during N-starvation induced TAG accumulation in *Chlamydomonas*. *Chlamydomonas* is a key reference organism for the chlorophyte algae because there is a draft genome with over 17,000 gene models (many of them manually curated and functionally annotated) and there are resources for classical and reverse genetics.

We had also proposed to undertake RNA Seq analysis of *Chlorella* (for another grantee Richard Sayre at the Donald Danforth Plant Science Center at the time of the award) and to assemble a transcriptome and genome for a diatom *Cyclotella cryptica* (for another grantee Mark Hildebrand at Scripps / UCSD). *Chlorella* was of interest because it grows to high density and the diatom had been noted previously to be a high producer of TAGs. *Cyclotella* is a promising alga for biofuel production, but to date virtually nothing is known about its genome and genes.

Approach

We used RNA-Seq on the Illumina platform for quantitative determination of the *Chlamydomonas* transcriptome. Deep coverage over multiple time points in nearly a dozen different experiments in at least 3 strains allowed us to distinguish early responses to the stress signal and to unequivocally identify relevant changes in RNA abundance. Gene models were corrected based on RNA Seq coverage, which allowed the proteins to be correctly expressed in heterologous systems to validate the activity of the gene product. We also used a reverse-genetic strategy as a form of validation of the role of individual genes in the TAG accumulation pathway. The success on this project prompted us to use the RNA-Seq coverage to annotate new (previously-unobserved) transcripts from the *Chlamydomonas* genome, many of which correspond to long non-coding RNAs (lncRNAs).

Significance

The fact that multiple distinct acyltransferase enzymes are upregulated during N and other stress starvation in *Chlamydomonas* indicates that there may be several sites / foci of TAG synthesis.

The transient pattern of expression of lncRNAs suggests that they may be regulatory. Hence we are interested in pursuing this work to understand how lncRNAs impact the metabolic program, especially during the transition from carbon fixing to carbon utilizing stage of the cell cycle.

Collaborators

Christoph Benning (Michigan State University) for chemical analysis of fatty acids in TAG and TAG quantitation

Arthur Grossman (Carnegie Institution) for screening for loss of function mutations in various genes.

Mark Hildebrand (UCSD/SIO) for biology of *Cyclotella*

Key findings

1. RNA-seq coverage-based annotation of gene models generates more reliable and functional gene models.
2. We identified several TAG synthesis enzymes that had not previously been described in *Chlamydomonas* and documented their activities by functional complementation in yeast.
3. We showed that TAG accumulation occurred in several different stress situations that impact cell growth and division, with evidence for increased expression of the diacylglycerol acyltransferase-encoding genes in each case.

Recognition of the investigators and the work

Post-doctoral researcher Blaby has received a post-doctoral award from the Molecular Biology Institute at UCLA.

Former post-doctoral researcher Boyle is now an independent investigator at Colorado School of Mines.

Merchant has been honored by election to the American Academy of Arts and Sciences and the US National Academy of Science. She has also been appointed as an external member of the Max Planck Society.

The publications from this project are very well-cited (> 240 to date).

Archival publications

Ferris, P., Olson, B.J., de Hoff, P.L., Douglass, S., Casero, D., Prochnik, S, Geng, S., Rai, R, Grimwood, J., Schmutz, J., Nishii, I., Hamaji, T., Nozaki, H., Pellegrini, M., Umen, J.G. (2010) Evolution of an expanded sex-determining locus in *Volvox*. *Science* 328: 351-354.

<http://www.sciencemag.org/content/328/5976/351.long>

cited 51 times

Kropat, J., Hong-Hermesdorf, A., Casero, D., Ent, P., Pellegrini, M., Merchant, S.S., Malasarn, D. (2011) A revised mineral nutrient supplement increases biomass and growth rate in *Chlamydomonas reinhardtii*. *Plant J.* 66:770-80.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-313X.2011.04537.x/full>

cited 39 times

Boyle, N.R., Page, M.D., Liu, B., Blaby, I.K., Casero, D., Kropat, J., Cokus, S.J., Hong-Hermesdorf, A., Shaw, J., Karpowicz, S.J., Gallaher, S.D., Johnson, S., Benning, C., Pellegrini, M., Grossman, A.R., Merchant, S.S. (2012) Three acyltransferases and a nitrogen responsive regulator are implicated in nitrogen starvation-induced triacylglycerol accumulation in *Chlamydomonas*. *J. Biol. Chem.* 287: 15811-25.

