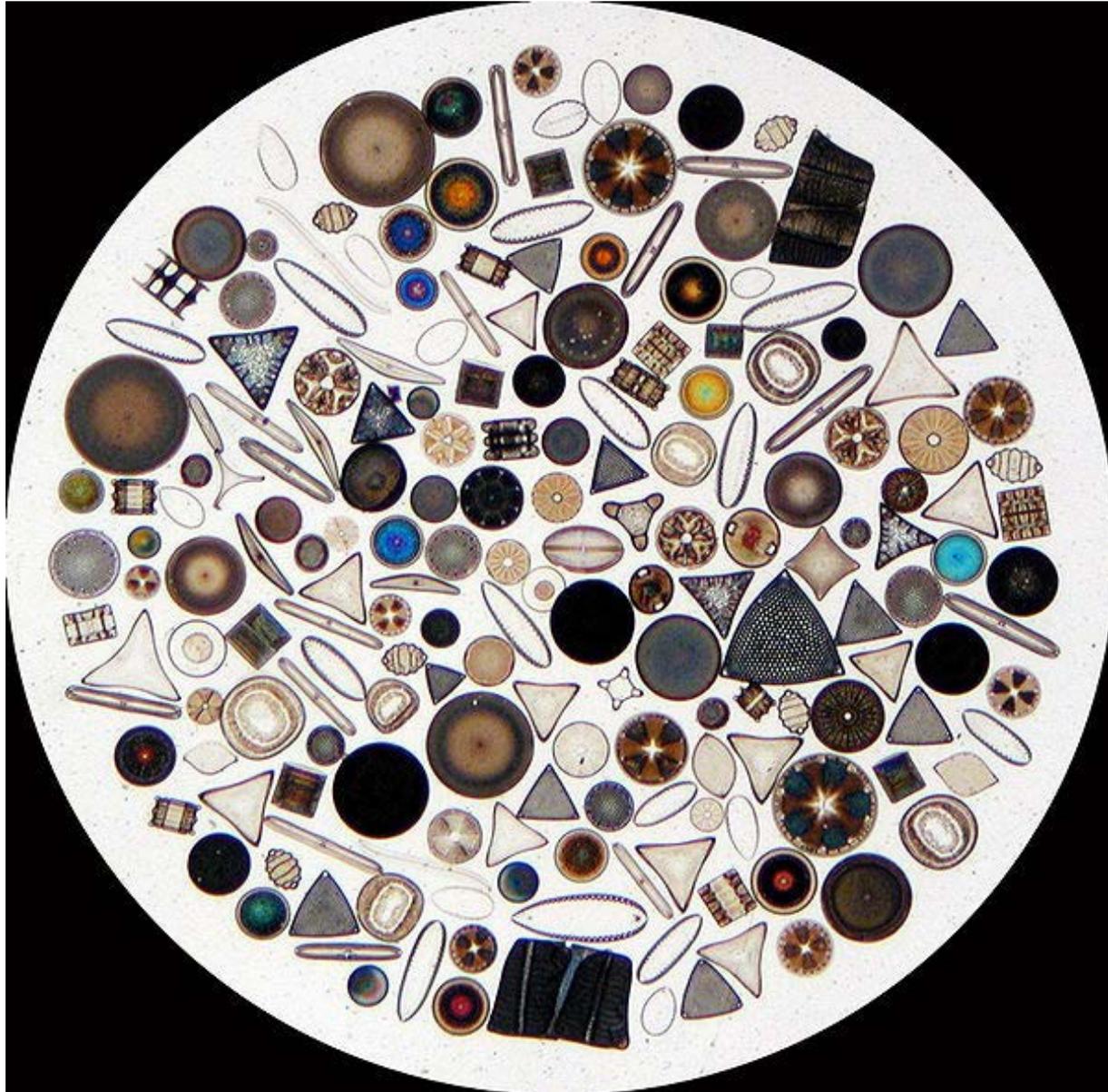


Rebels with a cause: engineering diatoms into fuel factories

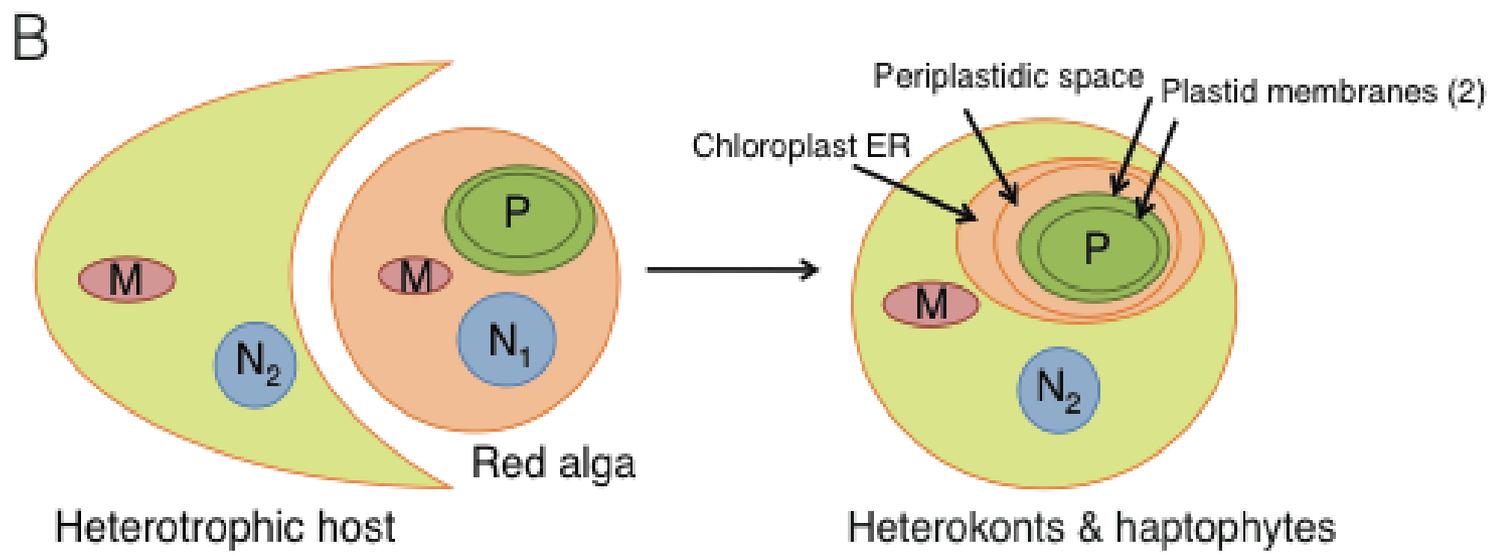
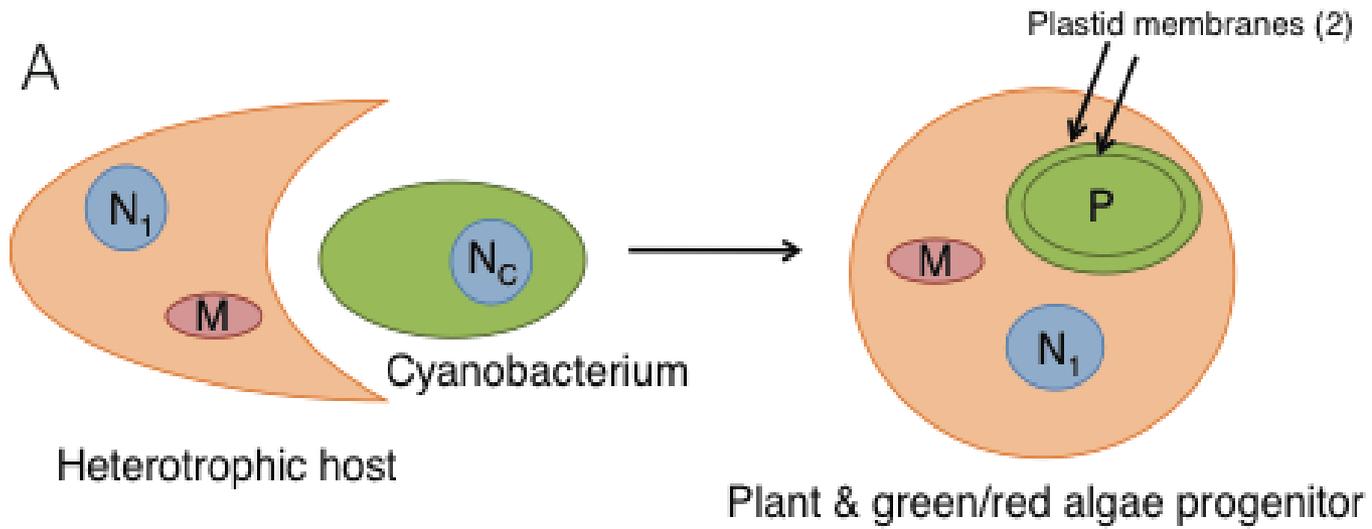
Emily Trentacoste
NOAA Office of Aquaculture
Scripps Institution of Oceanography
8/21/14

Roshan Shrestha, Sarah Smith, Corine Glé, Aaron Hartmann, Mark Hildebrand, Bill Gerwick

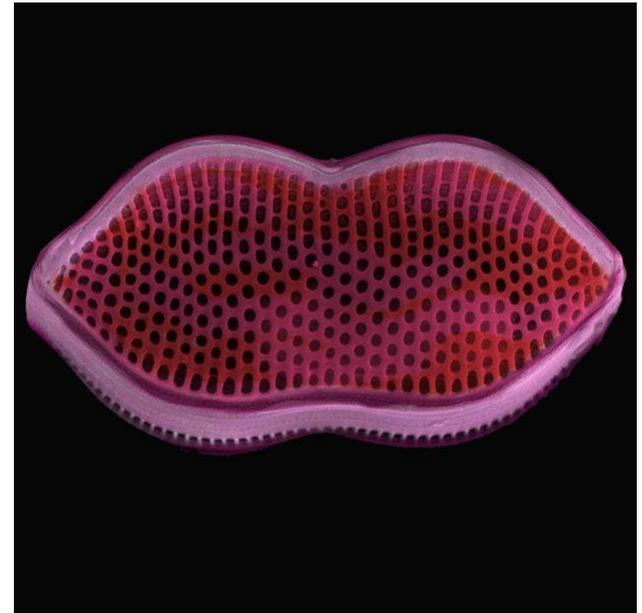
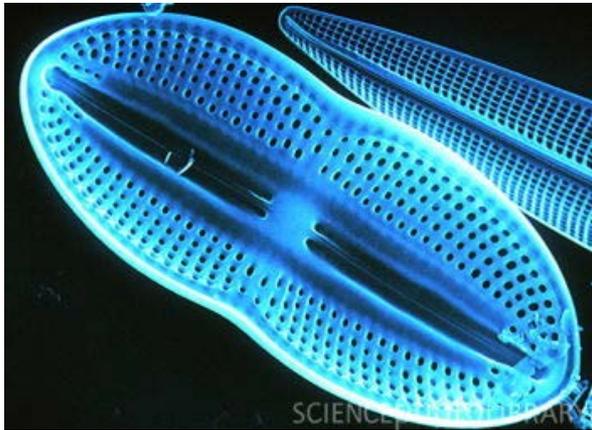
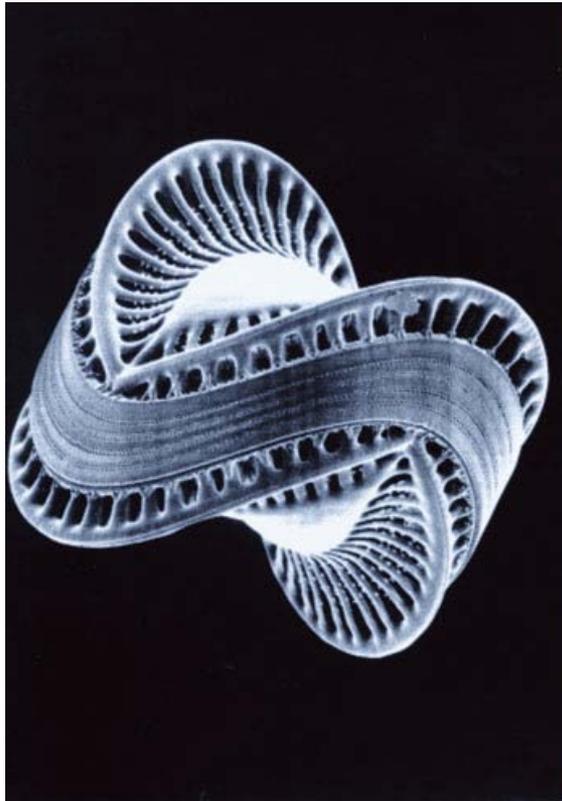
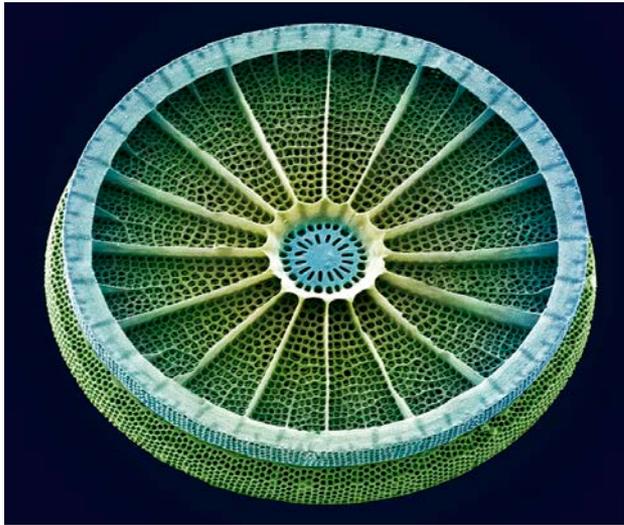
Diatoms: algal rebels



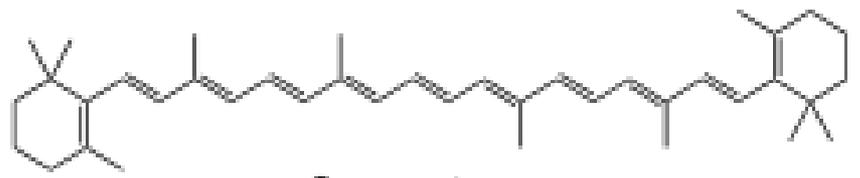
Diatoms: algal rebels



Diatoms: algal rebels



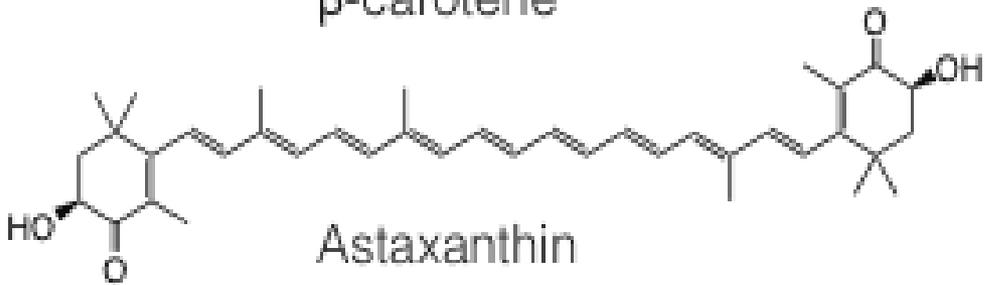
Algae produce molecules of anthropogenic interest



