UNIVERSITY OF CALIFORNIA, SAN DIEGO

Manipulation of Lipase Activity to Increase Lipid Yields in *Thalassiosira pseudonana*

A Thesis submitted in partial satisfaction of the

requirements for the degree Master of Science

in

Biology

by

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2013

The Thesis of Jennifer Rachel Hull is approved and it is acceptable in quality and form for publication on microfilm and electronically:

Co-Chair

Chair

University of California, San Diego

2013

I dedicate this thesis to my parents Dan and Jan Hull,

Jason, Kunal and Alyssa for their love and support. I'm encouraged by your insistence to follow my passions.

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ABSTRACT OF THE THESIS

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by

Jennifer Rachel Hull

Master of Science in Biology

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Professor William Gerwick, Chair

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The accumulation of fuel precursor-containing lipid droplets in microalgae provides a sustainable alternative to conventional fossil fuels. As large-scale development is pursued, strains that efficiently accumulate triacylglycerol (TAG) without compromised growth rates are of significant interest. We pursued

increasing neutral lipid droplet yields in microalgae by inhibiting lipase activity in the diatom *Thalassiosira pseudonana*. Transcriptomics data identified enzymes likely involved in lipid catabolism, Thaps3_264297 (CGI-58 Tp1) and Thaps3_269487 (CGI-58 Tp2), which were downregulated during lipid accumulation. Lipase, phospholipase and acyltransferase activity were detected in CGI-58 Tp1, a homolog of Cgi-58, while this study found lipase activity in CGI-58 Tp1. To inhibit the activity of this specific lipase in *T. pseudonana*, antisense transgenic lines for single knock-downs of CGI-58 Tp1, and double knock-downs of CGI-58 Tp2 and CGI-58 Tp1 were generated. Both single and double knock-down transformants showed comparable growth rates in replete media and two single knock-down transformants showed twice the BODIPY fluorescence of wild-type, indicating higher lipid accumulation in stationary phase. The results indicate that downregulation of lipase activity can result in increased lipid content without affecting growth, suggesting that strains expressing the knock-down could be useful for improved biofuel production.

I:

Introduction

The combustion of fossil fuels since the Industrial Revolution has resulted in rising atmospheric carbon dioxide levels, causing global climate change that is of significant concern as temperatures and weather patterns dramatically shift. Current energy usage from fossil fuels includes coal from fossilized plants, and petroleum from fossilized algae. Both of these fuel sources were buried under earth millions of years ago and converted under high heat and pressure into carbon-rich energy sources that are used today. However, since fossil fuels are a limited resource, it's vital to develop sustainable alternatives.

Microalgae provide advantages over other biofuel crops, while similarly reducing carbon emissions. Rather than releasing carbon fixed millions of years ago, microalgae provide a sink for existing atmospheric carbon. When this CO_2 is later released by combustion, the net atmospheric gain of carbon dioxide can be lower than conventional energy sources. Alternatively to other biofuel crops, including soy and palm, microalgae do not require arable land and can be grown in wastewater or saltwater ponds (Hannon et al., 2010). As a fuel-relevant crop that can reduce carbon emissions without requiring freshwater or agricultural land, microalgae provide a promising source of sustainable energy.

Microalgae fix carbon dioxide from the atmosphere for use in various metabolic processes. They typically use fixed carbon to promote growth and division, however when microalgae are under nutrient stress, a series of reactions produce triacylglycerol (TAG), energy-rich lipid molecules stored in neutral lipid droplets. Under these conditions, glucose is converted to pyruvate via glycolysis and then into Acetyl-CoA, a precursor to fatty acid synthesis. These

fatty acids are esterified to glycerol to form the energy-storage molecule TAG, which can easily be converted into a fuel source. Thus, increasing TAG yields is crucial to the optimization of microalgae for fuel production.

Metabolic engineering of carbon pathways in microalgae has been utilized to increase TAG levels, yet previous attempts have had negative effects on growth. Primary carbon pathways have been targeted by upregulating fatty acid synthesis or preventing carbohydrate formation (Wang et al., 2009; Li et al., 2010; Work et al., 2010; Radakovits et al., 2011). While cells may accumulate higher levels of TAG than wild-type, overall yields are not improved due to compromised growth rates. However, inhibiting lipid catabolism (rather than focusing on shunting carbon away from carbohydrate synthesis) may provide a means of increasing TAG yields. Since lipid catabolism is not a central metabolic pathway, we hypothesize that inhibiting lipid breakdown in microalgae will increase TAG levels without compromising growth rates.

Diatoms are a specific type of microalgae that are especially attractive in the development of algae biofuel for their lipid accumulation, genetic diversity, and environmental flexibility (Hildebrand et al., 2012). Since diatoms have a silaceous outer covering, known as a frustule, their growth is dependent on silicic acid concentration. Under silicon limiting conditions diatoms have decreased growth rates and are known to accumulate lipids. While placed under silicon-limited conditions, diatoms shift lipid anabolism from producing membrane lipids to neutral lipids in the form of triacylglycerols for carbon storage (Hu et al., 2008). The shift to lipid anabolism under silicon-limited conditions may be in preparation

for a resting stage of development. Diatoms sink into cold, dark water for survival when surface waters are depleted of valuable nutrients necessary for growth (Smetacek, 1985). The accumulation of TAG under these conditions likely provides a carbon source at depths where photosynthesis to fix CO_2 is not possible. This process of naturally shunting carbon metabolism toward lipid accumulation can be used to study genes involved in lipid synthesis and catabolism.

Genes with altered transcription during lipid droplet formation may play a vital role in regulating lipid accumulation and breakdown. Because lipid catabolism is a competitive process to lipid biosynthesis, lipases and lipase activators may be downregulated during lipid accumulation. A recent study in the diatom *Thalassiosira pseudonana* monitored RNA transcript levels of genes containing a lipase domain during control and silicon-limited conditions (Trentacoste et al., in review). This study identified two genes significantly downregulated during lipid accumulation: CGI-58 Tp1 and CGI-58 Tp2 . These genes are likely involved in lipid catabolism by releasing fatty acids from the glycerol backbone of TAG.