<http://www.ncbi.nlm.nih.gov/pubmed/22403401>

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Merchant, S.S., Kropat, J., Liu, B., Shaw, J., Warakanont, J. (2012) TAG, You're It! *Chlamydomonas* as a reference organism for understanding algal triacylglycerol accumulation. *Curr. Opin. Biotechnol.* 23:352-363.

<http://www.sciencedirect.com/science/article/pii/S0958166911007440>

cited 64 times

Blaby, I.K, Casero, D., Glaesener, A.G., Fitz-Gibbon, S., Mettler, T., Gallaher, S., Liu, B., Boyle, N.R., Kropat, J., Stitt, M., Johnson, S., Benning, C., Pellegrini, M., Merchant, S.S. (2013) Systems-level analysis of N-starvation induced TAG accumulation in a *Chlamydomonas* starchless mutant. *Plant Cell* 25: 4305-4323.

<http://www.plantcell.org/content/25/11/4305.full.pdf+html>

cited 15 times

Urzica, E.I., Vieler, A., Hong-Hermesdorf, A., Page, M.D., Casero, D., Gallaher, S.D., Kropat, J., Pellegrini, M., Benning, C., Merchant, S.S. (2013) Remodeling of membrane lipids in iron starved Chlamydomonas. **J. Biol. Chem.** 288:30246-30258.

<http://www.jbc.org/content/288/42/30246.long>

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Organization / Institution name

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Genome annotation and transcriptomics of oil-producing algae

Grant/Contract Number

AFOSR assigned control number. It must begin with "FA9550" or "F49620" or "FA2386".

FA9550-10-1-0095

Principal Investigator Name

The full name of the principal investigator on the grant or contract.

sabeeha merchant

Program Manager

The AFOSR Program Manager currently assigned to the award

Patrick Bradshaw

Reporting Period Start Date

04/01/2010

Reporting Period End Date

12/31/2014

Abstract

Most algae accumulate triacylglycerols (TAGs) when they are starved for essential nutrients like N, S, P (or Si in the case of some diatoms). We had proposed to use whole transcriptome analyses to detail the changes in gene expression that occur during N-starvation induced TAG accumulation in Chlamydomonas. We used RNA-Seq on the Illumina platform for quantitative determination of the Chlamydomonas transcriptome. Deep coverage over multiple time points in nearly a dozen different experiments in at least 3 strains allowed us to distinguish early responses to the stress signal and to unequivocally identify relevant changes in RNA abundance. This led to the functional identification of 8 acyltransferases, several of which were documented to have a role in TAG accumulation in stressed algal cells, and candidate regulators of the N starvation response. We used our RNA-Seq data analysis pipeline for building and quantitating transcripts from Chlorella and Cyclotella in TAG-producing conditions and we assembled a draft genome for Cyclotella. During a period of project extension, we generated new strand-specific RNA-Seq data from ribosomal RNA-depleted preparations of RNA for the purpose of identifying regulatory lncRNAs. Some of these candidates, which map to

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Archival Publications (published) during reporting period:

Ferris, P., Olson, B.J., de Hoff, P.L., Douglass, S., Casero, D., Prochnik, S., Geng, S., Rai, R., Grimwood, J., Schmutz, J., Nishii, I., Hamaji, T., Nozaki, H., Pellegrini, M., Umen, J.G. (2010) Evolution of an expanded sex-determining locus in *Volvox*. *Science* 328: 351-354. <http://www.sciencemag.org/content/328/5976/351>.long
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Blaby, I.K., Casero, D., Glaesener, A.G., Fitz-Gibbon, S., Mettler, T., Gallaher, S., Liu, B., Boyle, N.R., Kropat, J., Stitt, M., Johnson, S., Benning, C., Pellegrini, M., Merchant, S.S. (2013) Systems-level analysis of N-starvation induced TAG accumulation in a *Chlamydomonas* starchless mutant. *Plant Cell* 25: 4305-4323. <http://www.plantcell.org/content/25/11/4305.full.pdf+html>
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<http://www.jbc.org/content/288/42/30246.long>
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Changes in research objectives (if any):

Not applicable

Change in AFOSR Program Manager, if any:

Dr. Kozumbo was replaced by Dr. Bradshaw during the course of the project

Extensions granted or milestones slipped, if any:

We received a 2 year extension to initiate work on lncRNAs.

AFOSR LRIR Number

LRIR Title

Reporting Period

Laboratory Task Manager

Program Officer

Research Objectives

Technical Summary

Funding Summary by Cost Category (by FY, \$K)

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