Transcriptional Response of Chlamydomonas reinhardtii Treated with Lipid-inducing Small Molecules

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Abstract

A large scale in vivo high throughput screen was performed to identify small molecules that induce lipid accumulation in the model organism *Chlamydomonas reinhardtii*. Three compounds were selected for gene expression analysis using next-generation sequencing technique. Samples were collected after 72h of treatment with 3 biological replicates for each compound. Our previous work has shown that compound treatment can induce lipid droplet accumulation by up to 6 fold and does not severely compromise growth. mRNA was isolated and sequenced on Illumina Hi-seq 2000. The sequencing reads were mapped to the genome using Tophat2 and assembled as transcriptome using Cufflinks. Out of 14520 successfully assembled genes, 789, 908 and 2079 genes were differentially expressed when treated with compound 30, 42 and 84, respectively, with expression patterns different among treatment and control. Pathway analysis revealed significant metabolic shift under compound treatment. Changes TCA cycle possibly shift central energy metabolism towards lipid biosynthesis related pathways. Down-regulation of anabolic pathway and up-regulation of ER protein processing/degradation suggests the majority of TAG is not synthesized *de novo*, but via recycling of cellular components. Up-regulation of nitrogen assimilation suggests that increased nitrogen intake may reverse the suppressive effects of compound on growth.

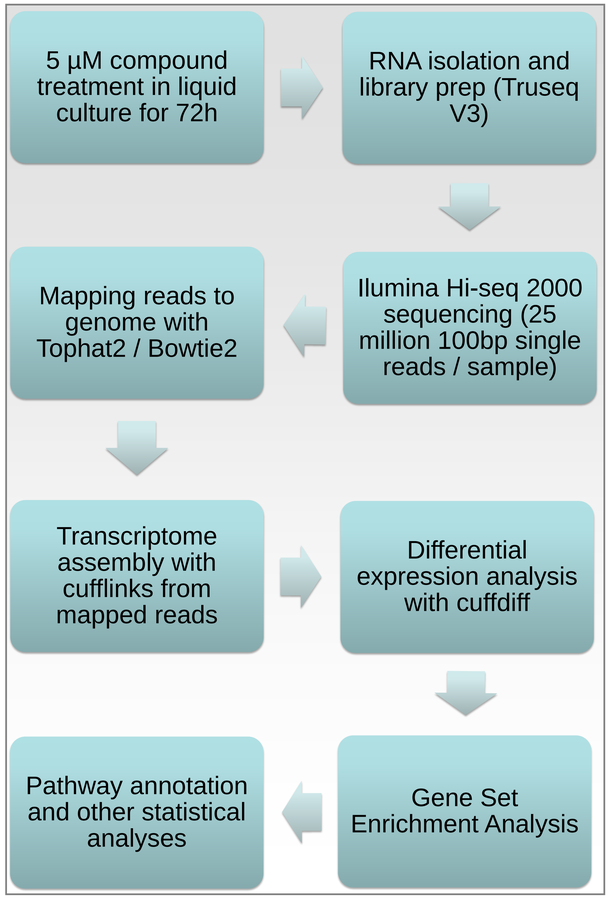
Background

Microalgae, a very large and diverse group of photosynthetic organisms, have attracted global attention as a renewable energy feedstock. Previous studies conclude that nutrient stresses, especially nitrogen starvation, induce significant lipid accumulation that might be used for the production of biofuels. However, nitrogen starvation also causes in degradation of the photosynthetic apparatus, severely limiting the rate of growth. Additionally as a lipid induction method, nitrogen limitation is impractical in large-scale production due to technical and financial drawbacks. This led us to develop a high throughput screening (HTS) system, which we have employed to identify synthetic chemical compounds that increase lipid production without severely compromising cell growth or photosynthetic capacity.

In recent years, RNA next-generation sequencing (RNA-seq) has been increasingly used in transcriptome studies for differential gene expression analysis. Compared to traditional microarray techniques, RNA-seq provides higher accuracy and larger dynamic range of the transcript levels and does not require premade microarray chips, which are not readily available for algae. Several well-accepted software tools adopted by the bioinformatics community for optimized pipelines were used in this study for computationally intensive data processing.

In this study, we focused on the transcriptional response of green algae Chlamydomonas reinhardtii on the level of pathway shifts in an attempt to elucidate the mechanisms of action of these lipid-inducing small molecules.

