Chromatin landscaping in algae reveals novel regulation pathway for biofuels production

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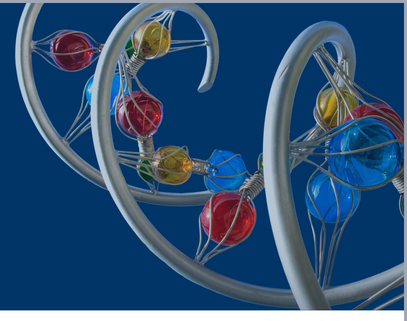
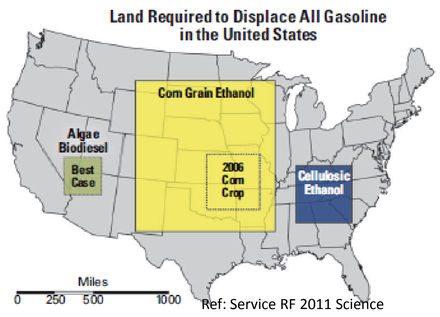


Summary

The diminishing reserve of fossil fuels calls for the development of biofuels. Biofuels are produced from renewable resources, including photosynthetic organisms, generating clean energy. Microalgae is one of the potential feedstock for biofuels production. It grows easily even in waste water, and poses no competition to agricultural crops for arable land. However, little is known about the algae lipid biosynthetic regulatory mechanisms. Most studies relied on the homology to other plant model organisms, in particular Arabidopsis or through low coverage expression analysis to identify key enzymes. This limits the discovery of new components in the biosynthetic pathways, particularly the genetic regulators and effort to maximize the production efficiency of algal biofuels. Here we report an unprecedented and de novo approach to dissect the algal lipid pathways through disclosing the temporal regulations of chromatin states during lipid biosynthesis. We have generated genome wide chromatin maps in chlamydomonas genome using ChIP-seq targeting 7 histone modifications and RNA polymerase II in a time-series manner throughout conditions activating lipid biosynthesis. To our surprise, the combinatory profiles of histone codes uncovered new regulatory mechanism in gene expression in algae. Coupled with matched RNA-seq data, chromatin changes revealed potential novel regulators and candidate genes involved in the activation of lipid accumulations. Genetic perturbation on these candidate regulators further demonstrated the potential to manipulate the regulatory cascade for lipid synthesis efficiency. Exploring epigenetic landscape in microalgae shown here provides powerful tools needed in improving biofuel production and new technology platform for renewable energy generation, global carbon management, and environmental survey.

Chlamydomonas as model for algal biofuel feedstock



Microalgae potentially produces the highest oil yield with the smallest land area required.

Unique Advantages of Algae as Biofuel Feedstock

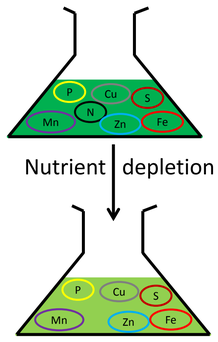
- High area productivity
- Non-seasonal, fast growing
- Minimum competition with agriculture
- Flexibility on water quality
- Renewable energy
- Recycles stationary emissions of CO_2

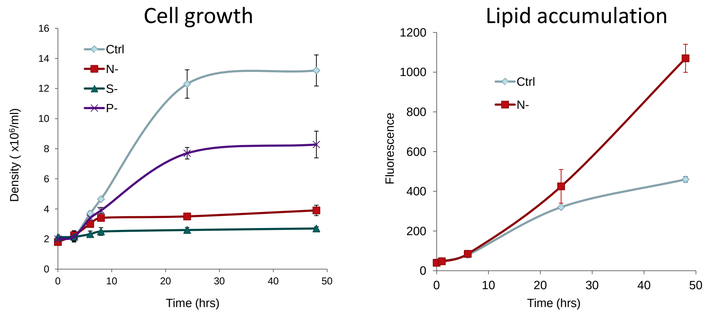
Ref:DOE National Algal Biofuels Technology Roadmap 2010

