Correlation of periodic changes in the transcriptome and proteome in the Chlamydomonas cell cycle

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Project goals: The green alga *Chlamydomonas reinhardtii* has been a reference organism for addressing many questions in biology, and it also serves as a reference organism for commercial applications in photosynthetic eukaryotes, including biofuel production. We have reported previously on a multilayer data set of gene expression, metabolomics, proteomics and physiology during the Chlamydomonas cell cycle, utilizing a flat panel bioreactor system for reproducible synchronization of *Chlamydomonas reinhardtii*. We further expanded our understanding of the cell cycle by generating an additional full-scale proteomic data set augmented by a concept called the "proteomic ruler", which allows for absolute protein quantification and copy number estimates. We integrate this additional dataset to review the relationship between the proteome and the transcriptome during the course of one day/night cycle.

Chlamydomonas reinhardtii is a unicellular green alga that has been widely used as a plant reference system, it has a short generation time and its three genomes are sequenced and well-annotated. Previously, we analyzed expression patterns of all three genomes of *Chlamydomonas reinhardtii* over the course of a day in cultures synchronized by a 12 h dark 12 h light period. Nearly 85% of transcribed genes show differential expression, with different sets of transcripts being up-regulated during each phase of the cell cycle. Parallel measurements of select metabolites and pigments, physiological parameters and a subset of proteins offered the opportunity for inferring metabolic events and for evaluating the impact of the transcriptome on the proteome. Assessment of starch, total organic carbon and respiratory activity suggested that fermentative metabolism may dominate during the night. This multi-omics approach offers an unprecedented high-resolution, systems-level view of cellular processes as cells grow in the light period and divide in the dark from one to two cells (1).

In this study, we generated a high resolution proteomic data set of synchronized Chlamydomonas cells. Over the course of the day we could identify 10696 different proteins, ~ 2120 proteins were identified with at least two peptides in each of the 16 time points. We further expanded our proteomic data set by a concept called the "proteomic ruler" (PR), which allows for absolute protein quantification and estimates of protein copy number per cell (2). This method is based on the assumption that the MS signal of histones can be used as an internal standard, the so-called "proteomic ruler". Since the amount of histones is proportional to the DNA content of a sample and correlates with cell number, this internal normalization allows absolute protein quantification without external spike-in standards. In order to validate protein quantification of our cell cycle data set using the Proteome Ruler (PR) method, we first compared the quantitative readout for total protein content per cell obtained using PR with results generated independently experimentally from BCA positive material. We obtained excellent correlation between these data, with a ratio of total protein content per cell PR:BCA of 1:1.11 (±0.15). We also validated correct absolute scaling of our proteomic data by comparison with earlier studies using either a spike-in

of Quantification Concatamers (QconCATs) (3), spike-in of purified proteins combined with heavy-labeled Chlamydomonas cell extracts (4) or spiked-in synthetic, isotope-labeled peptides and LC-MS analysis by SRM (5). Since other proteomic data was obtained using asynchronous cultures, we used the averaged protein quantifications over the diurnal cycle for comparison. In the proteomic data set using QconCATs it was estimated that unsynchronized Chlamydomonas cells contained an average of 2.9 amol PS1 per cell (3). Using the proteome ruler approach, we observed an average of 2.7 amol PS1 / cell, ranging from 2.3 (night) to 4.4 (day) amol PS1 / cell over the course of the day. We used this quantitative dataset to confirm ratios of proteins in protein complexes involved in photosynthesis. Our observed ratio of PS1 to plastocyanin of 1:1.17 compares well with the 1:1.12 ratio observed by the method based on quantification concatamers (3) and the 1:1.4 ratio that was determined using a spike-in of purified proteins (4).

Rubisco is composed of eight chloroplast encoded large subunits (rbcL) and eight nucleus-encoded small subunits (RBCS) (6). Based on known mechanisms for the regulation of RbcL and RBCS gene expression (7,8), we assumed both subunits to be present in equimolar amounts to allow a 1:1 subunit stoichiometry of the holoenzyme. Thus, ratios between RbcL and RBCS of 11–44:1 (4), 5:1 (9), determined in earlier studies were quite surprising, with a ratio of 1.56:1 obtained using QconCATs being closest to expectations (3). Using the proteome ruler approach, we estimated that the large subunit of Rubisco is present in average 8.9 amol / cell during the cell cycle, with a ratio of rbcL to RBCS of 1:0.98.

We further use these data to review the relationship between the proteome and the transcriptome during the course of a full diurnal cycle. Changes in transcript and protein abundances correlate in the majority of cases, if a delayed response at the protein level is taken into account.

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