β -carotene



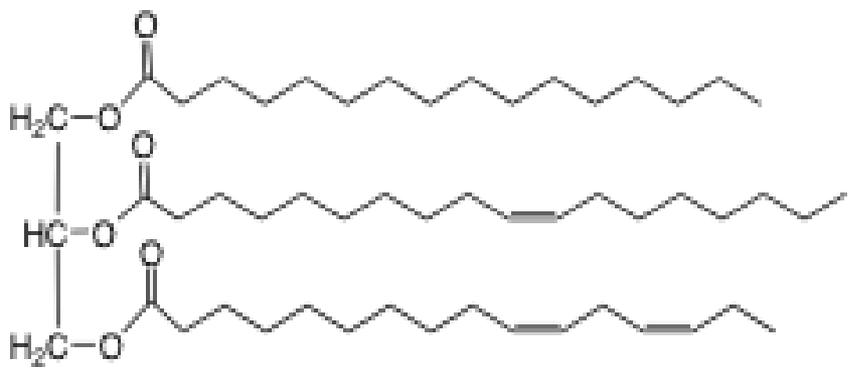
Eicosapentaenoic acid (EPA)



Astaxanthin

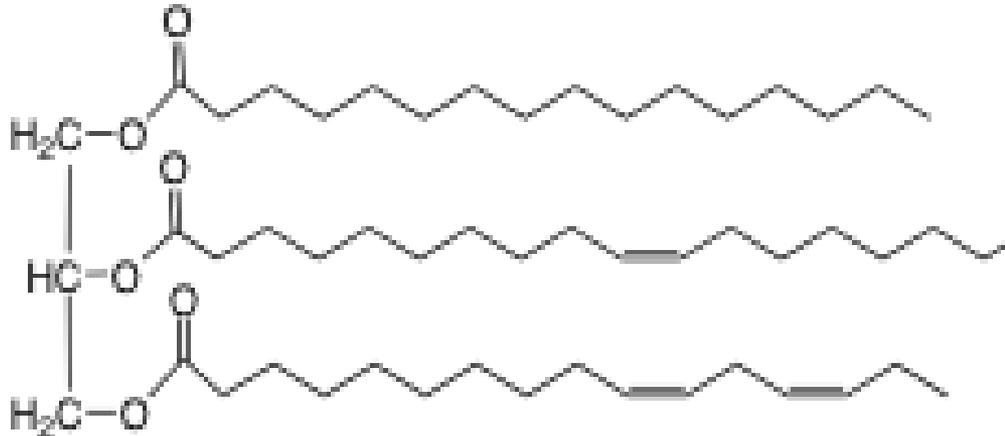


Docosahexaenoic acid (DHA)

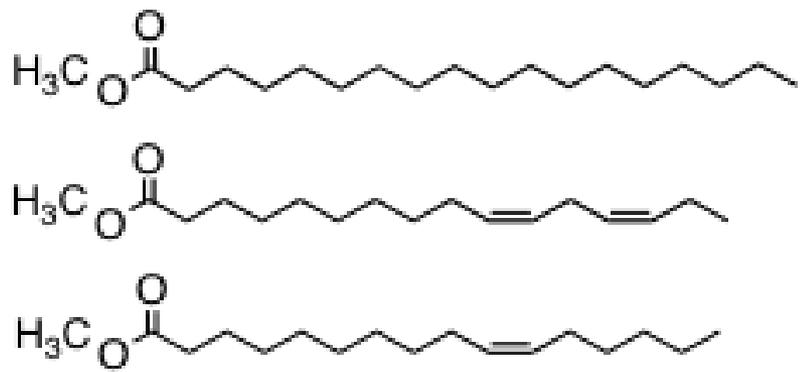


Triacylglycerol (TAG)

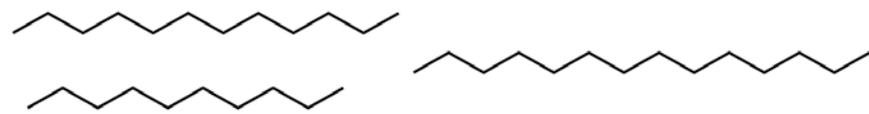
Algae produce molecules similar to liquid fuels



Algal TAG



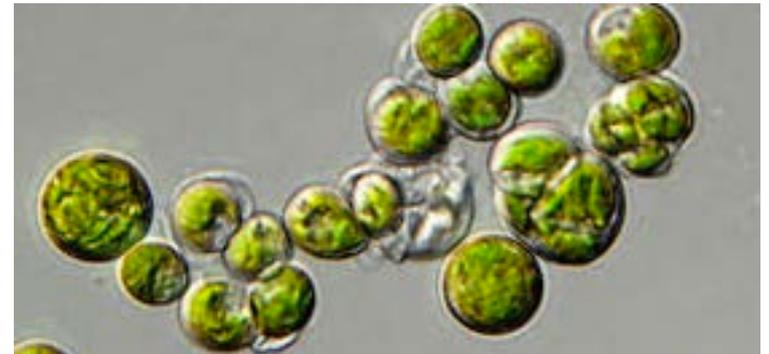
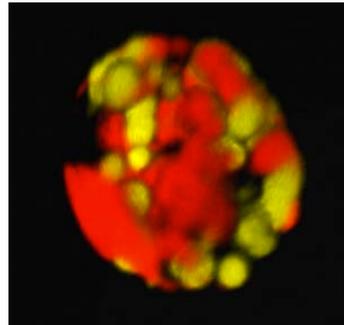
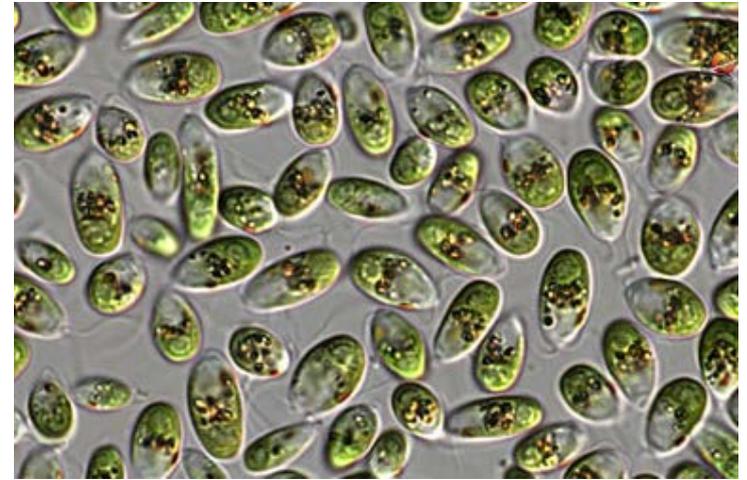
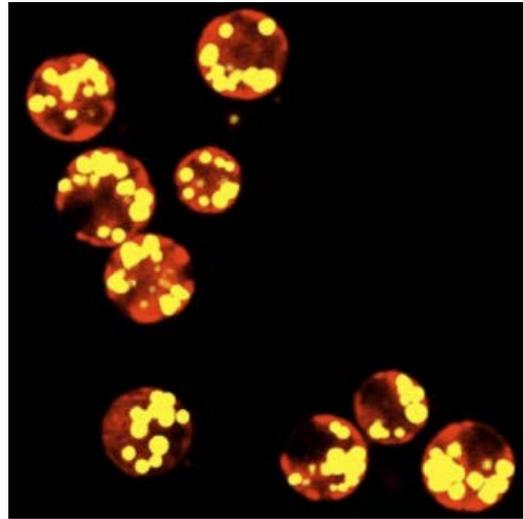
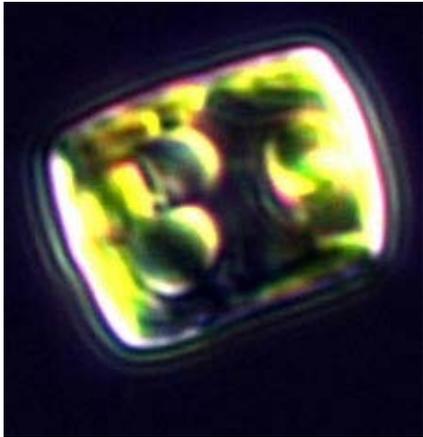
Fatty Acid Methyl Esters (FAMES)
Biodiesel



