A day in the life of Chlamydomonas

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Project goals: The green alga *Chlamydomonas reinhardtii* is a reference organism for many questions in biology, especially photosynthesis. To better understand this important model organism, we generated a rich functional genomics dataset, combining transcriptomic data with physiological measurements over the course of a Chlamydomonas cell cycle. Cultures were grown in a flat panel bioreactor system for reproducible synchronization. RNA-seq data was collected in triplicate at regular intervals over the course of a 12-h light/12-h dark cycle. In order to obtain a global view of expression, the transcriptomic data was sorted using a K-means clustering strategy. We observed that 81% of all transcribed genes were differentially expressed at one or more time points, and these were grouped into 16 major expression patterns describing the sequence of events during a day in the alga's life. Among the most interesting responses were the orchestration of cell division and fermentation during the night, and the balance between photoprotection and photosynthesis during the day.

During the cell cycle, DNA replication and cell division are initiated at the beginning of the dark period. The demand to duplicate the core histones during S phase is met by the coordinated expression of multiple genes encoding each of the histones (2). In metazoans, replication-dependent histone mRNAs differ from canonical mRNAs in that they lack a poly-A tail. In contrast, plants and many unicellular eukaryotes express only poly-Adenylated histone transcripts (3). The existence of non-poly-Adenylated histone mRNAs was assumed to be a unique to metazoans, until their recent discovery in the green algae Chlamydomonas (4) and Volvox (5). In our RNA-seq data, we observed coordinated expression of multiple genes encoding each of the core histones H2A, H2B, H3 and H4, as well as the linker histone H1. Interestingly, we also observed constitutive expression from two of each of the histone genes, suggesting that they provide an emergency supply of histones. The helicase of the mini-chromosome maintenance (MCM) complex involved in DNA replication, precedes the expression of core histone genes, ensuring that newly synthesized nucleosomes can be loaded on replicated DNA. The expression of the MCM complex increased more than 20-fold just prior to the onset of the dark phase.

After replication, the cells remain in G_0 for the remainder of the night. In Chlamydomonas, starch represents the major carbon storage molecule. We observed a maximum of starch accumulation at the end of the light period, directly prior to cell division. We also observed a dramatic reduction in the quantity of starch per cell occurring shortly after the onset of the night phase, which is consistent with redistribution of starch to daughter cells during mitosis. We measured the oxygen consumption of the cells during the cell cycle and found that the cells do not respire throughout the night. Instead, we observed that genes involved in anaerobic metabolism, such as the gene encoding an iron hydrogenase (*HYDA1*) were up-regulated.

The dark-light transition at the onset of a 12-hour photoperiod was concomitant with a rapid increase in stress related light harvesting genes involved in photo-protection. This increase was accompanied by reduced photosystem II capacity. Surprisingly, dusk-dawn experiments, in which the onset of light was gradually increased over a period of 2 hours, demonstrated that photosystem II capacity was still reduced during the dark light transition.

The plastid and nucleus encoded subunits of the chloroplast electron transfer chain were coordinately expressed, with peak expression occurring in the middle of the photoperiod. The increase in subunits of the electron transfer chain was also accompanied by an increased plastoquinone pool and more chlorophyll per cell, finally leading to increased oxygen evolution at the end of the day. This oxygenic photosynthesis was inevitably accompanied by the production of reactive oxygen species (ROS) that are generated within the electron transfer chain in chloroplasts. ROS production via photosystem I and photosystem II is known to be elevated, especially when carbon dioxide is limited, as it was in this experiment (1). Genes encoding ROS scavenging enzymes, such as ascorbate peroxidase 1 (*APX1*) and and Mn and Fe superoxide dismuates (*MSD1* and *FSD1*) were collectively up-regulated throughout the light period. In addition, there was increased expression of genes involved in the biosynthesis of a specific group of non-enzymatic anti-oxidants throughout the light period, namely tocopherols (Vitamin E). The major tocopherol, α-tocopherol was strongly induced after onset of light, but only the accumulation of γ-tocopherol increased proportionately with the increase in biomass.

References

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