The protein CGI-58 Tp1 is homologous to the characterized lipase Comparitive Gene Identification-58(CGI-58) found in humans. The involvement of CGI-58 in lipid catabolism was hypothesized because a loss of function mutation in CGI-58 is associated with the lipid storage disease Chanarin-Dorfman syndrome; which is characterized by TAG accumulation in non-adipose tissue (Ghosh et al., 2008). Homologues of CGI-58 found in plants, yeast, and

vertebrates all contain the highly conserved acyltransferase motif $H(X)_4D$, and plant and yeast homologues contain the lipase motif GXSXG (James et al., 2010).

To understand its physiological role, CGI-58 was transformed and overexpressed in *Saccharomyces cerevisiae*, resulting in decreased TAG and increased phospholipid levels (Ghosh et al., 2008). These findings suggest a role of CGI-58 in maintaining TAG and phospholipid homeostasis. When the CGI-58 locus was disrupted in *Arabidopsis thaliana*, loss of function mutants had larger amounts of TAG than wild-type plants (James et al., 2010). This indicates that knock-down of genes encoding lipases in algae may similarly result in lipid accumulation. To identify whether CGI-58 Tp1 (containing the $H(X)_4D$ and GXSXG motifs) was a potential candidate for knock-down in *T. pseudonana*, heterologous protein expression followed by activity assay confirmed lipase, phospholipase and acyltransferase activity (Trentacoste et al., in review). When knock-down strains of CGI-58 Tp1 were generated, transformants exhibited higher total lipid levels, including increased TAG and membrane lipids under nutrient-replete and deplete conditions.

Here we report the heterologous expression and characterization of CGI-58 Tp2. After confirming lipase activity, we generated five antisense RNA knock-down transformants. Two knock-down transformants of CGI-58 Tp2 showed increased TAG levels with comparable growth to the wild-type (WT) under nutrient-replete conditions. Three double knock-down transformants of CGI-58 Tp1 and CGI-58 Tp2 were generated with comparable growth to wild-type. These

results indicate that inhibition of this particular lipase activity can result in increased TAG levels without compromising growth.

II:

Materials and Methods

Optimization of heterologous soluble protein expression

Gene-specific primers were designed to amplify the gene CGI-58 Tp2 from *T. pseudonana* complementary DNA (cDNA) using PCR. The forward primer CGI-58 Tp2 NdeI-F (5'-GGGCATATGAACGTTGTTGA-3') includes a 5' NdeI restriction site, and the reverse primer, CGI-58 Tp2 SacI-R (5'-CCCGAGCTCCTAATCACCC-3') includes a 5' SacI restriction site. PCR was performed using Pfx high-fidelity DNA polymerase (Invitrogen) and ligation reactions using T4 DNA ligase (New England Biolabs). The vector used to heterologously express CGI-58 Tp2 in *E. coli* was constructed from the pet28b+ (Novagen) expression vector and expressed in BL21 *E. coli* (Stratagene).

Figure 1.Gene sequence of CGI-58 Tp1

Since the enzymatic activity of CGI-58 Tp2 was unknown, it was ideal to obtain CGI-58 Tp2 in the soluble fraction for further testing. Various conditions to optimize soluble expression were tested. Soluble proteins were separated using SDS-PAGE and gels visualized under UV (Nusep). Western blots using Thermo Pierce mouse 6XHis primary anti-His tag antibody and Thermo Pierce Goat anti-mouse horseradish peroxidase-conjugate secondary antibody were used to

detect recombinant protein.

Determination of CGI-58 Tp2 activity using lipase assay

Lipase activity was determined using the MarkerGene Fluorescent Lipase Assay Kit (Marker Gene), using purified *Candida albicans* lipase (Invitrogen) to generate a standard curve, and purified alcohol dehydrogenase (Invitrogen) as a negative control. Tested soluble fractions included CGI-58 Tp2 and pET-28b(+) empty vector treated for protein expression using 0.1 mM IPTG, 30°C, 1 hr, 225 RPM. The lipase inhibitor tetrahydrolipstatin (Orlistat) was used at 100 µM.

Gateway Cloning to generate plasmid for single knock-down of CGI-58 Tp2

PCR was performed to amplify DNA antisense to CGI-58 Tp2 gene, including the attB5 CGI-58 Tp2 antisense forward primer (5'-GGGGACAACTTTGTATACAAAAGTTGGCCCATTGAGTTGAGTGTTTTATAACT-3') and the attB2 CGI-58 Tp2 antisense reverse primer (5'-GGGGACCACTTTGTACAAGAAAGCTGGGTACTTCCAATGAACGAATAT-3'). The PCR product included 578 nucleotides of the coding region and 23 nucleotides of the 3' UTR flanked by the attB5 and attB2 recombination sequences. The PCR product was purified and knock-down vectors pA78_269487 and pA78_269487_264297 were constructed using Invitrogen's Multi-Site Gateway Cloning Protocol. First, a BP reaction was performed to clone the PCR fragment into the pDONR P5-P2 vector, generating the entry clone pDONR L5-L2 (containing CGI-58 Tp2 antisense). Then, an LR reaction was performed combining the entry vectors pMHL_107 (containing NAT resistance)

and pDONR L5-L2 with the destination vector pMHL_78. This LR reaction generated the following expression clone:

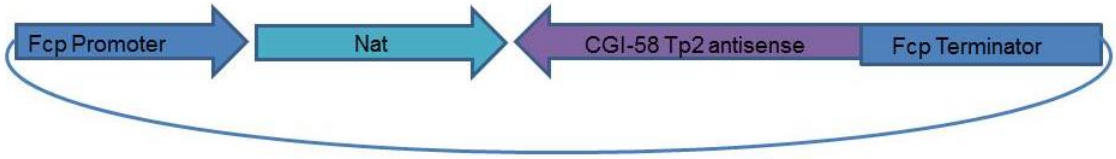


Figure 2. pA78_269487 expression clone for knock-down of CGI-58 Tp1

Gateway cloning to generate plasmid for double knock-down of CGI-58 Tp2 & CGI-58 Tp1