Hydrocarbons
Crude oil

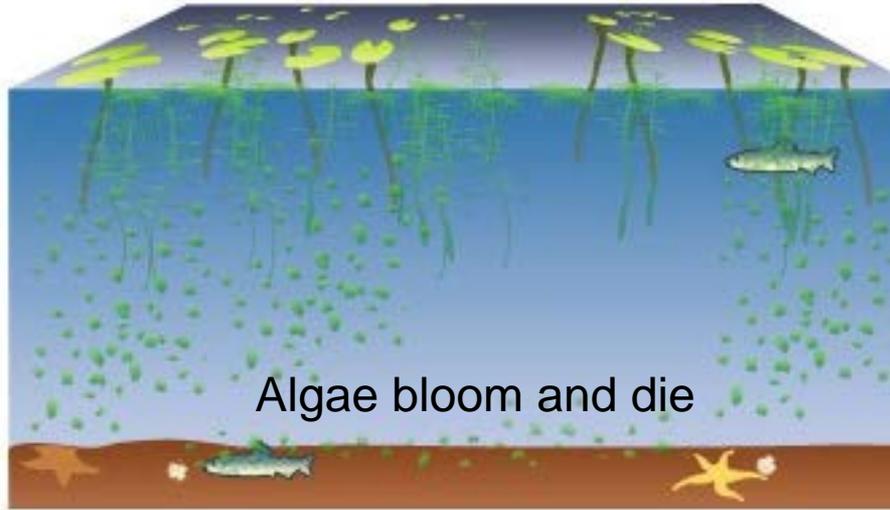
Lipid accumulation in microalgae

Many microalgae accumulate lipids that can be converted to fuel



Petroleum and algae are linked

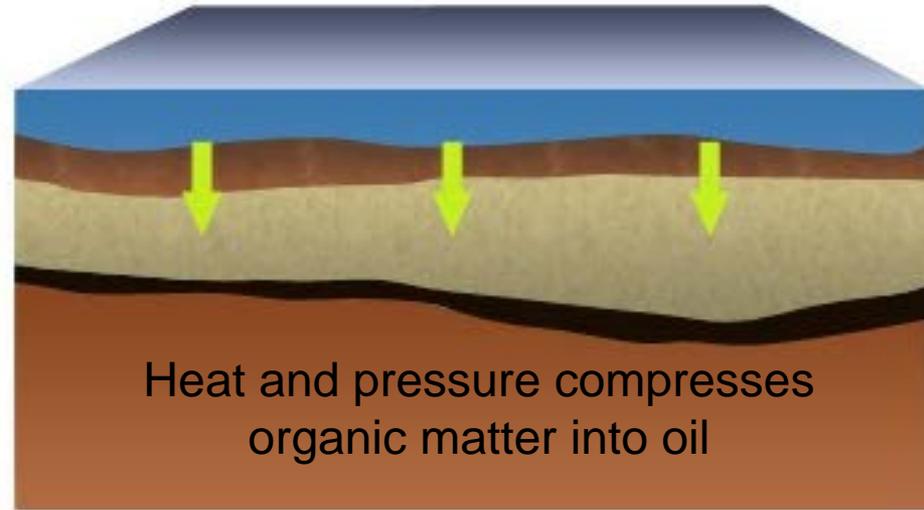
300-400 MYa



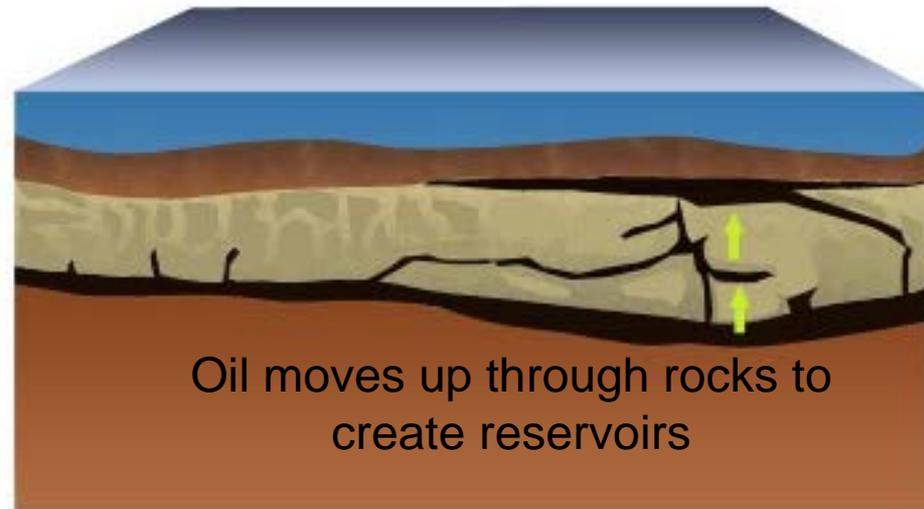
300-400 MYa



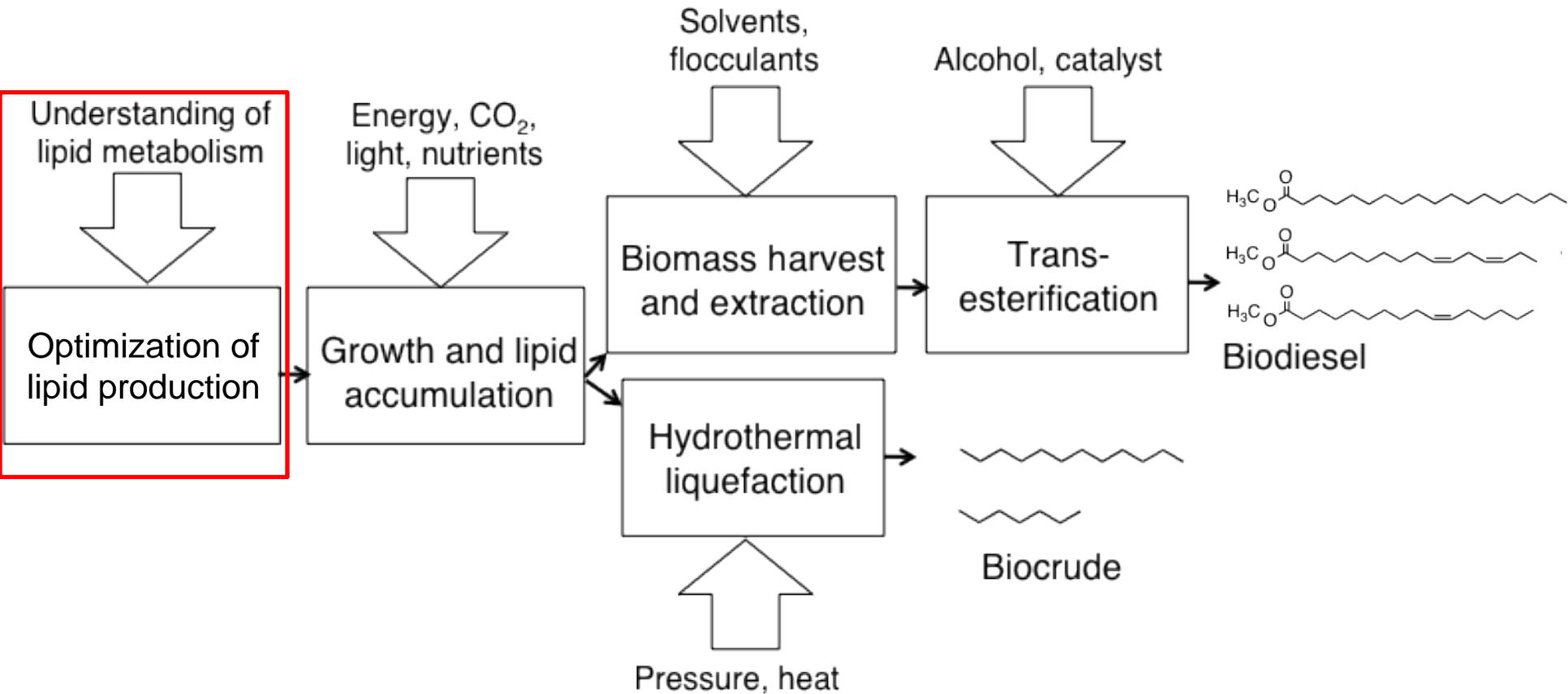
50-100 MYa



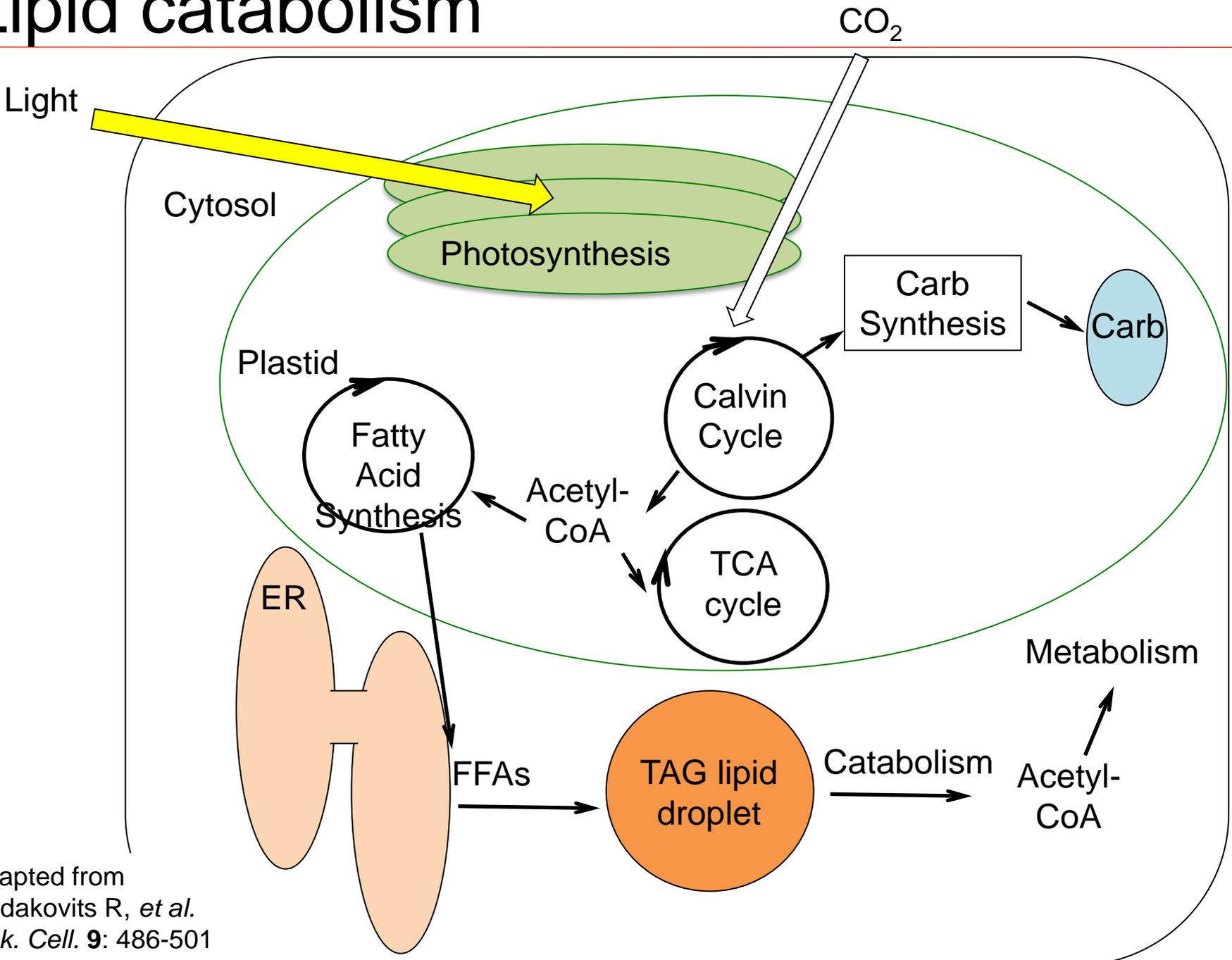
Today



Algal biofuel production



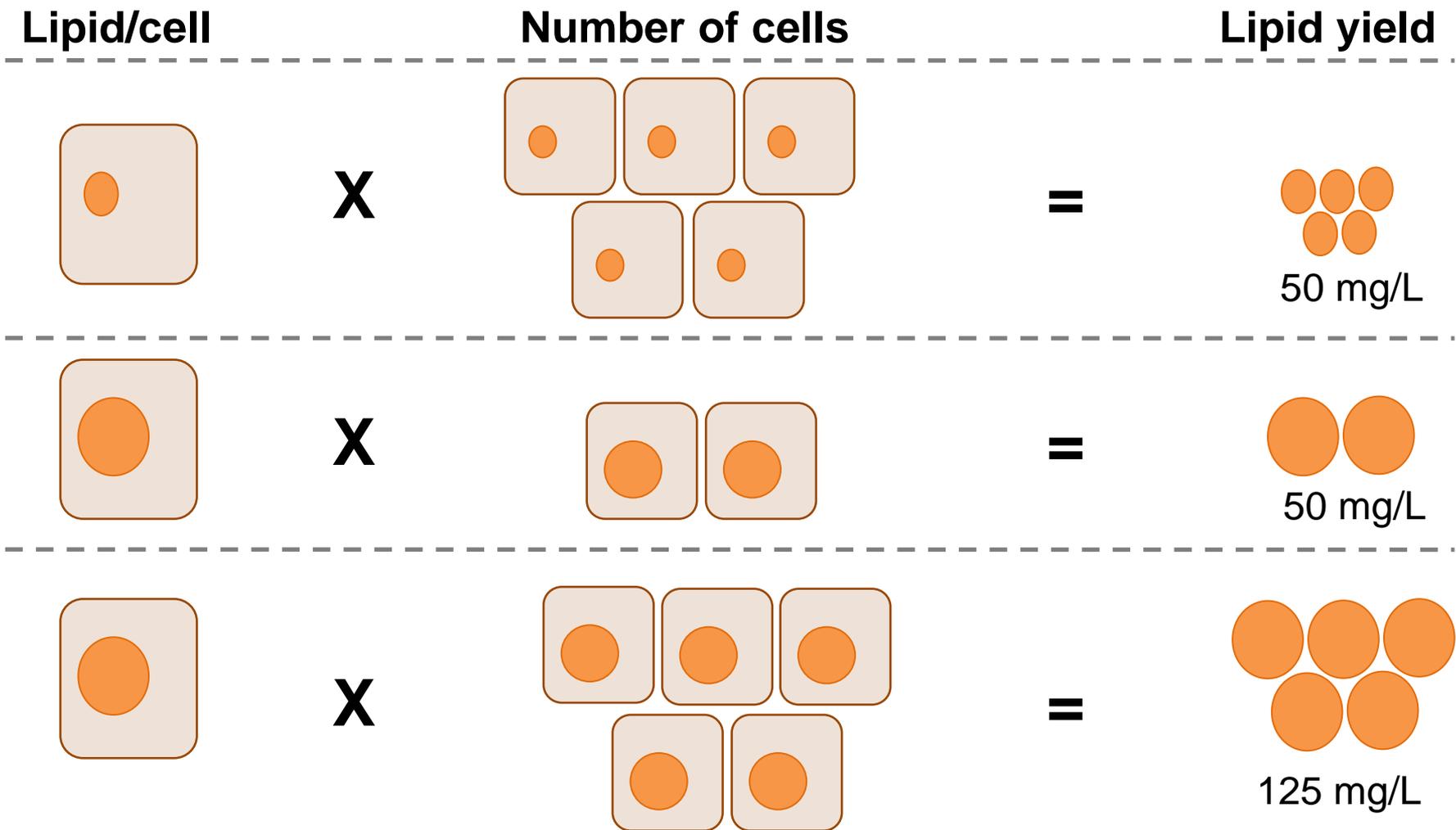
Lipid catabolism



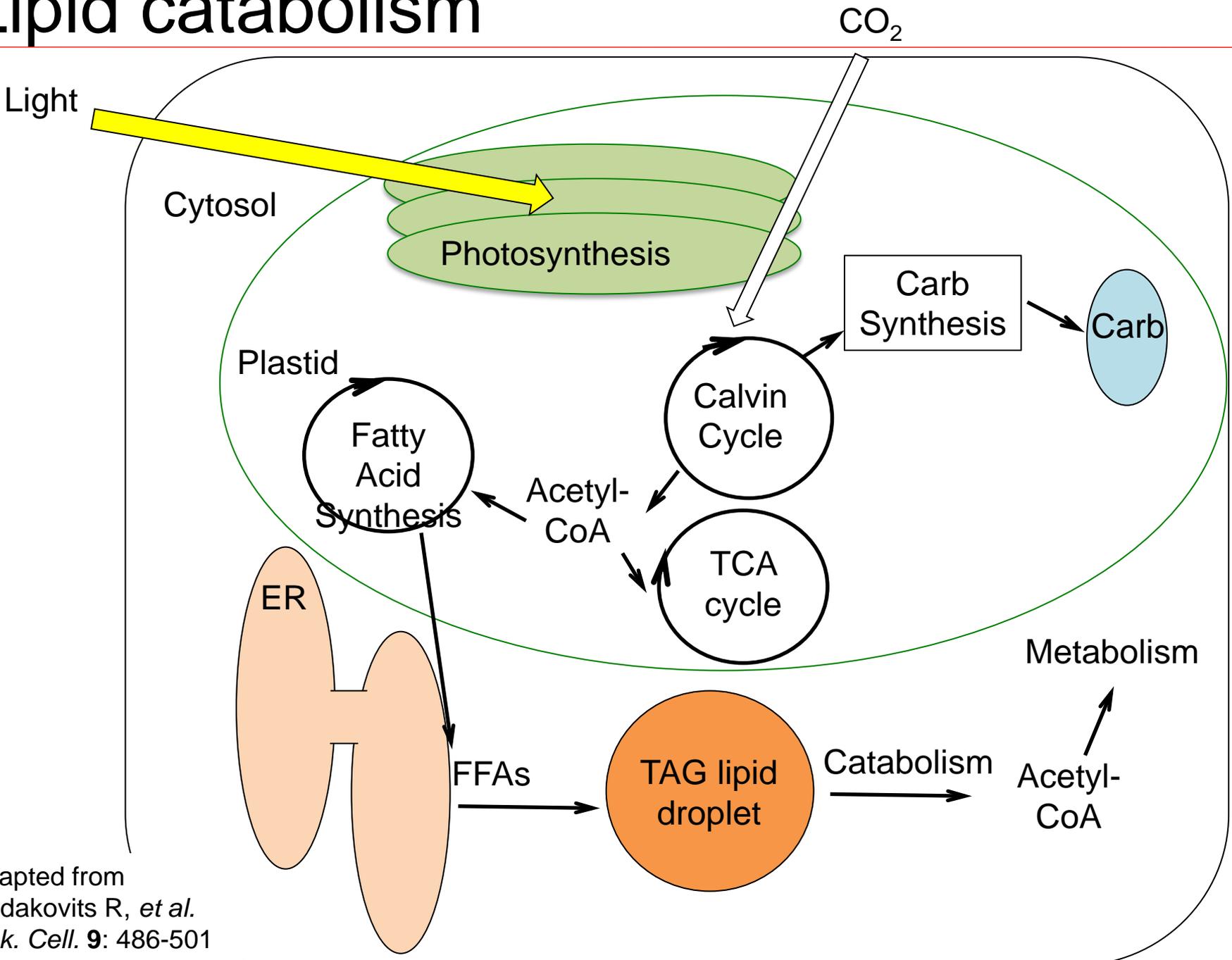
Adapted from Radakovits R, et al. *Euk. Cell.* 9: 486-501