Study Design



Results

1. Growth and lipid accumulation

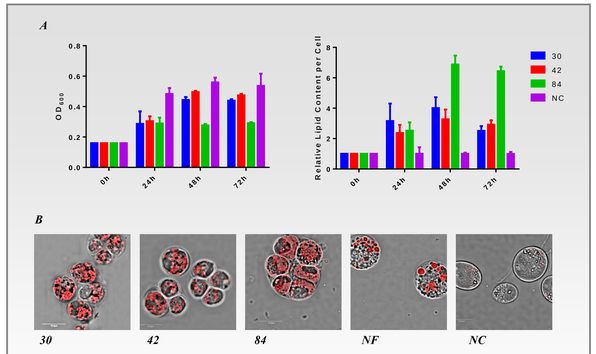


Figure 1. Growth and relative lipid content of cells with different treatments for 72h. (A) Cells in mid-log phase were washed 2× with TAP media. Compounds were added to a final concentration of 5 µM in the media. For each treatment and control, three biological replicates of 100 mL each in 250 mL flasks were grown with shaking under white light. Aliquots of 25 mL of cultures were collected at 72h of incubation. Cells were stained with Nile red and fluorescence signal measured with a BioTek Synergy plate reader. **(B)** Confocal images of algal cells stained with Nile Red to visualize lipid bodies. NF: nitrogen-free; NC: negative control.

2. Gene expression profile

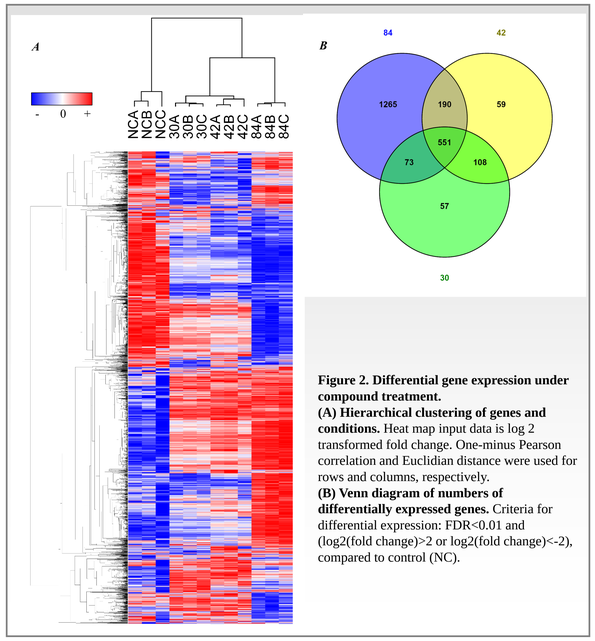


Figure 2. Differential gene expression under compound treatment.
(A) Hierarchical clustering of genes and conditions. Heat map input data is log 2 transformed fold change. One-minus Pearson correlation and Euclidian distance were used for rows and columns, respectively.
(B) Venn diagram of numbers of differentially expressed genes. Criteria for differential expression: FDR<0.01 and (log2(fold change)>2 or log2(fold change)<-2), compared to control (NC).

3. Transcriptional change in TCA cycle

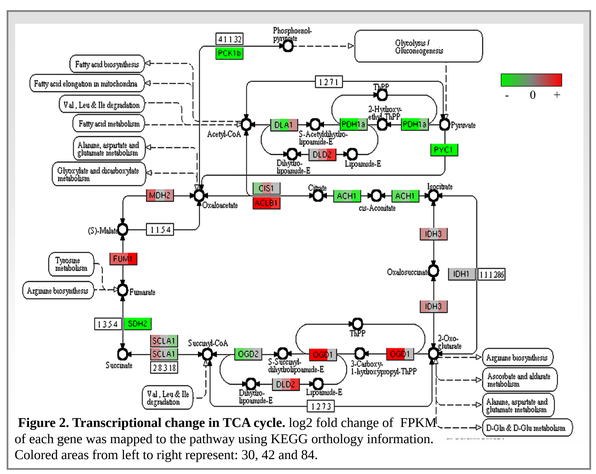


Figure 2. Transcriptional change in TCA cycle. log2 fold change of FPKM of each gene was mapped to the pathway using KEGG orthology information. Colored areas from left to right represent: 30, 42 and 84.

4. Transcriptional change in major anabolic pathways

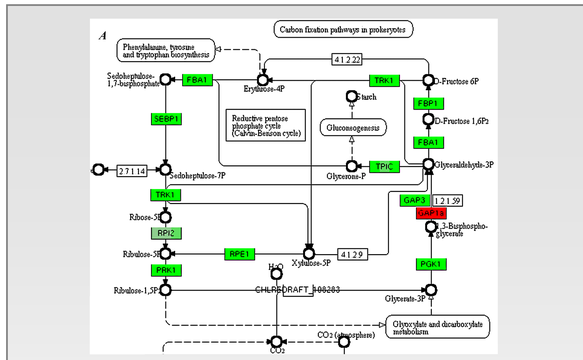


Figure 5B. (A) Transcriptional change in Calvin cycle. (B) Transcriptional change in pentose phosphate pathway. log2 fold change of FPKM of each gene was mapped to the pathway using KEGG orthology information. Colored areas from left to right represent: 30, 42 and 84.

