Transcriptome Analysis of Manganese-deficient *Chlamydomonas reinhardtii* Provides Insight on the Chlorophyll Biosynthesis Pathway

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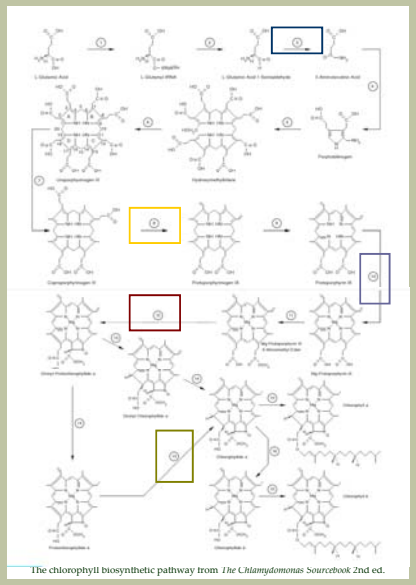
Abstract

The biosynthesis of chlorophyll and other tetrapyrroles is a vital but poorly understood process. Recent genomic advances with the unicellular green algae *Chlamydomonas reinhardtii* have created opportunity to more closely examine the mechanisms of the chlorophyll biosynthesis pathway via transcriptome analysis. Manganese is a nutrient of interest for complex reactions because of its multiple stable oxidation states and role in molecular oxygen coordination. *C. reinhardtii* was cultured in Manganese-deplete Tris-acetate-phosphate (TAP) media for 24 hours and used to create cDNA libraries for sequencing using Illumina TruSeq technology. Transcriptome analysis provided intriguing insight on possible regulatory mechanisms in the pathway. Evidence supports similarities of GTR (Glutamyl-tRNA synthase) to its *Chlorella vulgaris* homolog in terms of Mn requirements. Data was also suggestive of Mn-related compensatory up-regulation for pathway proteins CHLH1 (Manganese Chelatase), GUN4 (Magnesium chelatase activating protein), and POR1 (Light-dependent protochlorophyllide reductase). Intriguingly, data suggests possible reciprocal expression of oxygen dependent CPX1 (coproporphyrinogen III oxidase) and oxygen independent CPX2. Further analysis using RT-PCR could provide compelling evidence for several novel regulatory mechanisms in the chlorophyll biosynthesis pathway.

Introduction

Chlorophyll is a vital pigment that allows photosynthetic organisms to capture light. It is derived via a multistep biosynthetic pathway, many mechanisms of which are at the moment unclear.



The chlorophyll biosynthetic pathway from *The Chlamydomonas Sourcebook* 2nd ed.

To gain information on the mechanisms of this pathway, transcriptomes from cells starved of Manganese were compared with transcriptomes from cells grown under normal conditions. Mn was chosen because it has multiple stable oxidation states and therefore is an ideal cofactor for complex reactions. It is often implicated in reactions involving molecular oxygen.

The unicellular green algae *Chlamydomonas reinhardtii*, a precurser for microalgal biofuel research was chosen as the model organism for this study.

Methods & Materials

Cell Cultures

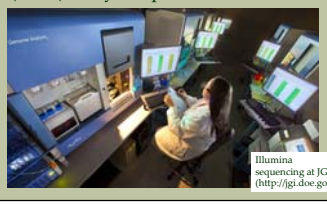
Chlamydomonas reinhardtii wild type strain CC4051 4a+ was cultured to mid-logarithmic phase in 50 ml Tris-acetate-phosphate (TAP) medium (26 μM Mn) . For Mn-deficiency, cells were harvested from normal growth conditions, resuspended in Mn-deficient TAP (0μM Mn) at 3.0×10^5 cells/ml, and grown for 24 hours.

cDNA Library Creation and Sequencing

RNA was purified from cell cultures and prepared for cDNA synthesis using an Absolutely mRNA Purification Kit (Stratagene, La Jolla, CA). Double stranded cDNA was synthesized according to the Illumina Truseq Library Creation Kit protocol. (Illumina, Inc., Hayward, CA). cDNA was sequenced using Illumina Truseq technology (50 cycles, single end reads).