Manipulations of microalgal lipid metabolism

Lipid yields are a product of both lipid accumulation and biomass

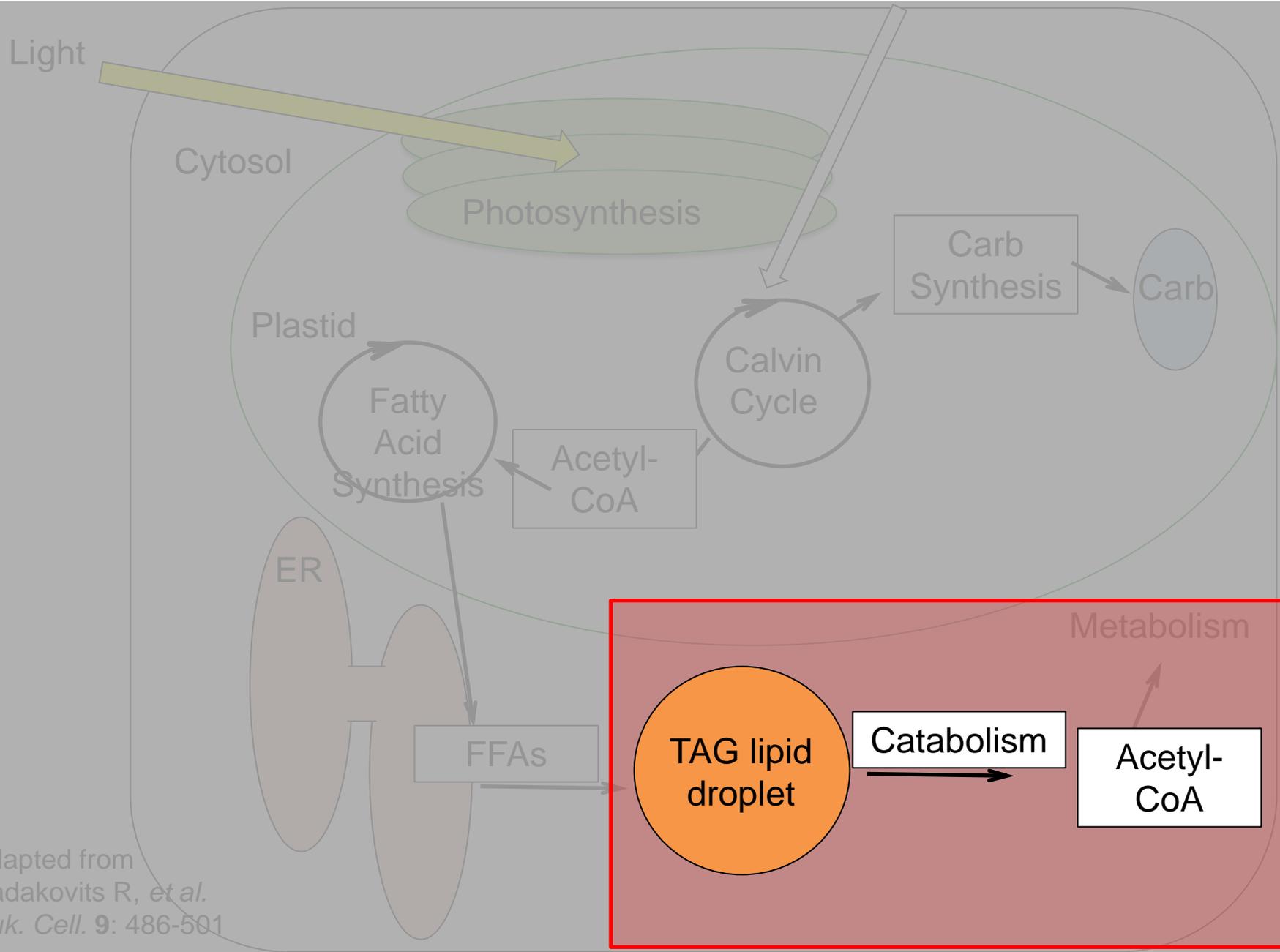


Lipid catabolism

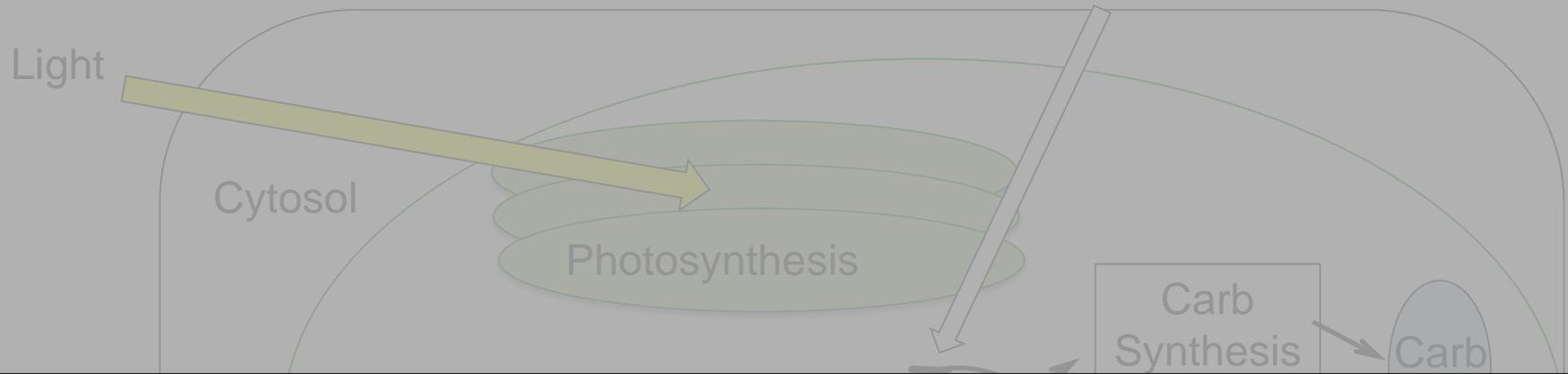


Adapted from Radakovits R, et al. *Euk. Cell.* 9: 486-501

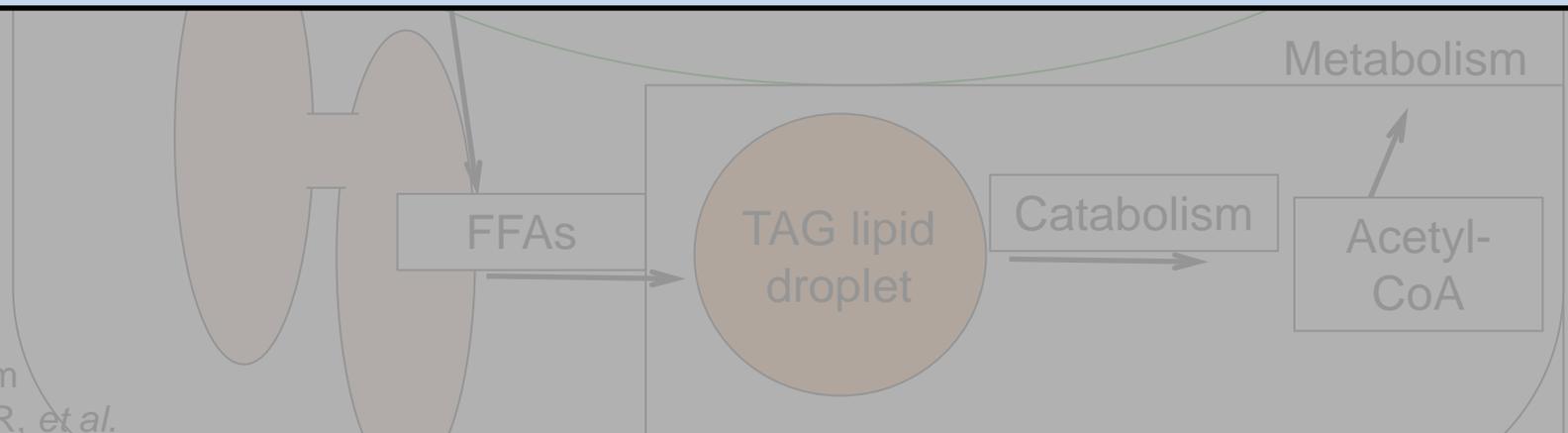
Lipid catabolism



Lipid catabolism



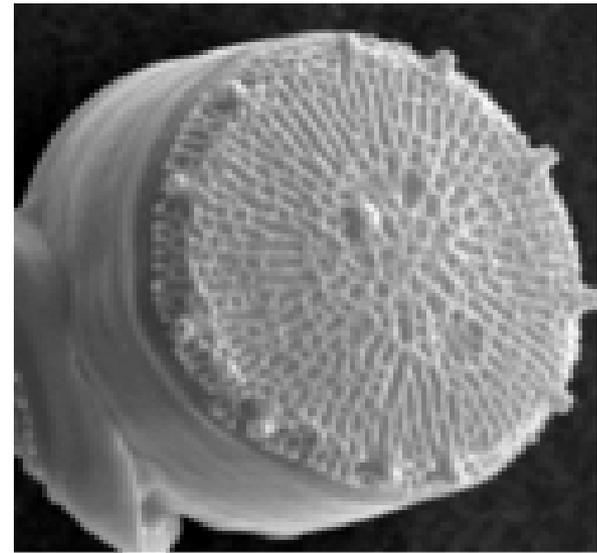
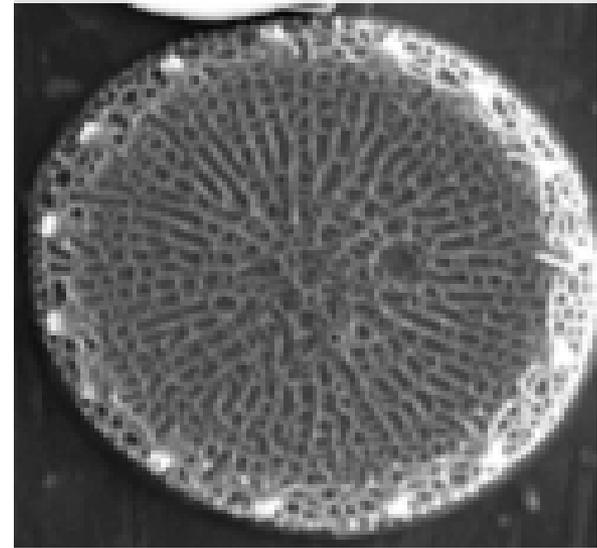
Disrupting lipid catabolism can lead to increased lipids without deleterious effects on growth



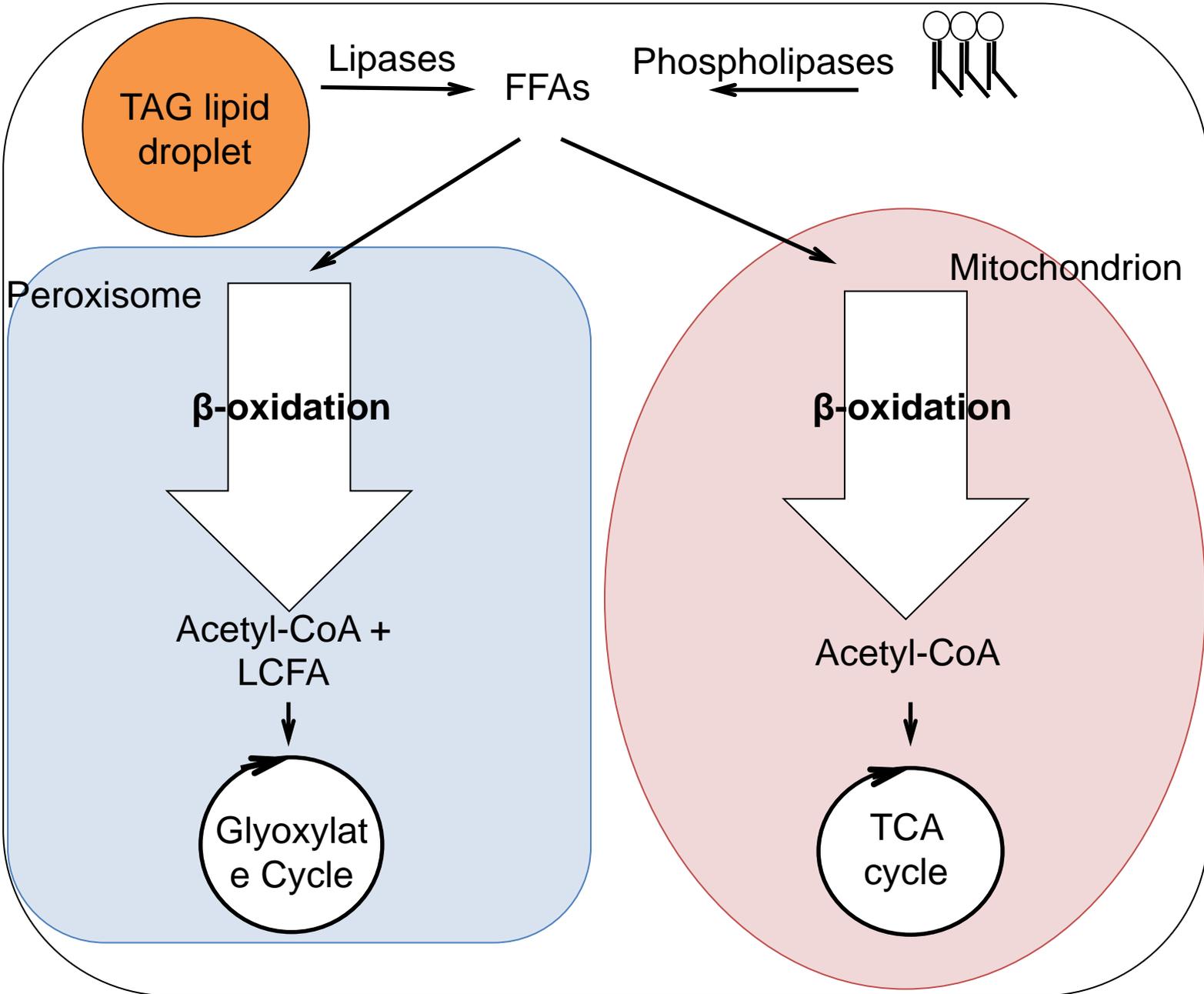
The organism: *Thalassiosira pseudonana*