Chlamydomonas reinhardtii **as a reference model**

- Unicellular green algae (Chlorophyta), found all over the world, in soil, fresh water, oceans
- The most widely used laboratory species is *Chlamydomonas reinhardtii*
- Cells can grow on a simple medium of inorganic salts, using photosynthesis to provide energy
- Reference genome available

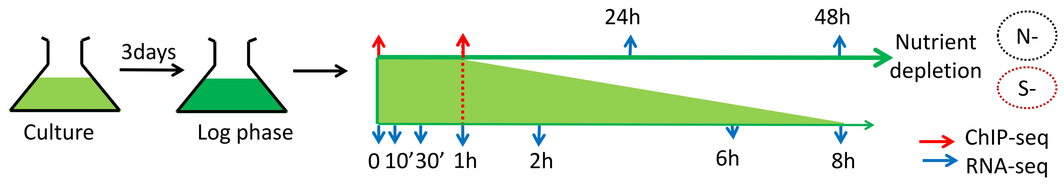
Lack of nutrients induces lipid accumulation chlamydomonas.





- N: Nitrogen, S: Sulfur, P: Phosphorus

Experimental Design

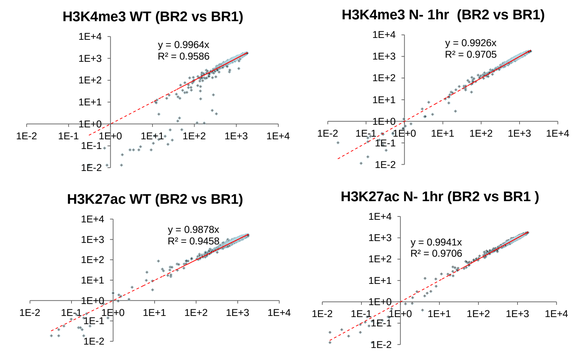


Chromatin Landscape

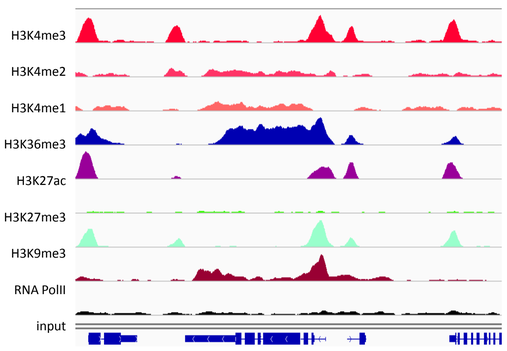
ChIP-seq Data summary

		Wild Type (0hr)			Nitrogen Depletion (1hr)			Sulfur Depletion (1hr)		
Target	Type	# of reads	# of dedupl unique reads	#MACS2 peaks*	# of reads	# of dedupl unique reads	#MACS2 peaks*	# of reads	# of dedupl unique reads	#MACS2 peaks*
H3K4me3	IP	6,991,217	5,146,811	**11,487**	3,977,733	3,074,149	**12,821**	6,860,877	4,651,112	**12,868**
	Input	6,743,218	4,040,258		3,719,293	2,216,073		6,382,232	3,638,293	
H3K27ac	IP	4,112,025	2,924,426	**11,992**	4,060,312	3,028,160	**12,244**	7,591,542	4,914,747	**12,623**
	Input	5,394,178	3,158,292		3,719,293	2,216,073		6,131,473	2,868,969	
H3K4me1	IP	4,897,309	4,067,191	106	4,798,606	3,844,505	2	6,858,495	4,895,951	808
	Input	4,090,951	2,714,973		3,757,818	2,404,500		7,755,521	3,867,641	
H3K4me2	IP	4,503,995	3,510,032	2,482	4,301,150	3,250,887	996	6,791,655	4,832,831	9,620
	Input	4,090,951	2,714,973		3,757,818	2,404,500		6,382,232	3,638,293	
H3K9me3	IP	5,174,636	3,214,285	10,575	4,091,263	2,851,236	8,120	8,704,508	3,107,982	2,293
	Input	3,797,702	2,558,492		4,369,910	2,942,246		7,722,693	3,787,568	
H3K27me3	IP	2,541,255	1,744,210	484	5,786,549	3,321,965	521	4,983,273	1,057,077	510
	Input	4,099,370	2,529,618		3,867,019	2,411,860		6,541,621	3,509,535	
H3K36me3	IP	4,339,861	3,371,955	8,873	4,710,003	3,554,242	9,188	8,481,474	4,456,946	8,528
	Input	4,070,390	2,681,361		4,369,910	2,942,246		7,722,693	3,787,568	
RNA Pol II	IP	3,791,640	2,077,194	**3,143**	3,877,267	2,119,854	3,138	6,729,049	1,945,188	**4,385**
	Input	2,475,900	1,550,148		3,649,425	2,221,803		6,382,232	3,638,293	