PCR was performed to amplify DNA antisense to the CGI-58 Tp2 gene. The primers utilized were the attB4r CGI-58 Tp2 antisense forward primer (5'-GGGGACAACTTTTCTATACAAAGTTGTTCCATTGAGTTGAGTGTTTTATAACT-3'), and the attB3r CGI-58 Tp2 antisense reverse primer (5'-GGGGACAACTTTATTATACAAAGTTGTCTTCCAATGAACGAATATCTGC-3'). The PCR product was purified and a BP reaction was performed to clone the fragment into the pDONR P4r-P3r vector, generating the entry clone pDONR L4r-L3r (CGI-58 Tp2 antisense). An LR reaction was performed combining the entry vectors pMHL_103 (NAT resistance), pDONR L4r-L3r (CGI-58 Tp2 antisense), pDONR L3-L2 (CGI-58 Tp1 antisense) with the destination vector pMHL_78. This LR reaction generated the expression clone diagramed below:

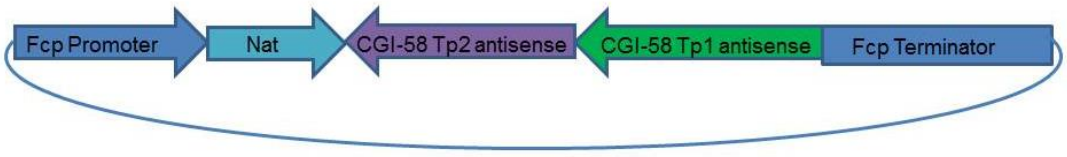


Figure 3. pA78_269487_264297 expression clone for knock-down of CGI-58 Tp1 and CGI-58 Tp2

Transformation of expression clones for knock-down into *T. pseudonana*

The expression clones for single and double knock-down were coated onto Tungsten particles and transformed into *T. pseudonana* using microparticle bombardment with BioRad PDS-1000 (Bio-Rad, Hercules, CA) using established procedures (Dunahay et al., 1995).

Strains and culture conditions

T. pseudonana was grown in batch culture with shaking or stirring and aeration under continuous illumination at 150 µmol m^{-2} s^{-1} at 18 to 20°C in sterile artificial seawater medium (ASW) supplemented with biotin and vitamin B12 at 1 ng L^{-1} (Darley and Volcani, 1969).

Enzymatic activity assay of knock-down strains

Phospholipase activity was determined using the EnzCheck Phospholipase A2 Assay (Life Technologies), using purified Phospholipase A_2 to generate a standard curve, and purified alcohol dehydrogenase (Invitrogen) as a negative control. The lipase inhibitor tetrahydrolipstatin (Orlistat) was used at 100 µM.

Imaging flow cytometry

Imaging flow cytometry data were collected on an AmnisImageStream^X at 40X magnification. Frozen cell pellets were resuspended in 2.3% NaCl solution and stained with 2.3 µg/mL BODIPY for 15 min, excited with a 488 nm laser at 10mW, and brightfield and fluorescent images were collected for 20,000 events. Amnis IDEAS 4.0 Software was used to analyze raw image files. Cutoffs for in-focus and single cells were determined manually, and images were screened to remove those of debris.

III:

Results

Heterologous expression and characterization of CGI-58 Tp1

CGI-58 Tp2 was hypothesized to play a role in lipid catabolism because it contained a lipase motif and was downregulated during lipid accumulation similarly to CGI-58 Tp1 (Fig. 4; Trentacoste et al., in review).

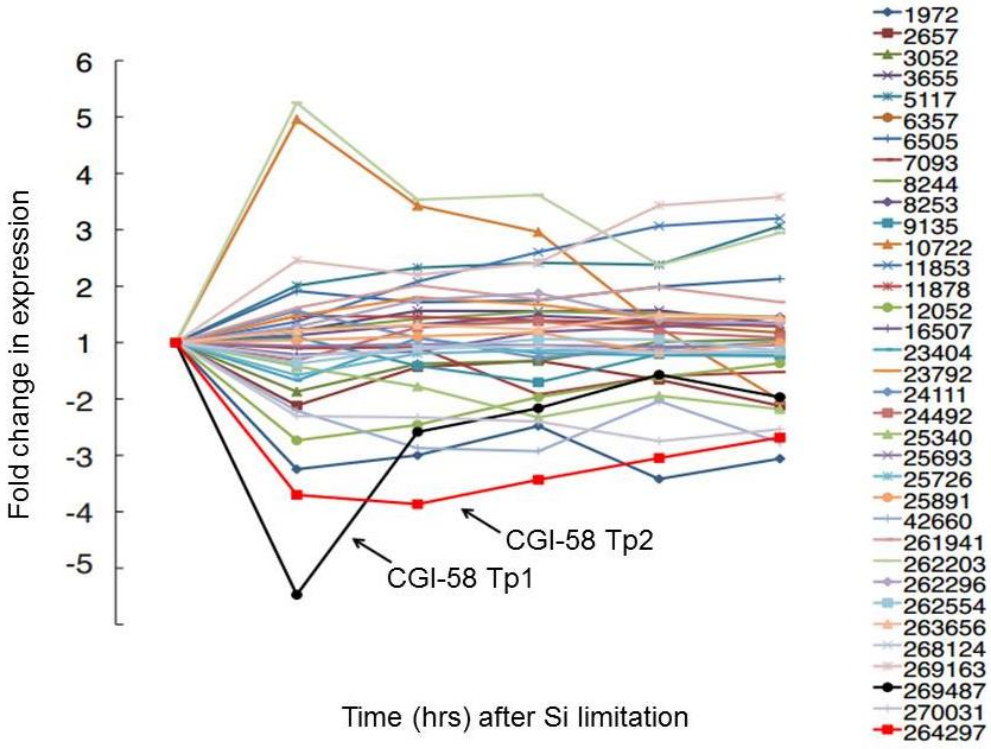


Figure 4. Transcript levels of enzymes containing a lipase motif during lipid accumulation (Trentacoste et al., in review)

Annotation of CGI-58 Tp2 indicated a 357 amino acid, 40-kilodalton protein product. A BLAST search revealed the protein was most similar to CGI-58 homologues in other organisms. CGI-58 Tp2 contains the acyltransferase motif $H(X)_4D$ (Heath and Rock, 1998) and a lipase motif. This lipase motif AXSXG found in CGI-58 Tp2 is a less common variation of the lipase motif GXSXG (Beranek et al., 2009), which is found in the CGI-58 yeast and plant

homologues. The similar combination of motifs in CGI-58 Tp1 and CGI-58 Tp2 suggests that these enzymes may have similar activity.