T. pseudonana (*Tp*)

- Accumulates lipids
- Sequenced genome
- Molecular techniques

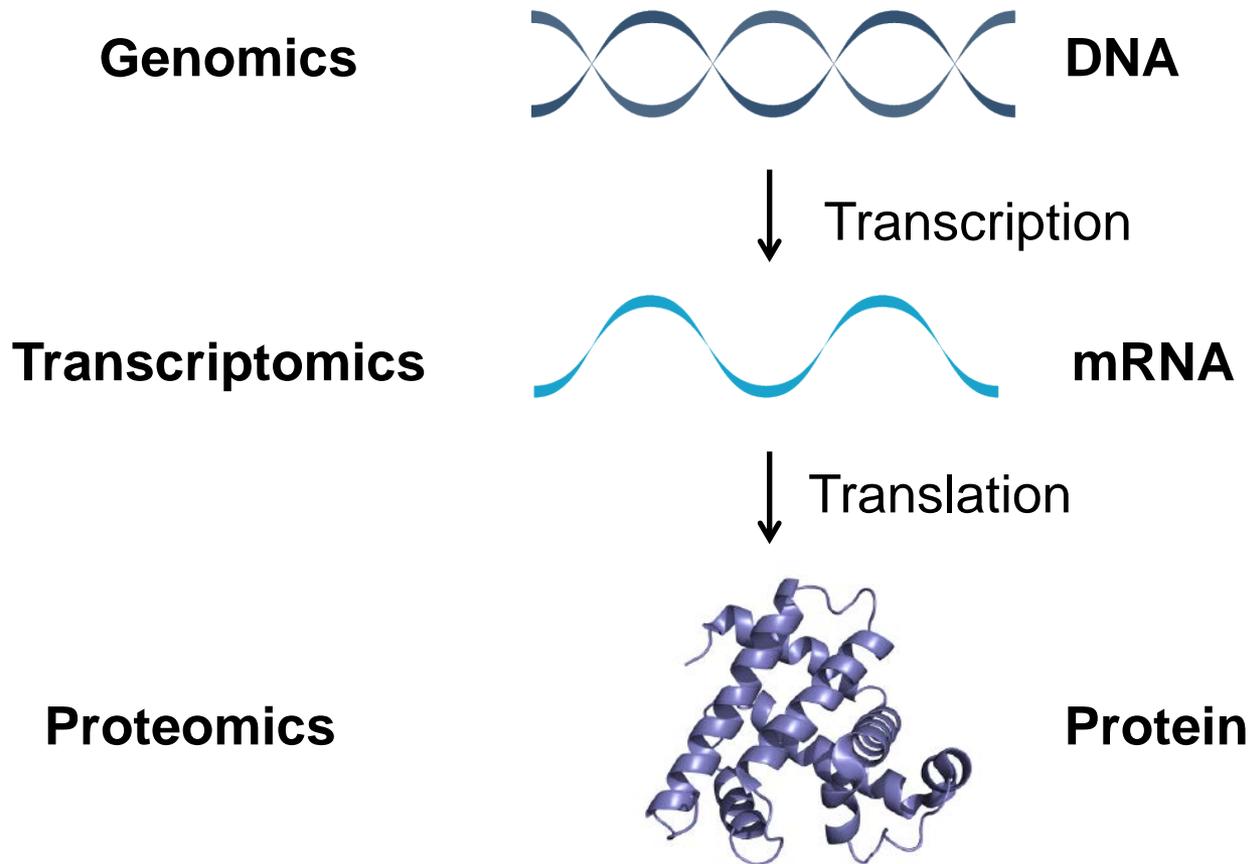


Lipid catabolism



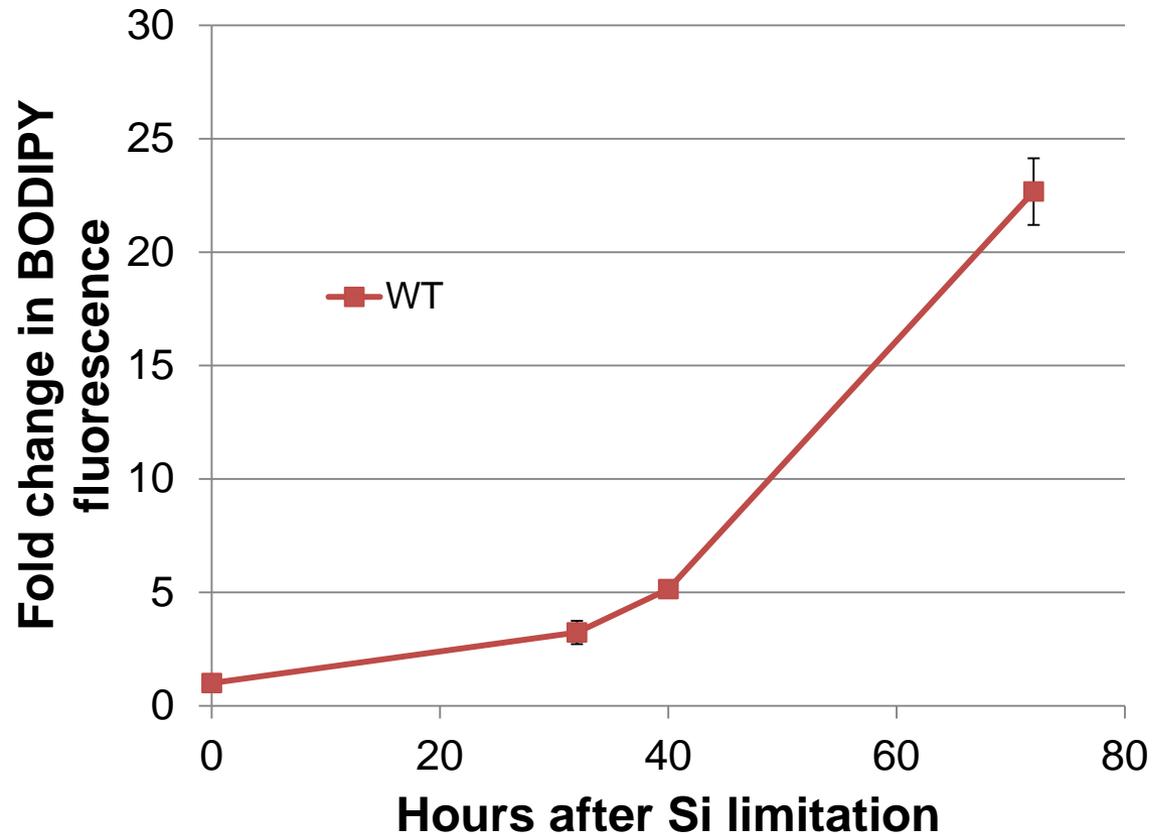
Transcriptomics

The central dogma of molecular biology

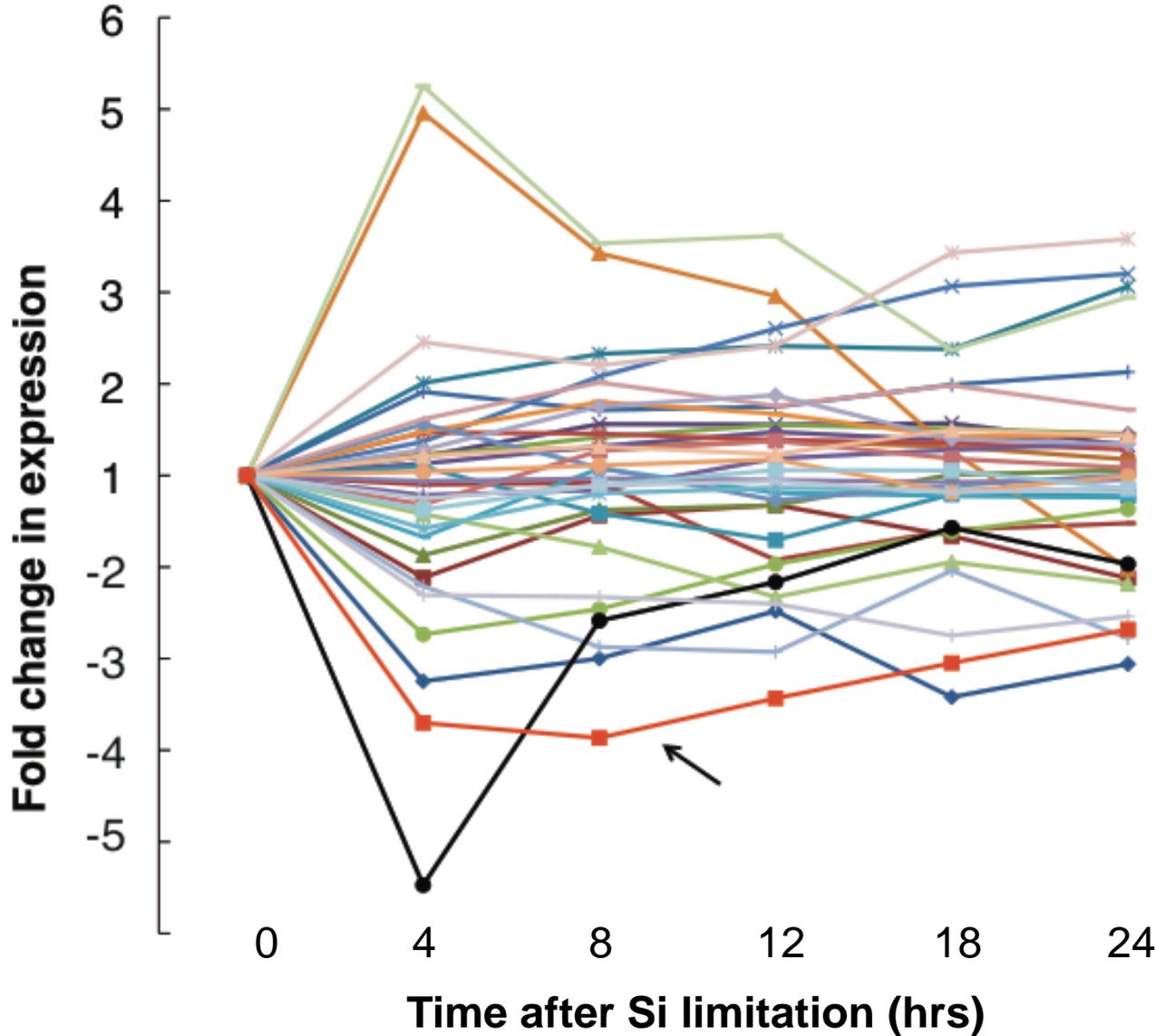


Transcriptomics-guided target identification

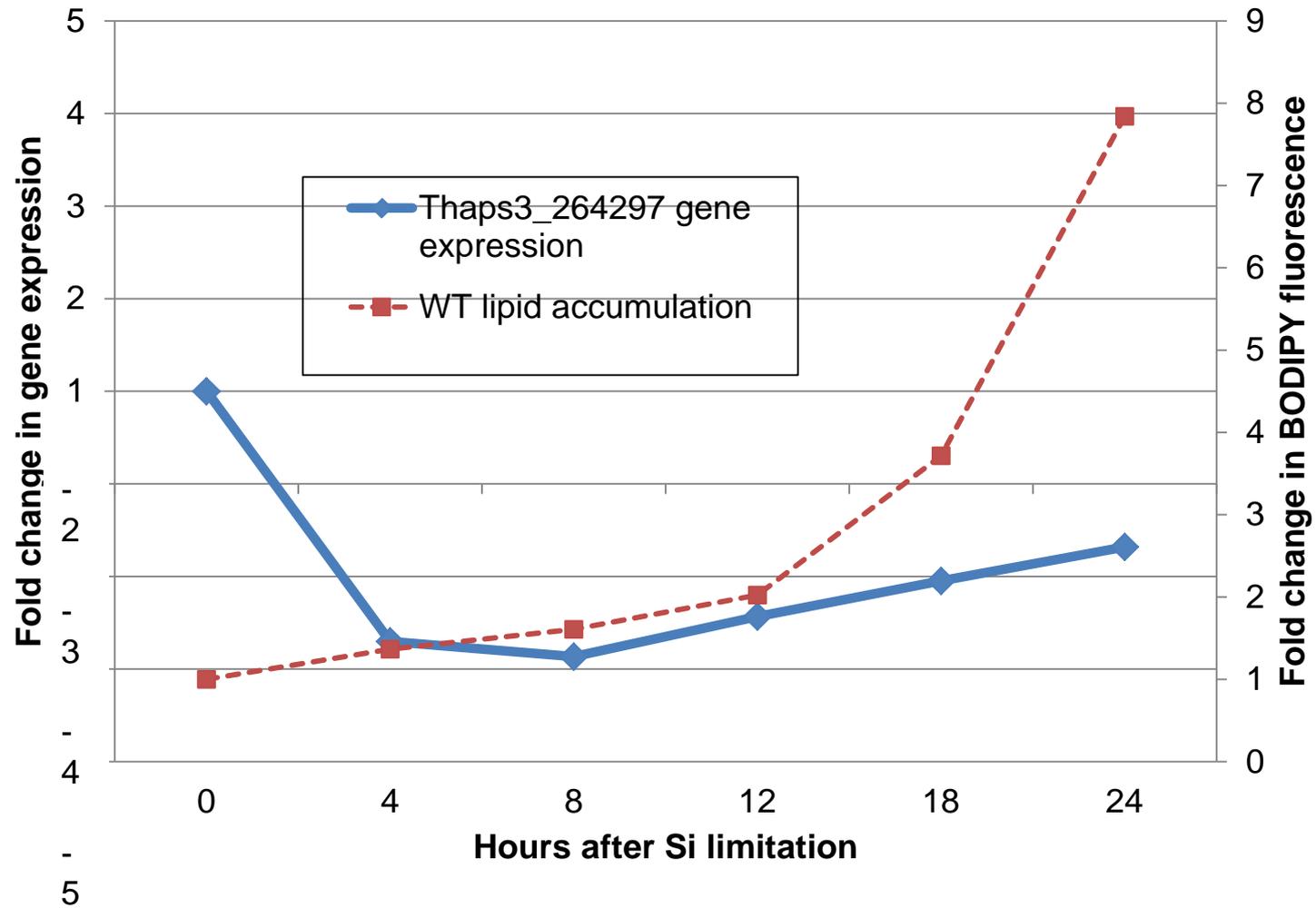
Lipid accumulation in *Tp* under Si starvation