Data Reproducibility

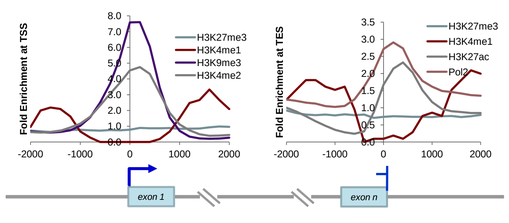


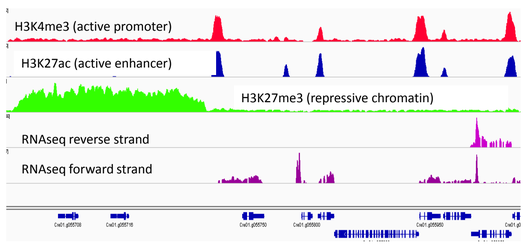
Genomic features



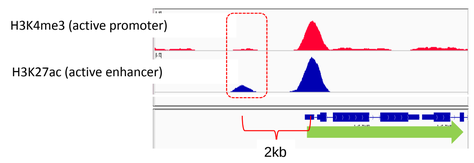
- Histone marks indicate a promoter-centric regulation.

		WT Fold Enrichment					
Mark	%genome	Intergenic	Gene	TSS	Exon	TES	Intron
H3K4me1	0.05	0.13	1.13	0.00	0.99	0.10	1.35
H3K27me3	0.62	1.58	0.91	0.77	0.92	0.71	0.90
Pol2	2.77	0.22	0.97	4.26	1.15	2.67	0.70
H3K4me2	1.51	0.23	0.90	4.47	1.06	1.80	0.64
H3K36me3	9.38	0.55	1.02	5.02	1.07	0.79	0.93
H3K4me3	10.94	0.29	0.94	6.78	1.08	1.55	0.72
H3K27ac	10.24	0.30	0.93	6.95	1.09	1.60	0.69
H3K9me3	8.32	0.22	0.97	7.48	1.12	1.33	0.73

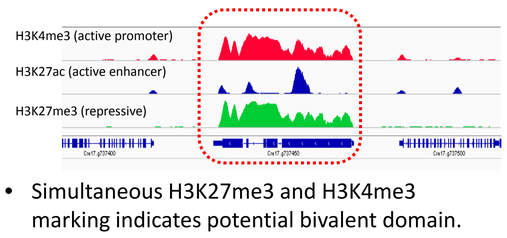




- H3K27me3 marks heterochromatin.



- H3K27ac-specific marks indicates potential distal regulatory elements/enhancers.



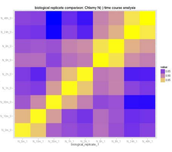
- Simultaneous H3K27me3 and H3K4me3 marking indicates potential bivalent domain.