Figure 5. ClustalW2 multiple sequence alignment depicting lipase motif (GXSXG or AXSXG) and acyltransferase motif (HX_4D) in CGI-58 homologs.

Optimization of heterologous soluble protein expression

To understand the role of CGI-58 Tp2, we expressed His6-tagged recombinant CGI-58 Tp2 protein in *Escherichia coli* under control of the T7 promoter. Soluble expression levels were highest using 0.1 mM IPTG, 30°C, 1 hr, with shaking of cultures at 225 RPM (Table 1, Figs. 5, 6) and an in vitro lipase assay revealed activity. In the presence of the lipase inhibitor tetrahydrolipstatin (Orlistat), enzymatic activity of CGI-58 Tp1 was reduced.

Table 1. Summary of conditions tested for soluble protein expression

Temperature	IPTG Concentration	Time	RPM	Soluble protein expression?
18°C	0	O/N	225	Y
	0.5	O/N	225	N
25°C	0	O/N	0	Y
	1	O/N	0	N
30°C	0.1	1 hr	225	Y
	0.1	3 hr	225	N
	0.5	3 hr	225	N
37°C	0.1	2 hr	225	N
	0.5	2 hr	225	N

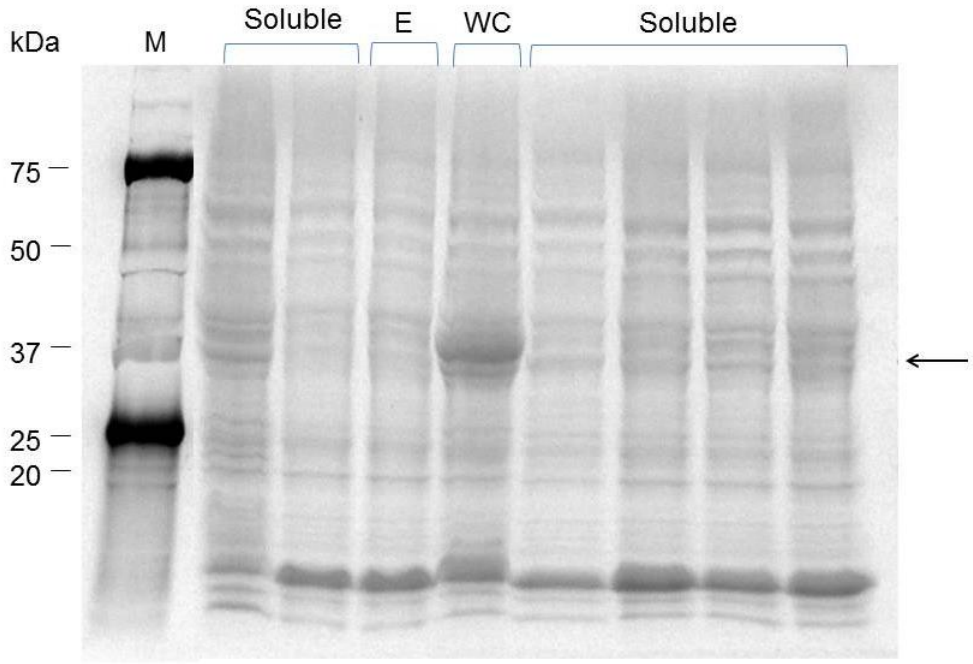


Figure 6. SDS-PAGE gel showing expression levels after treatment with 0.1 mM IPTG at 30°C for 1 hr. Soluble fraction with CGI-58 Tp2 (Soluble), soluble fraction pET-28b(+) empty vector (E), whole cells with CGI-58 Tp2 (WC)

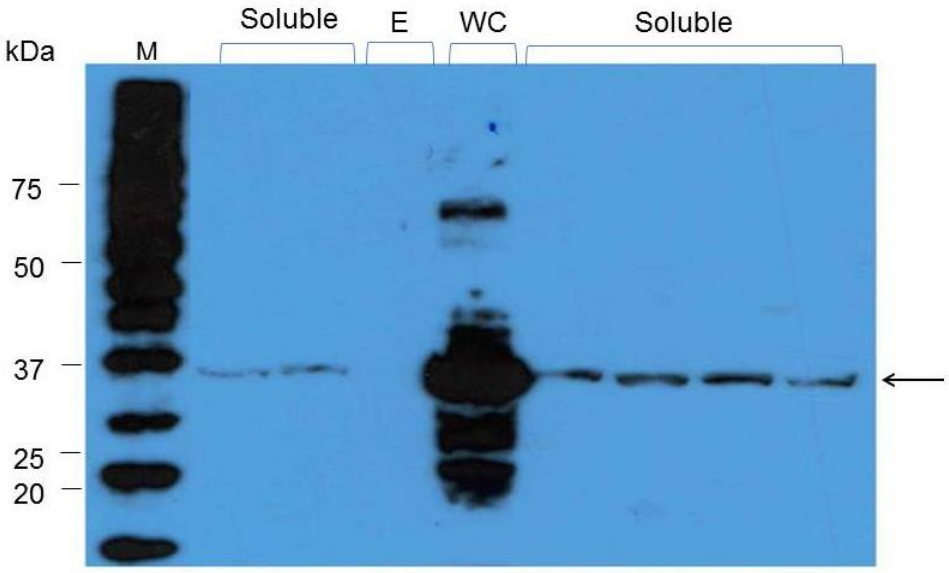


Figure 7. Western blot showing expression levels after treatment with 0.1 mM IPTG at 30°C for 1 hr. Soluble fraction with CGI-58 Tp2 (Soluble), soluble fraction pET-28b(+) empty vector (E), whole cells with CGI-58 Tp2 (WC)

In vitro assay of soluble lysates containing recombinant CGI-58 Tp2 protein revealed TAG lipase activity that was inhibited by tetrahydrolipstatin

(Orlistat), a known lipase inhibitor (Fig. 7). Statistical analyses were performed using student's t-test to compare CGI-58 Tp2 to the empty pet-28b(+) vector (* $P < 0.05$). These data indicated CGI-58 Tp2 is involved in lipid catabolism by releasing fatty acids from the glycerol backbone of TAG.