Overview of lipase expression

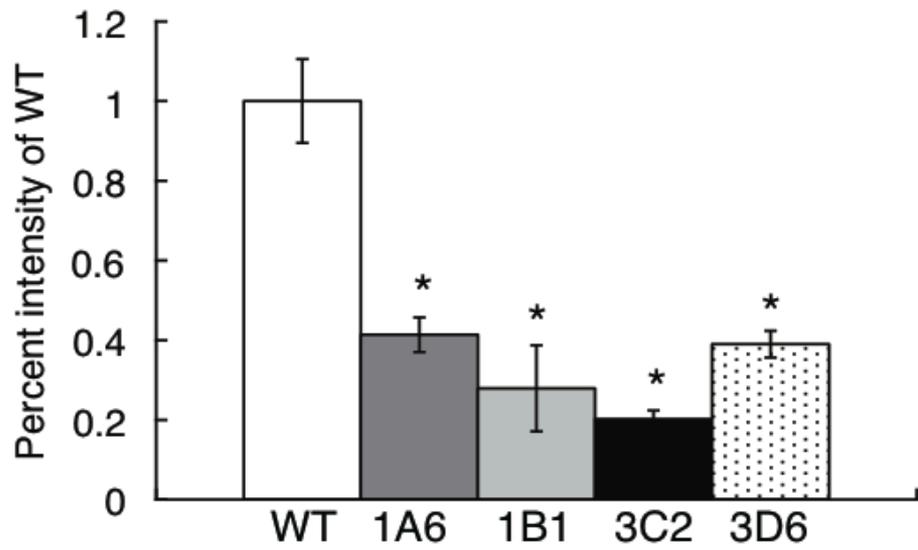


Target: Thaps3_264297

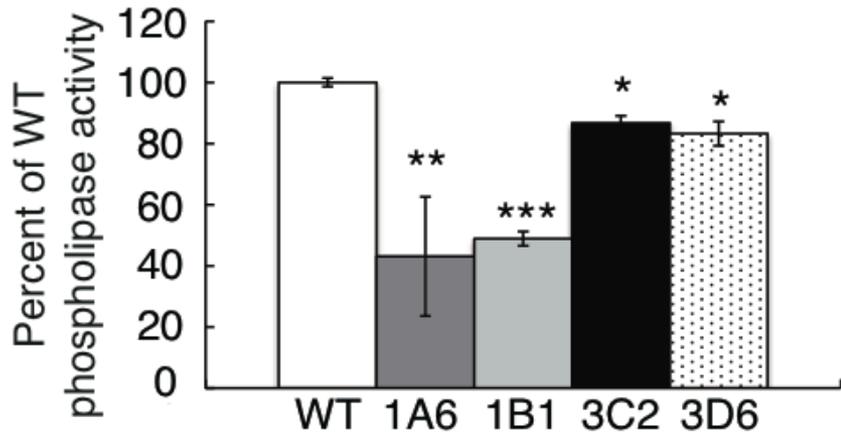


Knock-down of Thaps3_264297

Immunoblotting



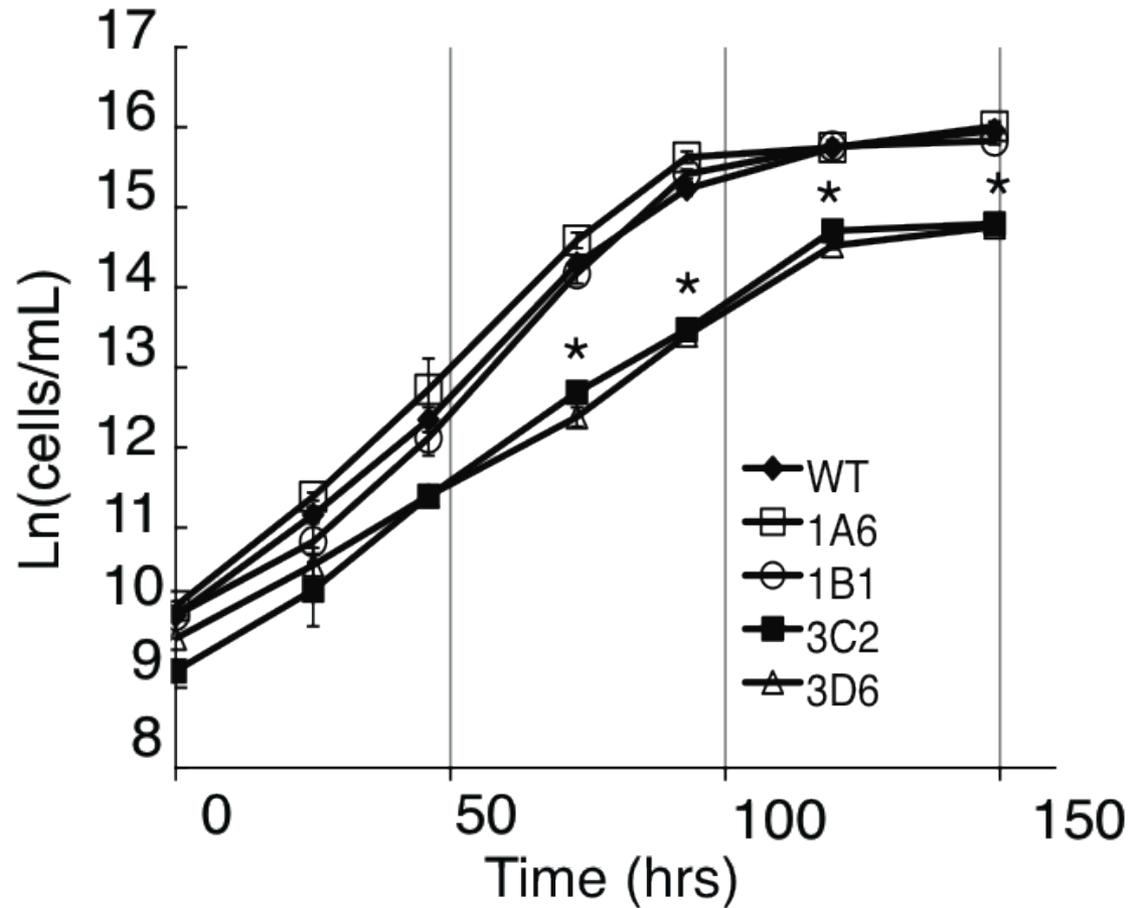
Functional assay



*p<0.05
**p<0.01
***p<0.001

Growth analysis

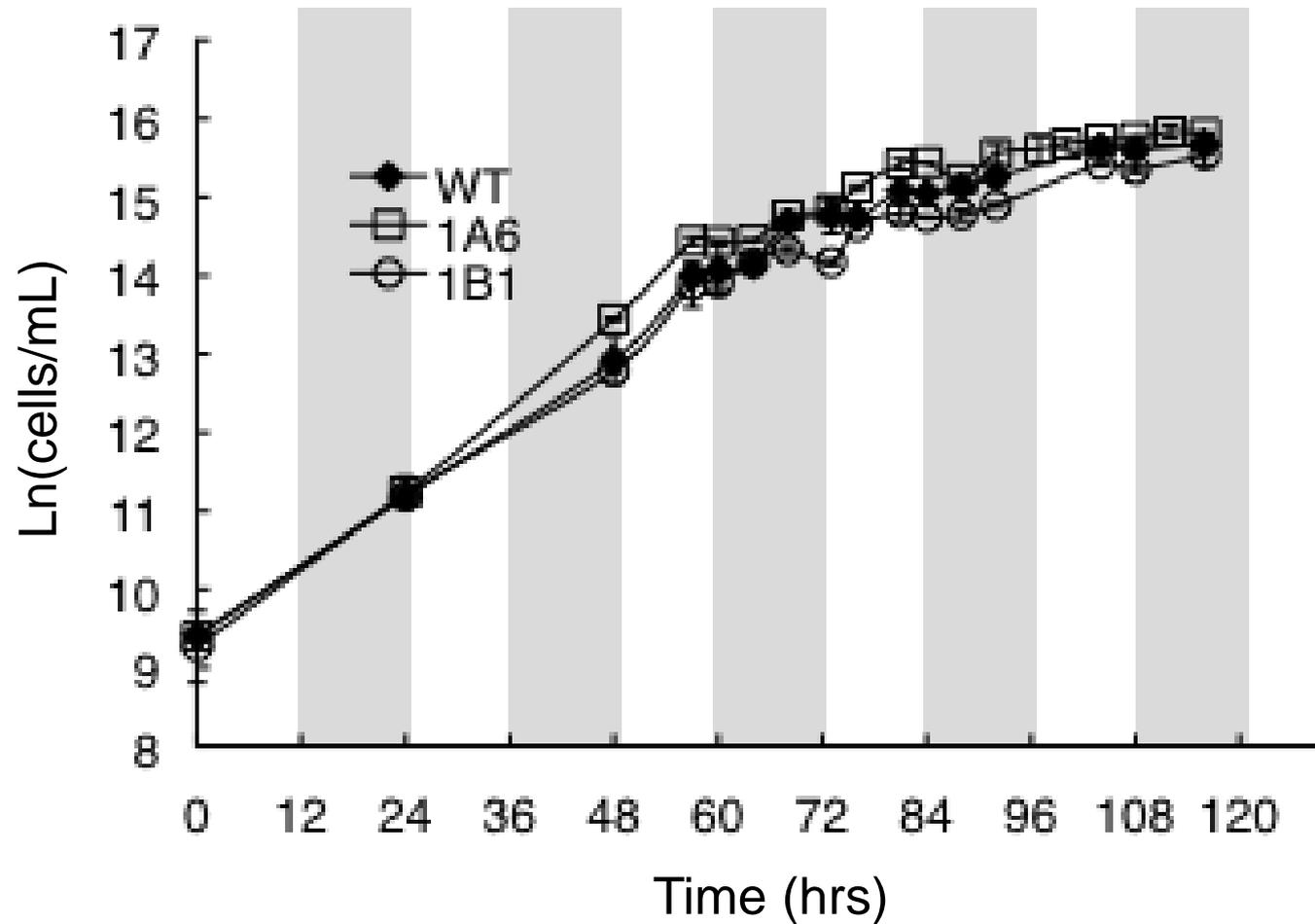
Antisense strains 1A6 and 1B1 show no decrease in growth



*p<0.05

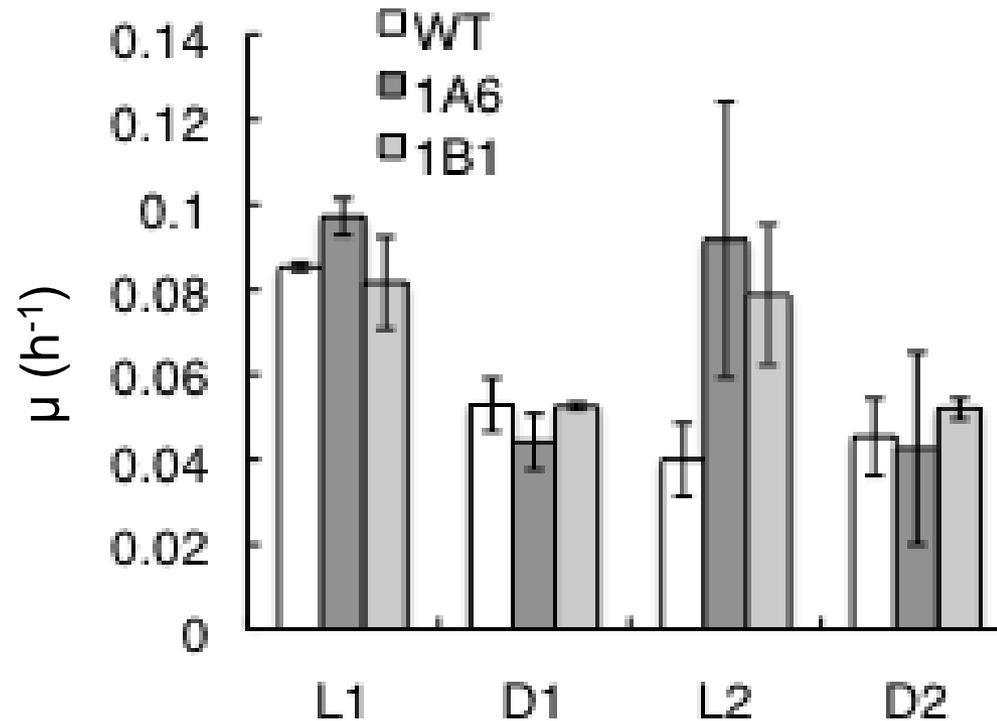
Growth analysis

Antisense strains 1A6 and 1B1 show no decrease in growth



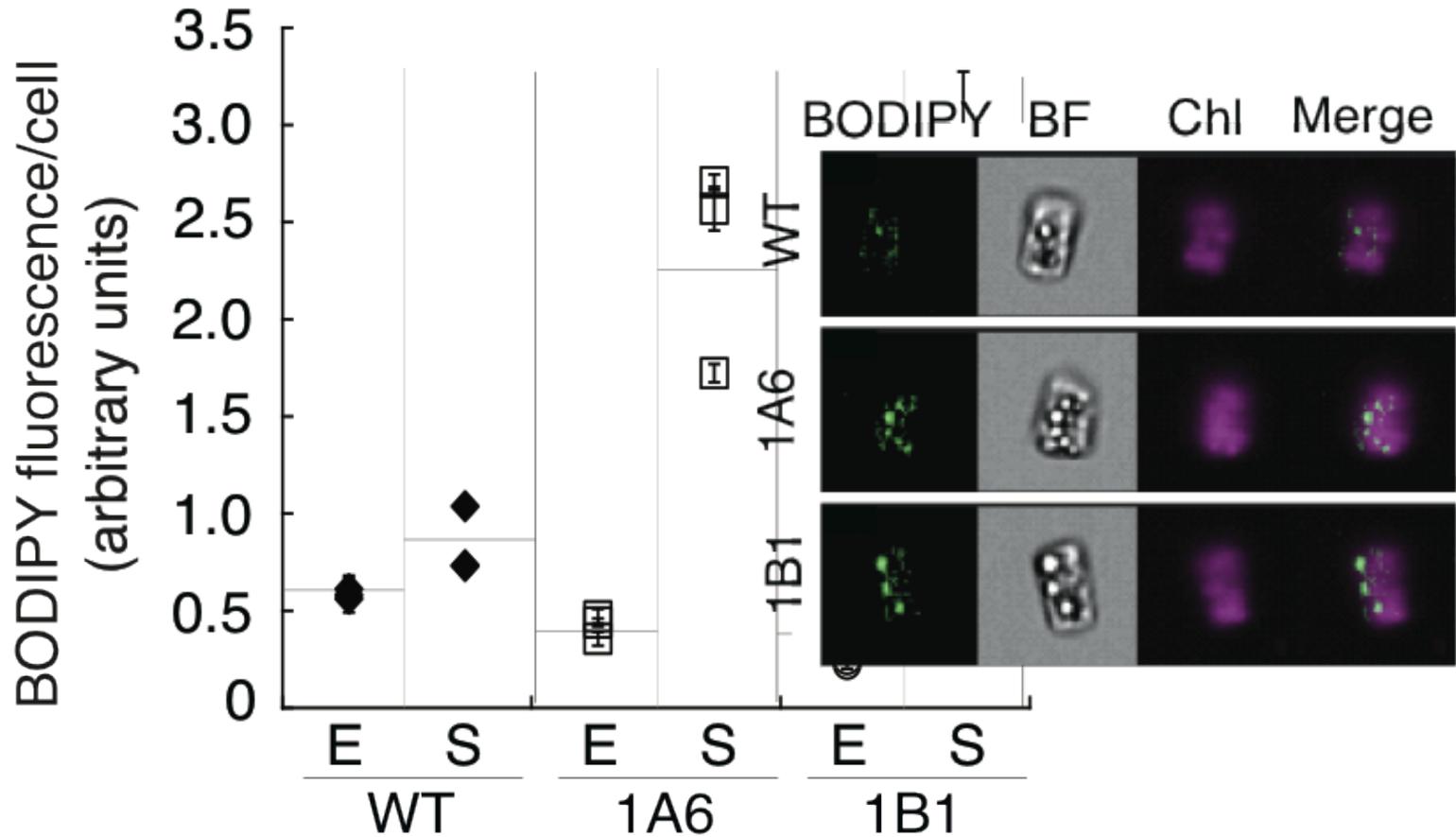
Growth analysis

Antisense strains 1A6 and 1B1 show no decrease in growth



Lipid analysis

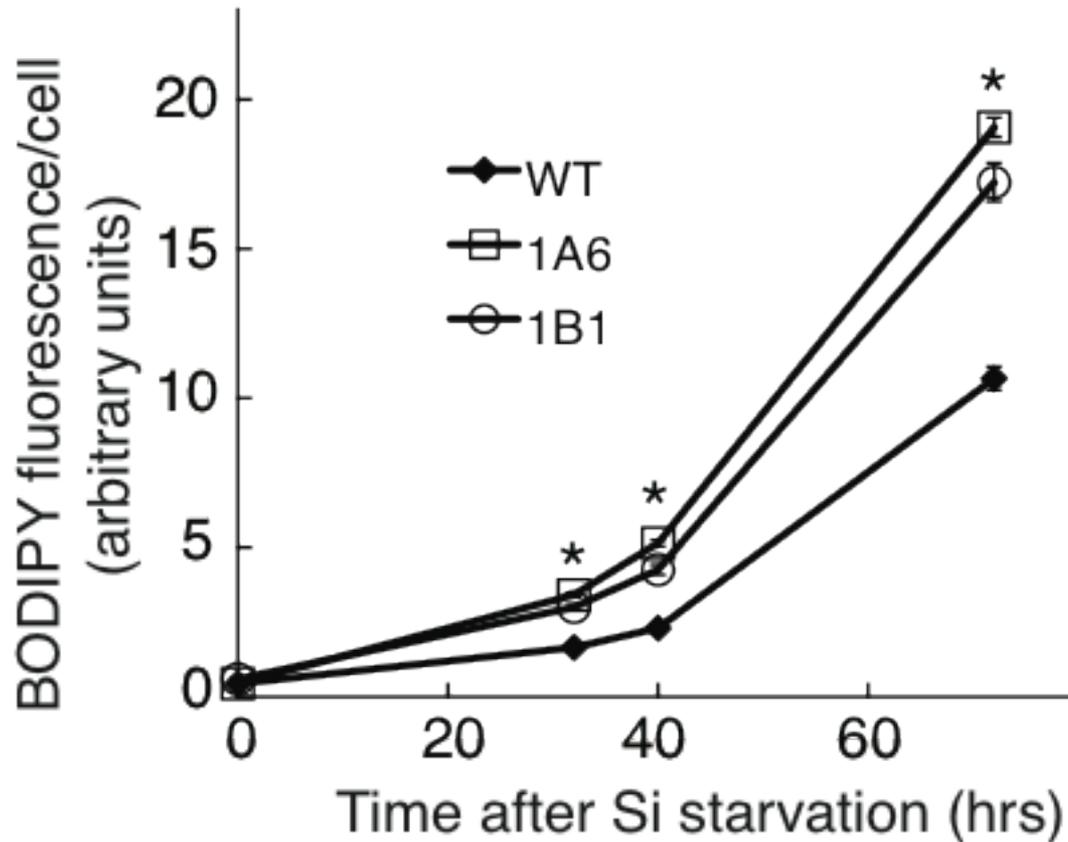
1A6 and 1B1 show increased TAG in stationary phase