Transcriptome

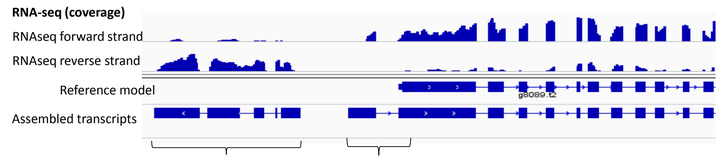
Data summary

Total 9 timepoints x 2 conditions, Total reads of >50m per lib

Genome ver5	Reference	Cufflink	Modified/filtered
Genes	18,773	17,730	16,467
Transcripts	19,529	33,929	32,395
Unique Transcriptome size (Mb)	94.6	97.8	96.1
Mean transcript length	5411	5922	6203

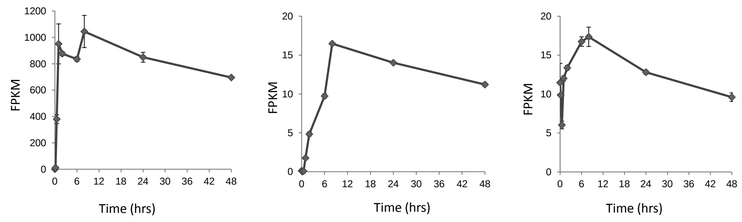


RNA-seq (coverage)



- Novel genes (184)
- Antisense genes (1727)
- Novel exon (544)
- Extension (6100)
- Merged (685)

Control genes expression profile



AMT4 (ammonium transporter): Immediate response to N depletion

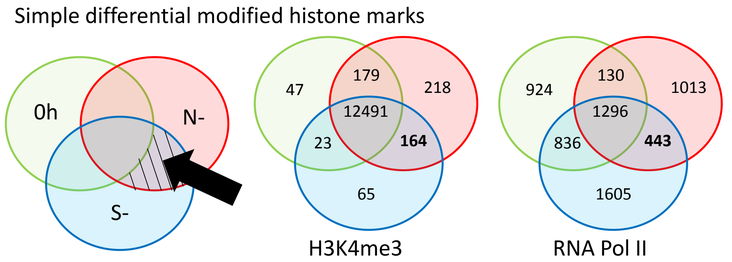
DGTT1 (diacylglycerol acyltransferase)

PDAT1 (phospholipid diacylglycerol acyltransferase)

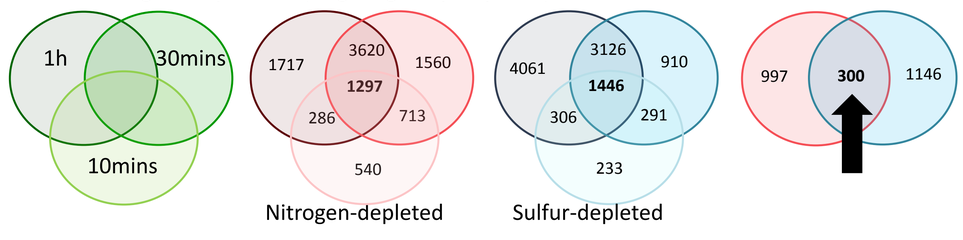
Data mining: Searching for common players in lipid biosynthesis pathway

Multiple approaches targeting common differentially regulated components in nitrogen and sulfur depleted conditions.

- Simple differential modified histone marks



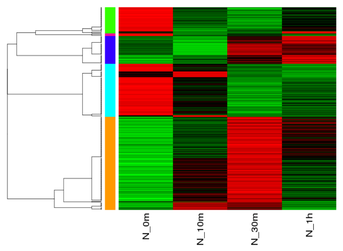
- Differential gene expression (targeting early response)



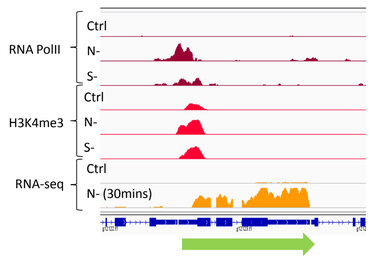
- Differential chromatin state (ChromHMM)