3. Transcriptional change in other selected pathways

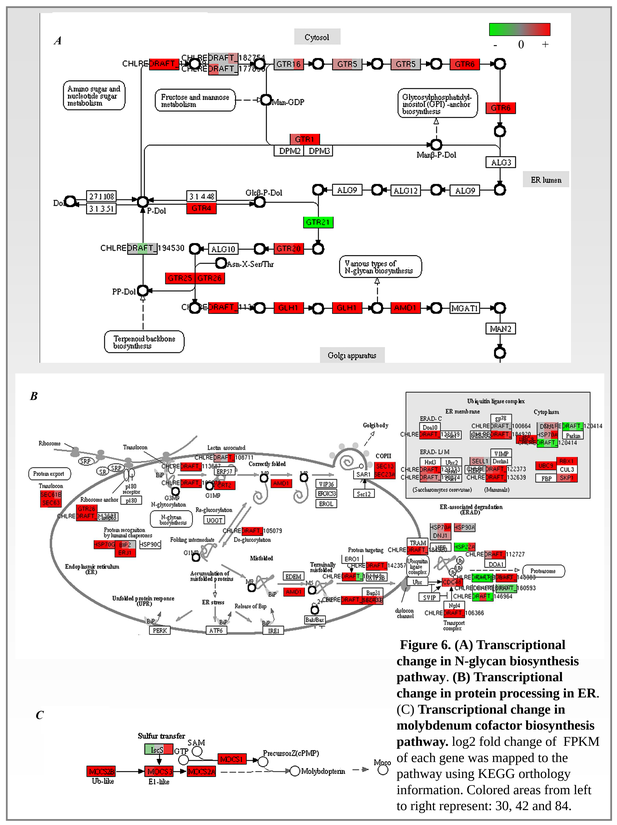


Figure 6. (A) Transcriptional change in N-glycan biosynthesis pathway. (B) Transcriptional change in protein processing in ER. (C) Transcriptional change in molybdenum cofactor biosynthesis pathway. log2 fold change of FPKM of each gene was mapped to the pathway using KEGG orthology information. Colored areas from left to right represent: 30, 42 and 84.

6. Expression of selected genes in lipid metabolism

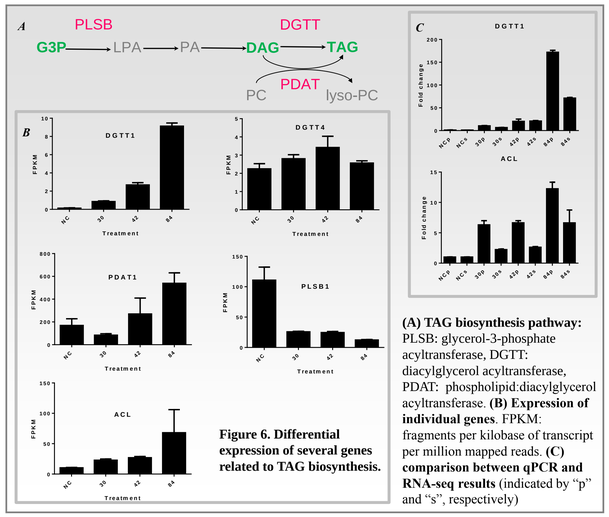


Figure 6. Differential expression of several genes related to TAG biosynthesis.

(A) TAG biosynthesis pathway: PLSB: glycerol-3-phosphate acyltransferase, DGTT: diacylglycerol acyltransferase, PDAT: phospholipid:diacylglycerol acyltransferase. **(B) Expression of individual genes**. FPKM: fragments per kilobase of transcript per million mapped reads. **(C) comparison between qPCR and RNA-seq results** (indicated by "p" and "s", respectively)

Conclusions

- Each compound induced lipid accumulation up to 6-fold higher than control
- No compound severely compromised growth, whereas nitrogen deprivation did
- Out of 14520 successfully assembled genes, 789, 908 and 2079 genes were differentially expressed when treated with compound 30, 42 and 84, respectively,
- Changes TCA cycle possibly shift central energy metabolism towards lipid biosynthesis related pathways
- .Down-regulation of anabolic pathway and up-regulation of ER protein processing/degradation suggests the majority of TAG is not synthesized de novo, but via recycling of cellular components
- Up-regulation of nitrogen assimilation suggests that increased nitrogen intake may reverse the suppressive effects of compound on growth
- Compound treatment may be useful for identifying components and mechanisms that regulate lipid synthesis and can be utilized for biofuel production

Future Directions

- Identified metabolic shifts on transcription level indicate that proteomic and metabolomics analyses are warranted
- Confirm the observed alterations in gene expression and their metabolic effects using qPCR, western blots and enzyme assays
- Perform bioinformatic analysis integrated with particle simulation to identify the direct targets of these compounds and their mechanisms of action
- Identify practical approaches to rescue the growth of algae while producing lipid

References

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