E: exponential S: stationary

Lipid analysis

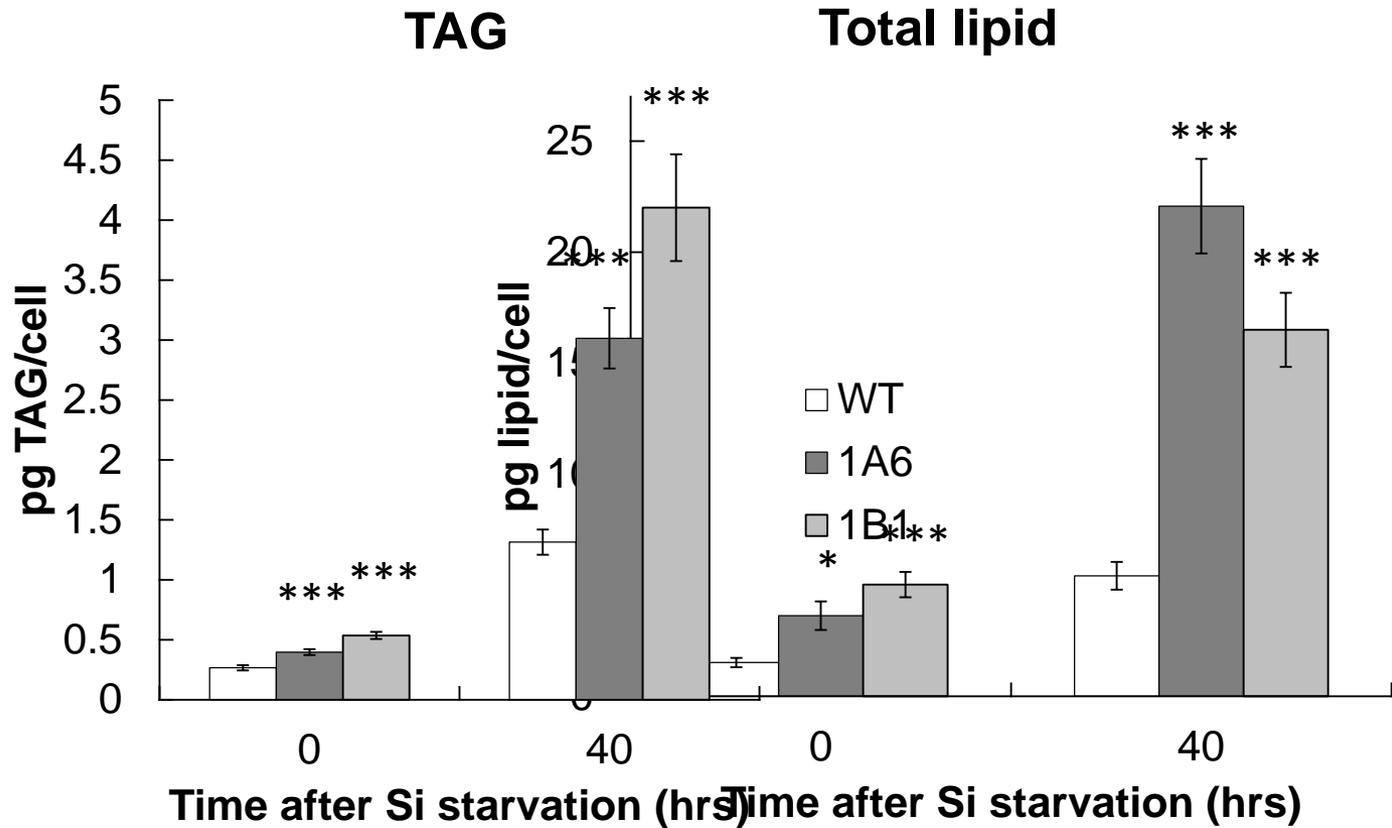
1A6 and 1B1 show increased lipid accumulation during nutrient starvation



*p<0.05

Lipid analysis

1A6 and 1B1 show increased lipid accumulation during nutrient starvation

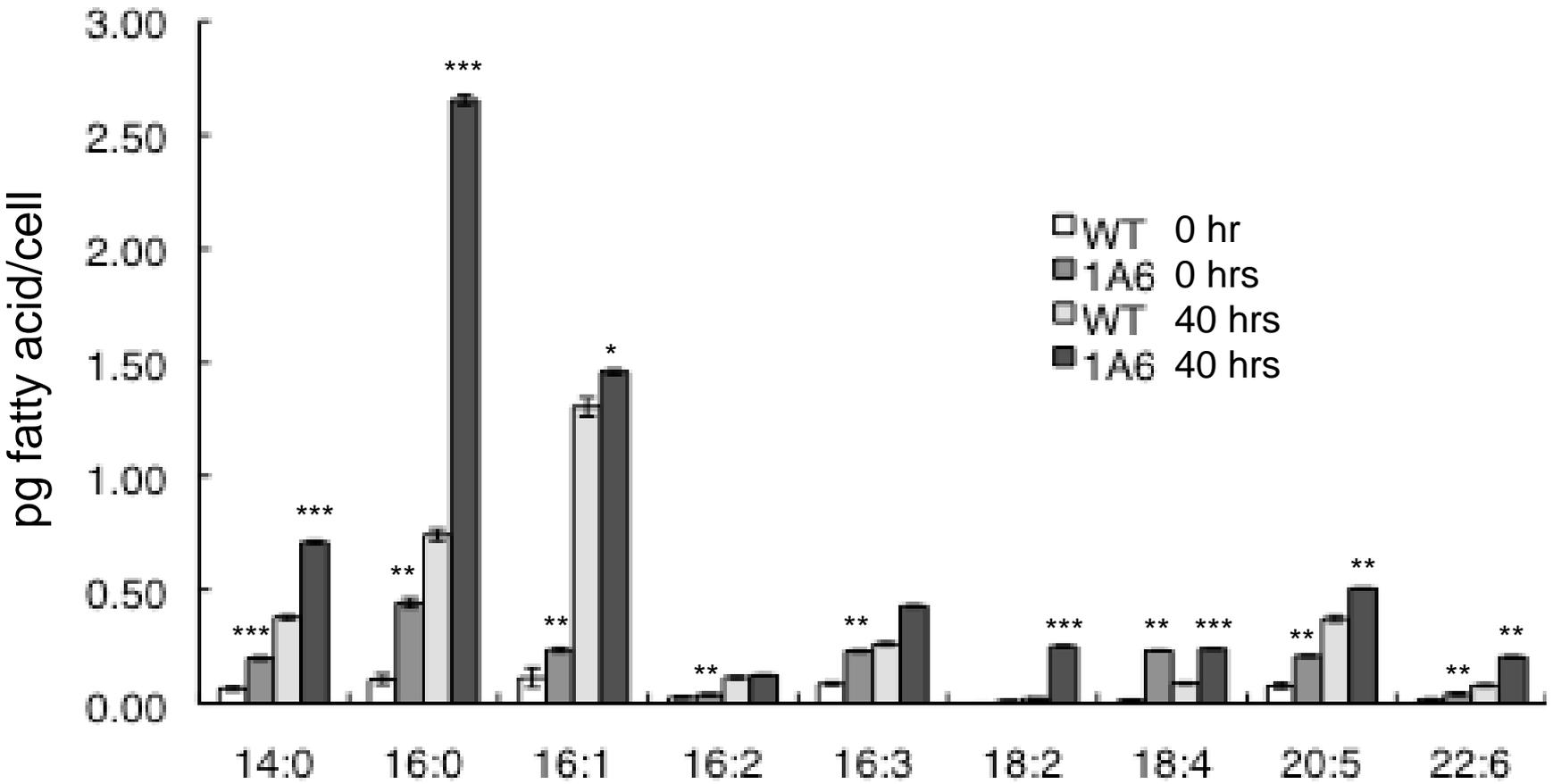


*p < 0.05
***p < 0.001

Corine Glé
Aaron Hartmann

Lipid analysis

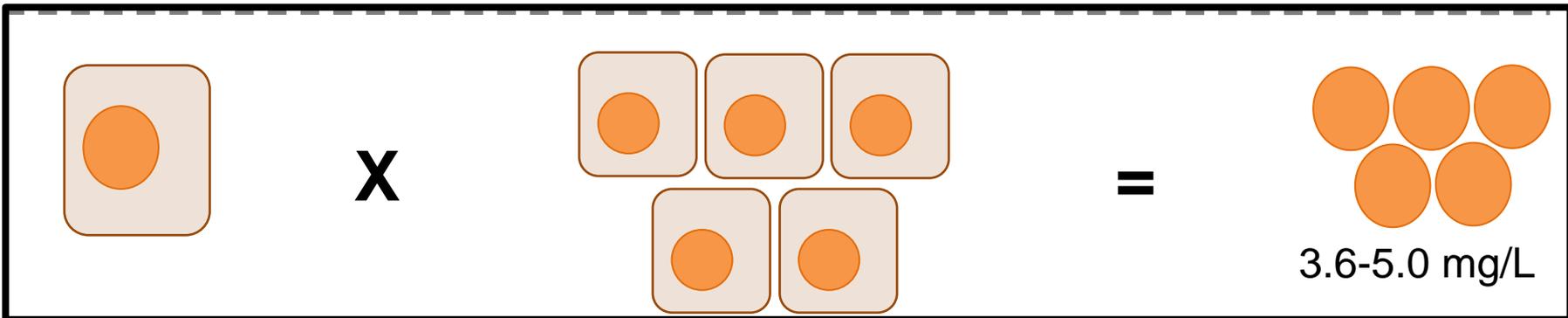
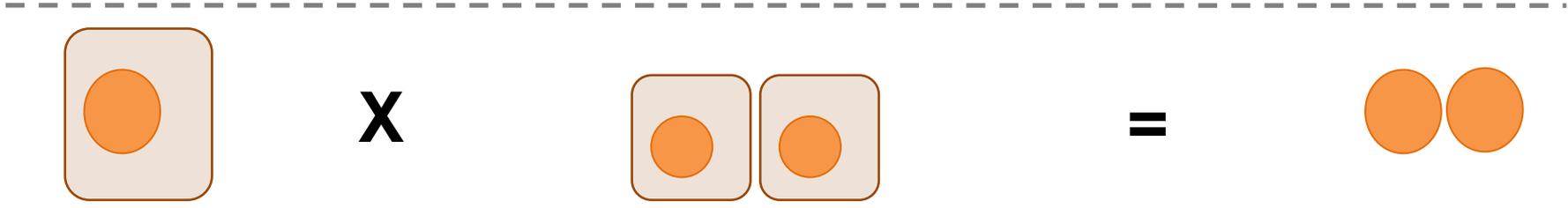
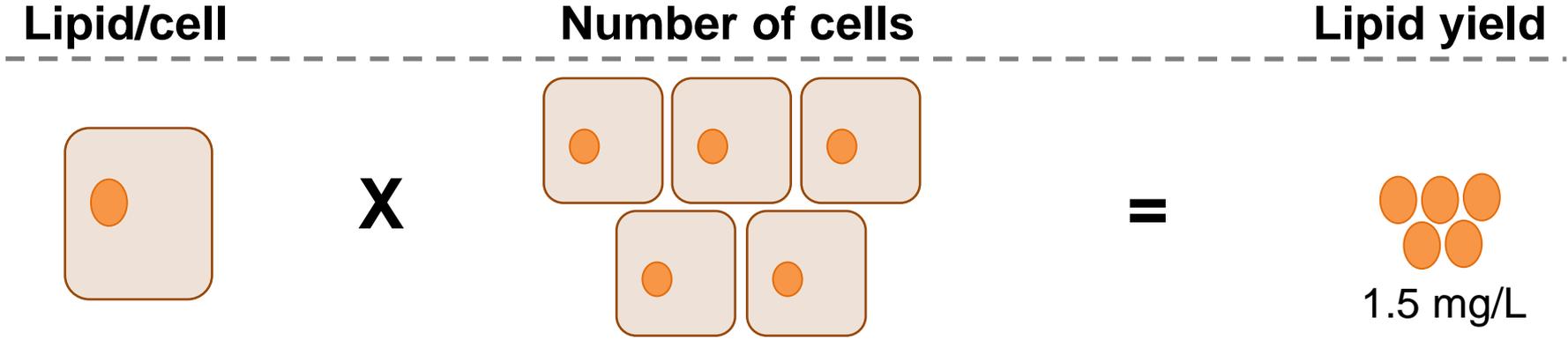
Fatty acid distribution differs slightly between 1A6 and WT