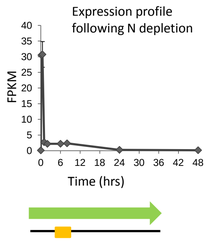
	H3K27	H3K4	H3K4	H3K4	H3K36	H3K27	H3K9		WT Fold Enrichment					
State	ac	me1	me2	me3	me3	me3	me3	Pol2	%genome	#segments	Intergenic	Gene	TSS	Exon	TES	Intron
14	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	84.32	13,641	1.39	1.01	0.26	0.99	0.89	1.04
3	0.6%	0.6%	1.0%	1.3%	98.8%	0.0%	2.6%	0.0%	1.96	3,982	1.53	1.12	0.07	1.00	0.16	1.31
4	3.2%	1.0%	3.1%	3.3%	100.0%	0.0%	5.4%	97.7%	0.31	613	0.10	1.08	0.27	1.01	0.91	1.21
1	0.1%	0.0%	0.5%	0.4%	0.0%	86.1%	10.4%	0.5%	0.19	302	0.29	0.88	0.54	0.91	0.78	0.84
2	0.1%	0.0%	1.8%	0.9%	98.8%	98.7%	11.9%	0.4%	0.42	204	0.25	0.93	0.66	0.93	0.74	0.93
5	2.0%	0.2%	0.9%	1.5%	0.0%	0.0%	0.4%	97.2%	0.91	1,205	0.12	0.95	0.91	1.29	5.14	0.41
10	84.8%	0.0%	0.0%	91.3%	78.8%	0.0%	0.0%	0.0%	0.33	1,707	0.15	1.01	1.22	0.84	0.67	1.28
13	89.4%	0.0%	0.0%	0.0%	0.0%	0.0%	2.3%	1.3%	0.52	2,385	0.33	0.71	2.41	0.85	2.65	0.49
12	24.0%	0.1%	95.5%	66.9%	0.6%	0.5%	0.0%	1.2%	1.07	2,085	0.63	0.88	4.15	1.04	1.97	0.62
9	80.1%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	3.1%	1.72	6,093	0.33	0.82	4.62	0.96	2.50	0.62
11	63.8%	0.0%	92.9%	99.3%	58.6%	10.3%	78.8%	8.0%	0.39	1,019	0.60	0.94	5.68	1.12	1.32	0.66
6	98.2%	0.0%	0.5%	99.7%	95.3%	0.0%	94.8%	99.8%	1.43	2,010	0.49	0.98	7.13	1.10	1.29	0.80
7	97.1%	0.0%	0.0%	99.7%	100.0%	0.0%	100.0%	0.0%	4.78	7,519	1.00	0.99	7.44	1.13	0.93	0.78
8	94.3%	0.0%	0.0%	100.0%	0.0%	0.0%	100.0%	0.1%	1.65	4,422	1.12	0.89	8.65	1.11	2.50	0.56
									Pol2	0.22	0.97	4.26	1.15	2.67	0.70	

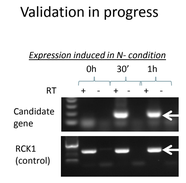
- Clustering (gene expression)



- Potential regulators







Discussion

Here, we report a pilot attempt to understand the regulation of algae lipid biosynthetic pathways by genome wide chromatin landscaping. Deep sequencing of transcriptome also provide supporting data to further dissect the regulatory pathways.

Computational analysis have enabled us to narrow into a smaller group of highly potential regulators. In-depth analysis is still on going to map out a potential interaction or inter-regulatory pathways. Effort to further validate these findings in the biological system is currently underway.

This approach shall provide a more feasible way of dissecting regulatory elements in algae and potentially overcome the limitation of the laborious and low-throughput mutagenesis screening approach.

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