RNASeq Analysis

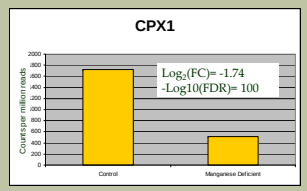
RNASeq reads were mapped to the *C. reinhardtii* transcriptome (http://genome.jgi-psf.org/chlre3/chlre3) with Burrows-Wheeler Aligner (BWA). Only unique reads were counted.

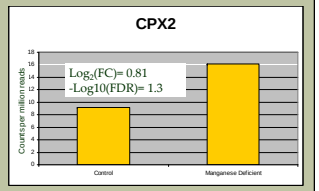


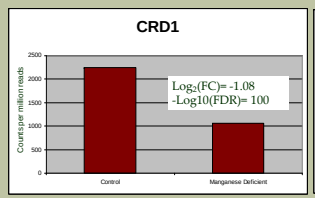
Illumina sequencing at JGI (http://jgi.doe.gov)

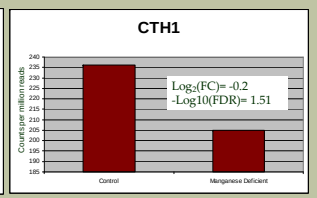
Results

Coproporphyrinogen III oxidases CPX1 and CPX2 catalyze step 8 of the pathway and are respectively oxygen-dependent and –independent.

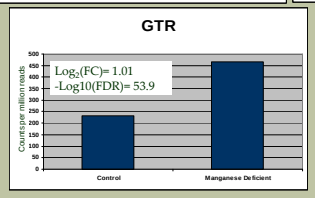


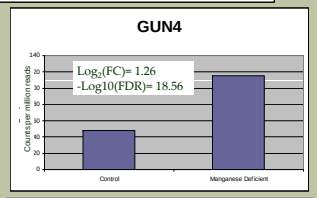


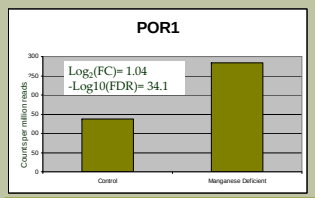




Magnesium-protoporphyrin IX monomethyl esters CRD1 and CTH1 catalyze step 12 of the pathway







Glutamyl-tRNA reductase (GTR) catalyzes step 3.

Magnesium chelatase activating protein (GUN4) catalyzes step 10 .

Light-dependent protochlorophyllide reductase (POR1) catalyzes step 13.

$Log_2(FC)$ is the fold change in expression. The –Log10(FDR) reports False Discovery Rate; values ≥ 3 are considered statistically significant.

Discussion

•Breckau, *et al.* (2003) reported stimulation of HemF, the *Escherichia coli* homolog of oxygen-dependent CPX1, by Mn and proposed an alternative anaerobic pathway catalyzed by HemN, the *E. coli* homolog of oxygen-independent CPX2. In the present study, CPX1 was siginificanly down-regulated, and CPX2 was slightly up-regulated suggesting potential reciprocal expression of the two genes. Longer Mn-starvation periods could lead to increased CPX2 production.

•Moseley, *et al.* (2002) reported a similar reciprocal expression pattern for CRD1 (up-regulated in hypoxic cells) and CTH1 (up-regulated in oxygenated cells) in *C. reinhardtii*, however both genes were down-regulated in the present study.

•Mayer, *et al.* (1994) reported a possible Mn requirement for GTR in *C. vulgaris* and *Synechocystis* sp.. Up-regulation of GTR in this study supports a similar requirement in *C. reinhardtii*.

•Up-regulation of GUN4 and POR1 in this study is indicative of a possible Mn requirement.

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