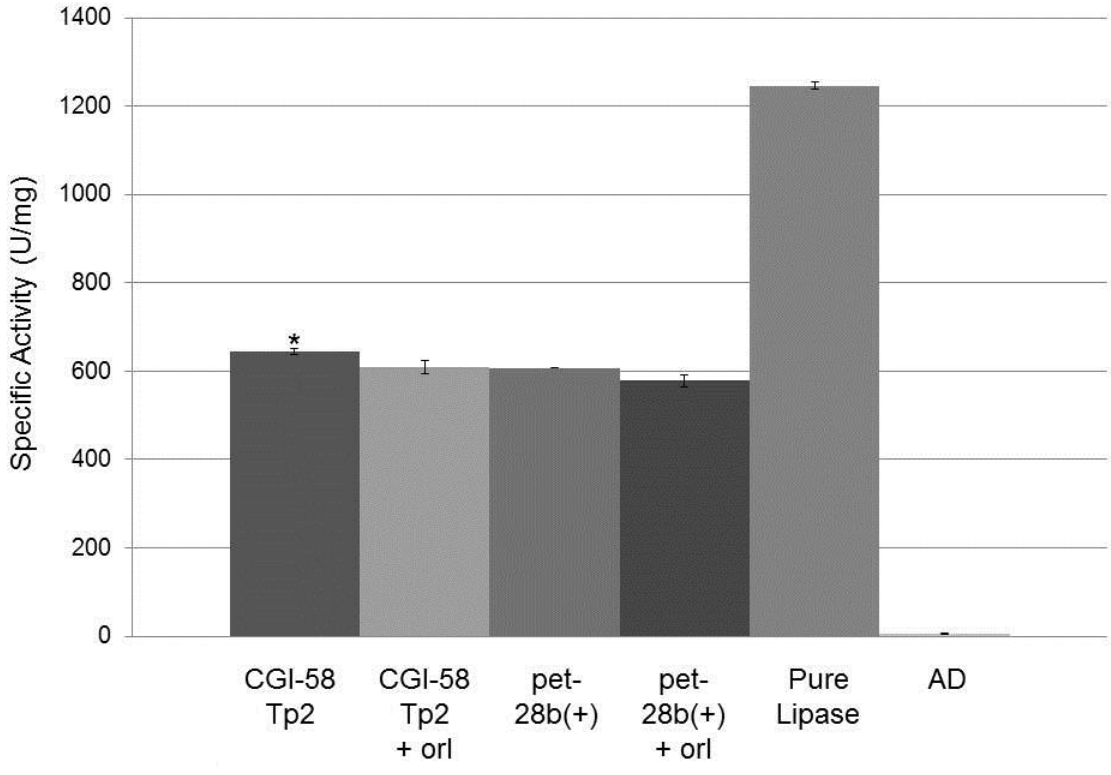


Figure 8. Lipase assay results for CGI-58Tp2 soluble fraction, CGI-58 Tp2 soluble fraction with orlistat, pet-28b(+) empty vector soluble fraction, pet-28b(+) empty vector soluble fraction with orlistat, pure lipase, and alcohol dehydrogenase (negative control). Statistical analyses were performed using student's t-test to compare CGI-58 Tp2 to the empty pet-28b(+) vector (* $P < 0.05$). Error bars, s.e.m.

Knock-down of CGI-58 Tp2 using antisense RNA

The knock-down procedure targeted functional CGI-58 Tp2 mRNA with antisense RNA to prevent translation. Two antisense RNA-encoding knock-down constructs were made, pA78_269487 encoding antisense RNA for single knock-down of CGI-58 Tp2, and pA78_269487_264297 encoding antisense RNA for

double knock-down of CGI-58 Tp2 and CGI-58 Tp1. Expression was driven by the fucoxanthin chlorophyll a/b-binding protein (fcp) promoter (Poulsen et al., 2006) and the nourseothricin resistance gene *nat1* was included as a selectable marker. The constructs were transformed into wild-type *T. pseudonana* and transformants selected using nourseothricin. The presence of integrated constructs was confirmed using PCR. Five transformants (two single knock-down and three double knock-down strains) were screened for growth and lipid accumulation under nutrient-replete conditions. All single and double knock-down strains showed comparable growth to WT (Figs. 9, 11). TAG levels were monitored using BODIPY staining and imaging flow cytometry. Single knock-down strains had higher TAG levels in stationary phase compared to WT under nutrient-replete conditions (Fig. 10). Knock-down strain 2.A3 had a 2-fold increase in BODIPY fluorescence per cell. Both replicates in one of the double knock-down transformants, 3.A2, exhibited higher TAG levels during exponential phase, while double knock-down strain 3.9 and 3.A6 had similar TAG levels as wild-type (Fig. 12).