*p<0.05
**p<0.01
***p<0.001

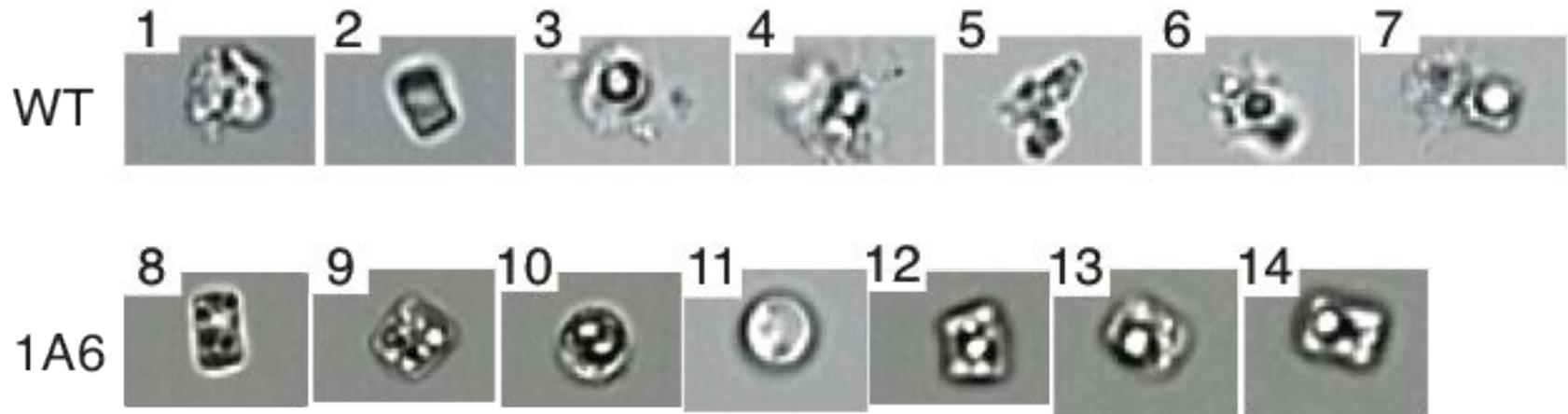
Lipid accumulation in microalgae

Lipid yields are a product of both lipid accumulation and biomass



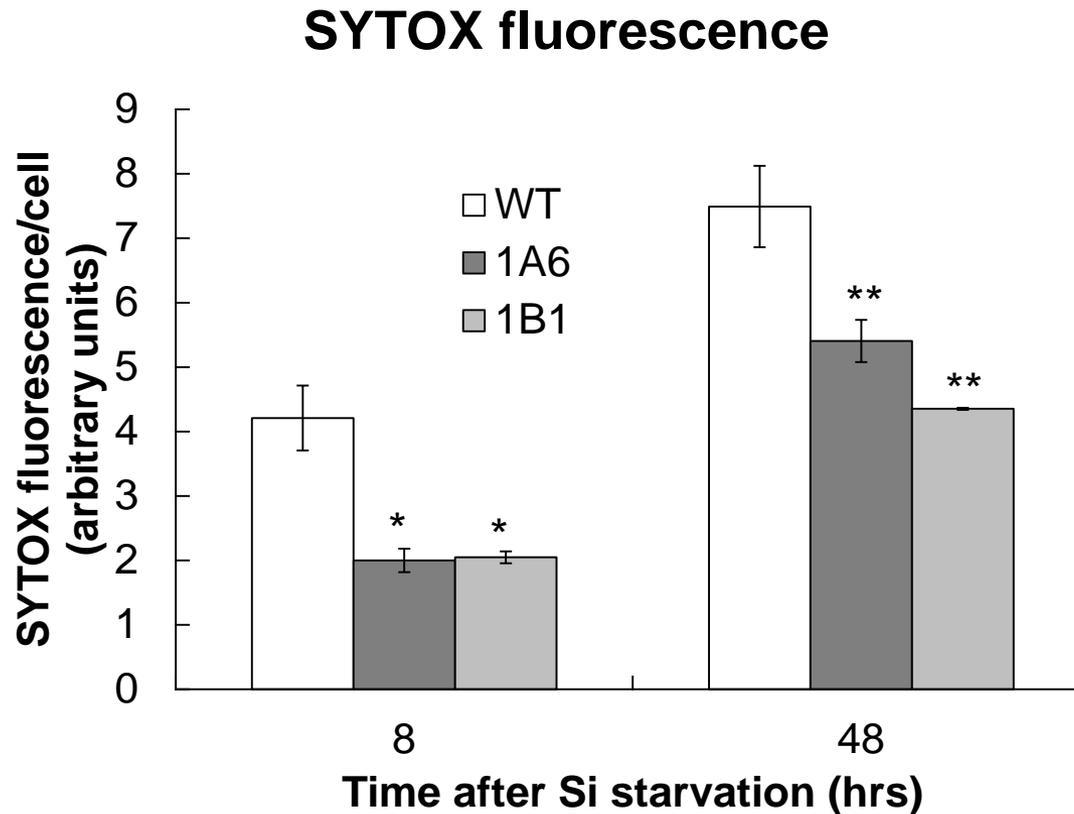
Cell intactness

Transgenic cells were visibly more intact after nutrient limitation



Membrane stability

1A6 and 1B1 show decreased membrane permeability during nutrient starvation

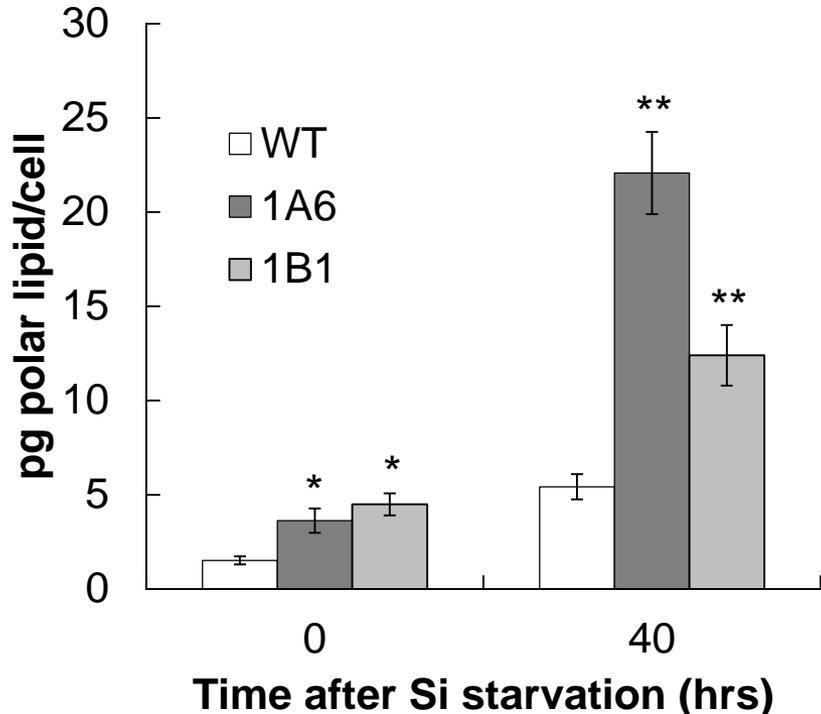


* $p < 0.05$
** $p < 0.01$

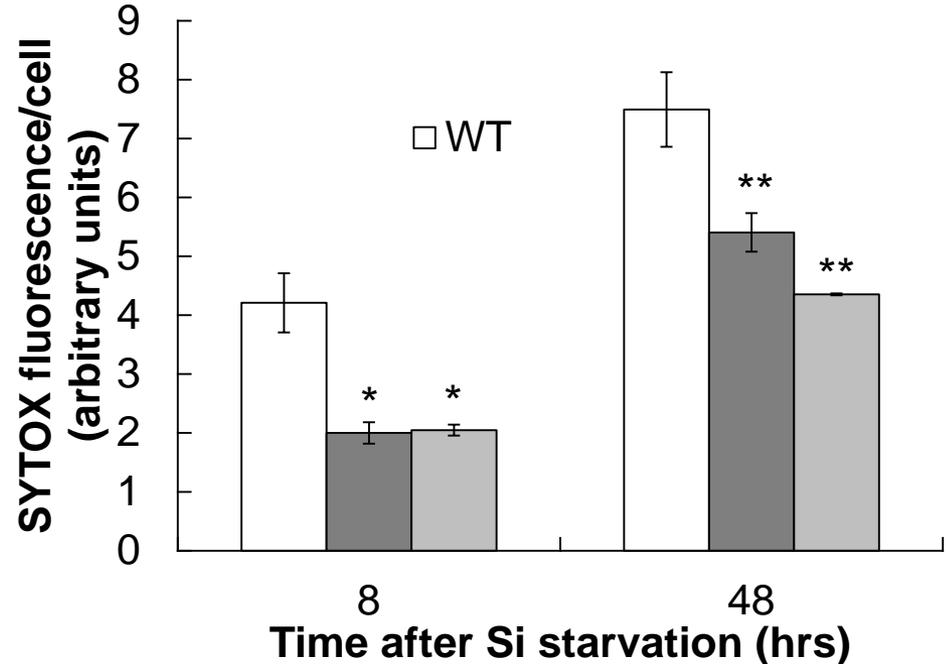
Membrane stability

1A6 and 1B1 show decreased membrane permeability during nutrient starvation

Polar lipids



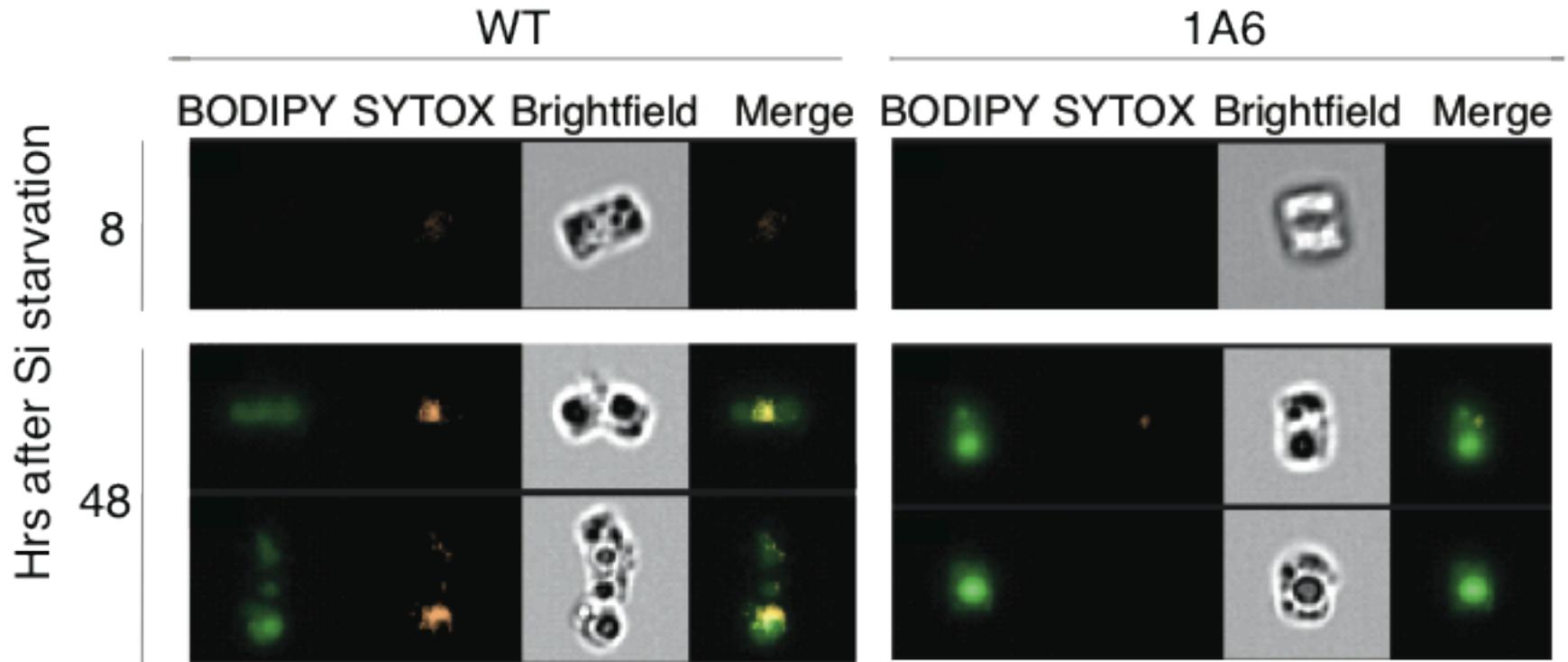
SYTOX fluorescence



*p < 0.05
**p < 0.01

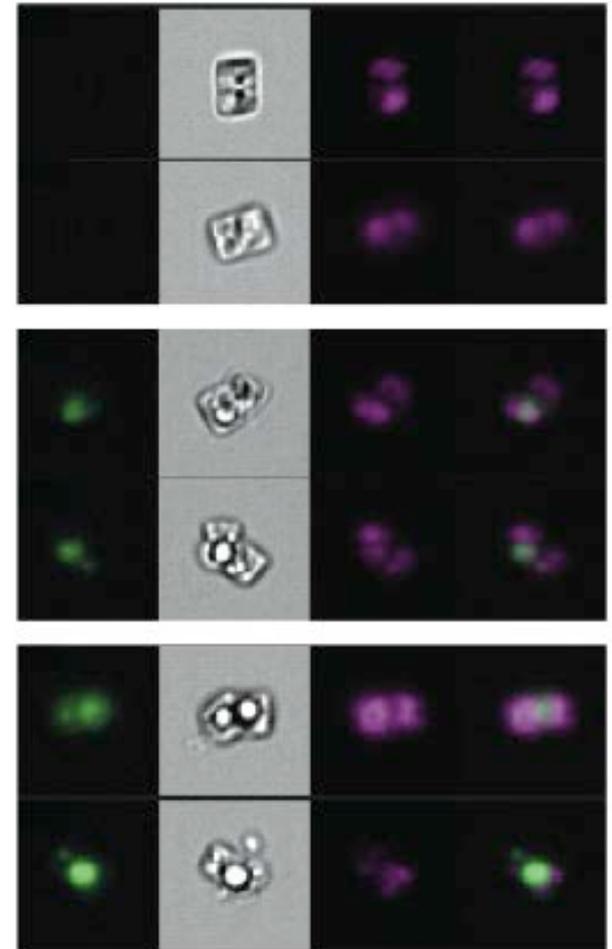
Membrane stability

1A6 and 1B1 show decreased membrane permeability during nutrient starvation



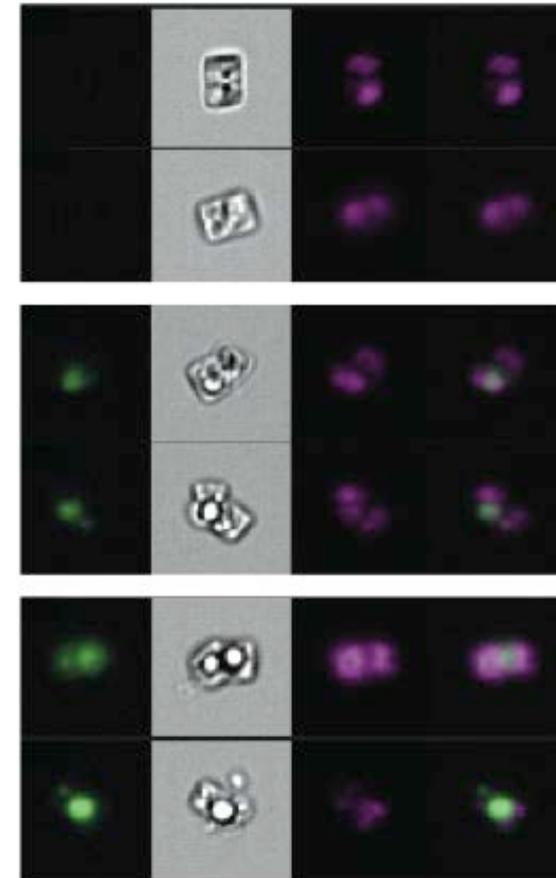
Phenotypes of knock-down strains

- Uncompromised growth
- Increased lipids in exponential and stationary phase
- Increased lipid yields during nutrient starvation
- More intact membranes (viability)



Conclusions

- Hypothesis: **Disrupting lipid catabolism can lead to increased lipids without deleterious effects on growth**
- What this means:
 - Lipid droplets are dynamic entities
 - Lipid accumulation and growth are not mutually exclusive
 - Other targets can exist that decouple growth from lipid production



Conclusions

- Energy is one of the major issues of our time
- Broadening our energy base with alternative, renewable and sustainable fuels is imperative for the future
- Algae are a source that can fulfill these goals



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The Gerwick Lab

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