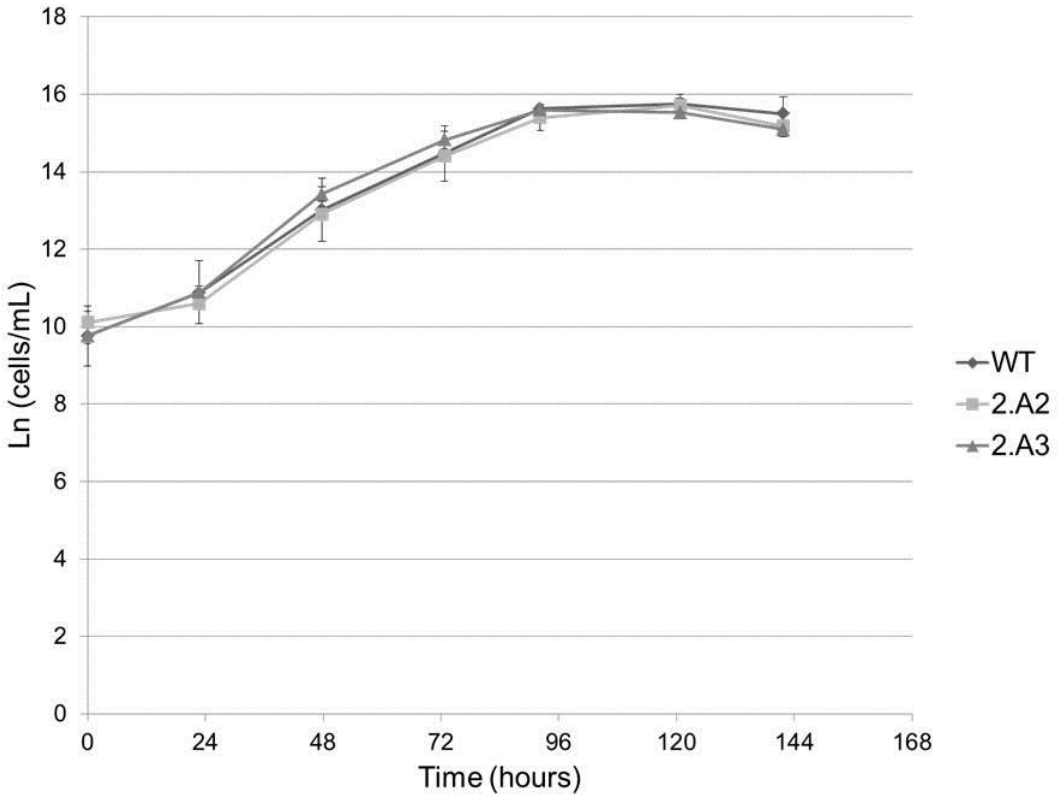
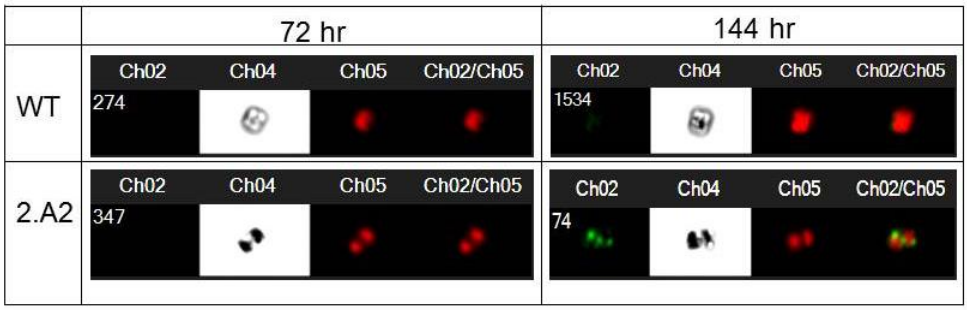


Figure 9. Growth curve comparing wild-type with the single knock-down strains 2.A2 and 2.A3. Each point represents the average of two biological replicates. Error bars, s.d., n=2.

A.



B.

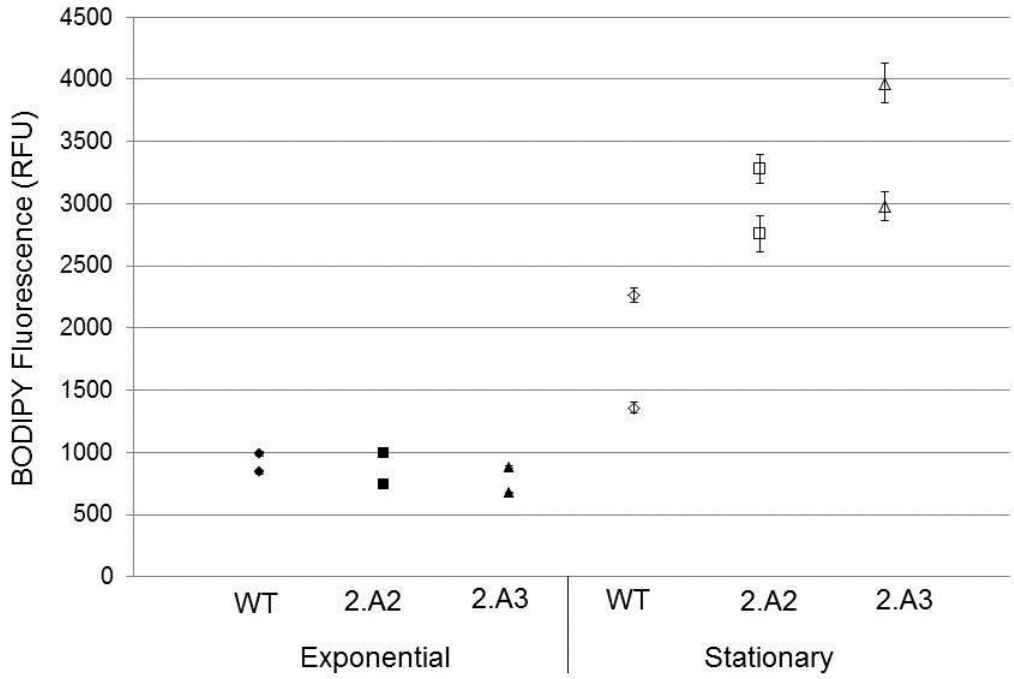


Figure 10. (A) Flow cytometric images of individual cells depicting BODIPY fluorescence (Ch02), brightfield image (Ch04), and chlorophyll fluorescence (Ch05) during exponential phase (72 hr) and stationary phase (144 hours) for WT and single knock-down strain 2.A2.
(B) BODIPY fluorescence per cell in Raw Fluorescence Units (RFU) vs. time. Each point represents the average of a population for a single biological replicate from WT, 2.A2, or 2.A3. Error bars, s.e.m.

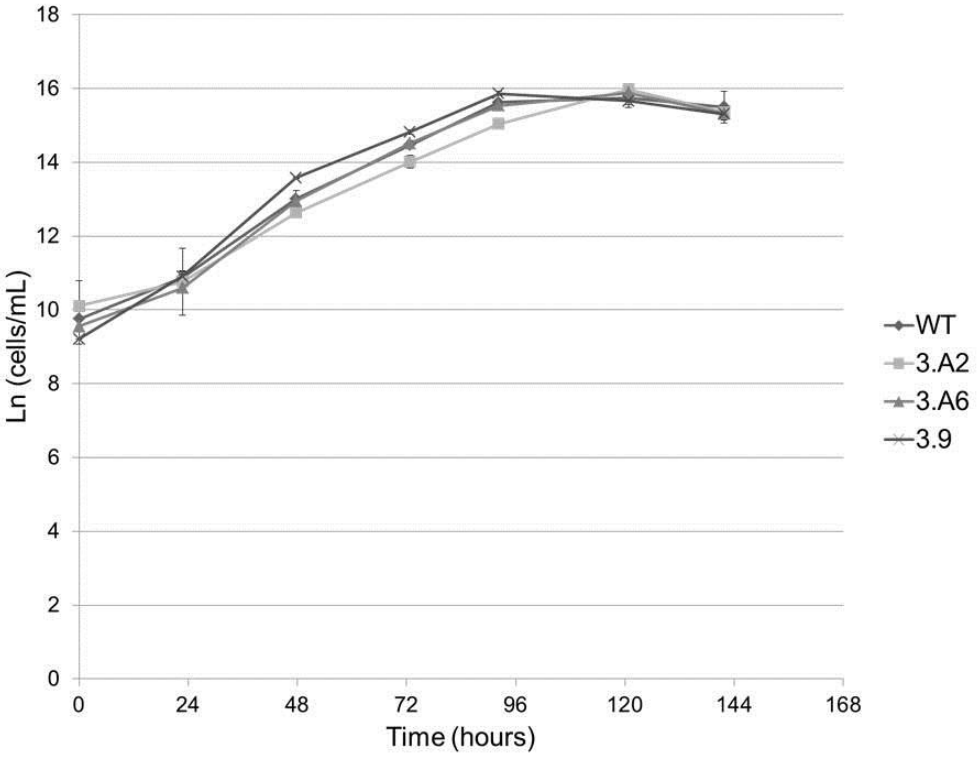


Figure 11. Growth curve comparing wild-type with the double knock-down strains 3.A2, 3.A6, and 3.9. Each point represents the average of two biological replicates. Error bars, s.d., n=2.

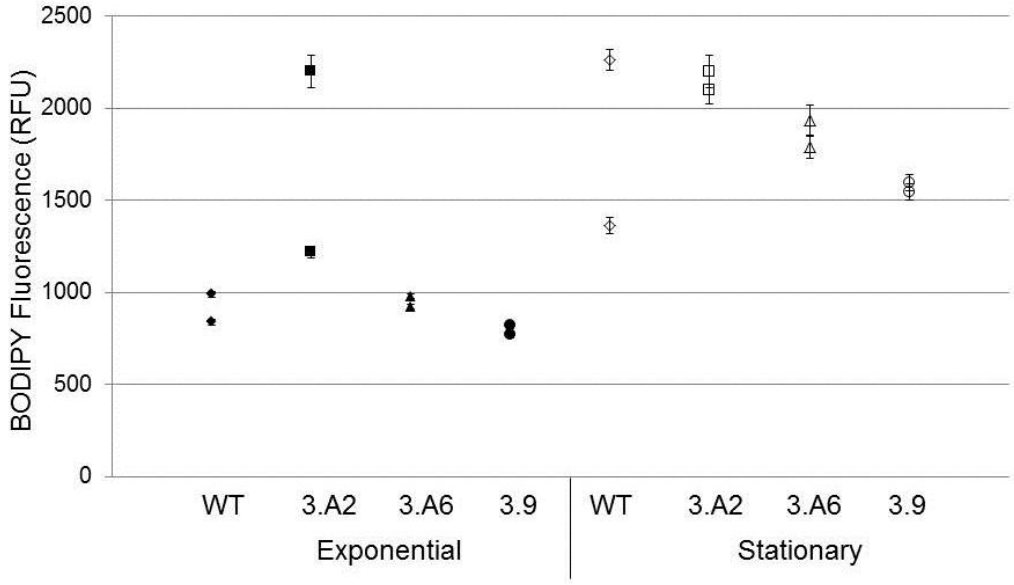


Figure 12. BODIPY fluorescence per cell in Raw Fluorescence Units (RFU) vs. time. Each point represents the average of a population for a single biological replicate from WT, 3.A2, 3.A6, or 3.9. Error bars, s.e.m.

Enzymatic activity assay

An in vitro enzymatic activity assay was used to compare phospholipase activity of single knock-down strains (2.A2, 2.A3) and double knock-down strains (3.A2, 3.9) to wild-type (Fig. 13). Alcohol dehydrogenase served as a negative control.

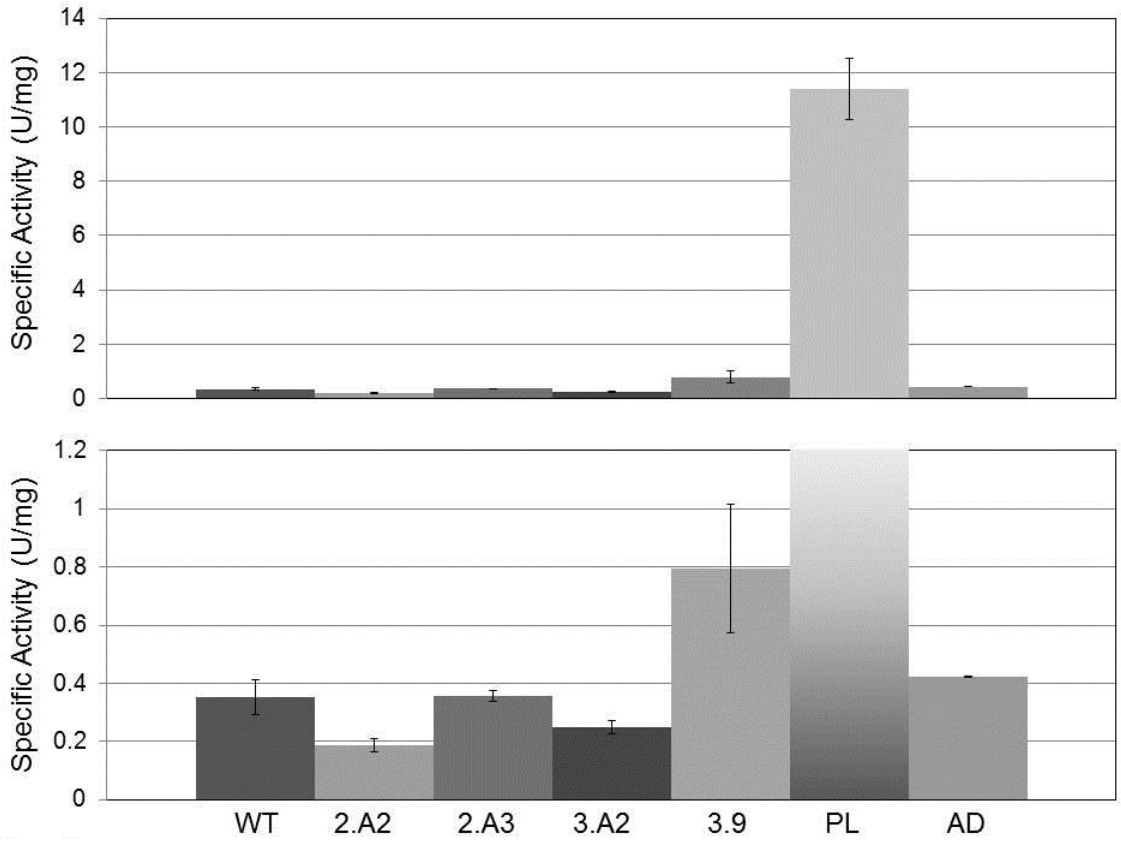


Figure 13. Phospholipase activity in U/mg for wild-type (WT), knock-down strains, and alcohol dehydrogenase (negative control). Error bars, s.e.m.

In the phospholipase assay, the negative control alcohol dehydrogenase showed comparable activity to wild-type. No conclusions were drawn from these unexpected results and we plan to re-run this assay with fresh soluble cell lysates and a new kit.

IV:

Discussion

Microalgae provide a sustainable fuel source that can be optimized for large-scale development by increasing lipid yields. Manipulating pathways to increase the amount of TAG per cell without compromising growth rates can significantly improve the recovery of fuel-relevant molecules from microalgae. In this study we hypothesized that knock-down of lipase activity would improve TAG yields without compromising growth. To test this hypothesis, we identified and characterized an enzyme with a proposed role in lipid catabolism, CGI-58 Tp2. After confirming lipase activity, antisense knock-down was used to target lipase-encoding transcripts. Single knock-down transformants, 2.A2 and 2.A3, showed higher TAG levels in stationary phase than wild-type, while the double knock-down transformant 3.9 showed higher TAG levels during exponential phase. While these results are likely from knock-down of lipase activity, further testing is required to confirm decreased transcript levels by qRT-PCR or decreased enzyme levels by Western blot.

In the phospholipase assay comparing activity from wild-type to knock-down strains, alcohol dehydrogenase showed comparable activity to wild-type. These results were unexpected and we plan to re-run this assay with fresh soluble cell lysates and a new kit. Since the activity of WT, 2.A2, 2.A3, and 3.A2 were all similar to the negative control we are unable to draw conclusions about reduced phospholipase activity in the knock-down strains. However, comparable activity of the knock-down strains to wild-type may result from the activity of other lipases masking the effect of knock-down. Furthermore, the positive control of purified phospholipase appeared to show significant activity and double knock-

down 3.9 seemed to have increased phospholipase activity compared to wild-type. Further testing is required to confirm this result but it could indicate that decreased levels of CGI-58 Tp1 and CGI-58 Tp2 resulted in upregulation of other enzymes with phospholipase activity.

Previous attempts to increase TAG yields have involved upregulating genes involved in lipid production or diverting carbon away from carbohydrate synthesis. The overexpression of an enzyme involved in TAG synthesis, Acetyl CoA Carboxylase, did not improve oil yields (Sheehan et al., 1998); heterologous expression of thioesterase genes in *Phaeodactylum tricornutum* to increase production of shorter chain length fatty acids resulted in compromised growth rates (Radakovits et al., 2011); when carbon was diverted from starch synthesis in low starch and starchless mutants, neutral and total lipid levels per cell increased but growth rates were compromised (Li et al., 2010). While these previous studies shed light on pathways associated with lipid production, significantly improving yields will require higher TAG per cell without inhibiting growth. Since carbohydrates, proteins, and lipids are all relevant to biofuel production, biomass yields are crucial to their economic viability (Barreiro et al., 2012). Thus, our study targeted lipid catabolism, a process downstream of lipid synthesis that should not interfere with central carbon pathways associated with growth.

Through knock-down of lipases involved in the breakdown of TAG, we hoped to improve lipid yields. To our knowledge, knockdown of CGI-58 Tp1 and CGI-58 Tp2 are the first targeted manipulations that have resulted in increased

TAG levels without compromising growth. The results of this study are consistent with knock-down transformants of CGI-5 Tp1, which exhibited comparable growth to wild-type and higher lipid levels under nutrient-replete and nutrient-deplete conditions (Trentacoste et al., in review). These results indicate that targeting lipid catabolism is a practical means of increasing lipid yields in microalgae.

In the development of tools for genetic manipulation, the ability to target multiple genes using a single vector for transformation is highly valuable. Previous attempts to achieve double knock-down using a single transcript in *T. pseudonana* have been unsuccessful. This study attempted simultaneous knock-down of the two lipases CGI-58 Tp2 and CGI-58 Tp1. We suspected that previous attempts of double knock-down were unsuccessful because antisense transcripts targeted genes necessary for growth, resulting in unviable transformants. Since lipase activity is not considered a central part of carbon metabolism, we did not expect compromised growth in double knock-down strains.

We have confirmed lipase activity in a previously uncharacterized enzyme and used antisense knock-down to increase TAG yields in *T. pseudonana*. These findings can be translated to other species, as all eukaryotes have homologues of CGI-58, which are likely to have lipase activity. Overall we have shown an approach to improve TAG yields without compromising growth to improve the economic viability of developing microalgal biofuels.

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