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Commentary

Short Commentary on: Comparative Proteomics of *Mesembryanthemum crystallinum* Guard Cells and Mesophyll Cells in the Transition from C3 to CAM

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ABSTRACT

In the work entitled "Comparative proteomics of *Mesembryanthemum crystallinum* guard cells and mesophyll cells in the transition from C3 to CAM", we presented an interesting diurnal/diel proteomics analysis in M. *crystallinum* during its photosynthesis transition using the label-free method. We identified the proteins showing inverse responding patterns at each time point between the control (no transition) and treatment group (undergoing transition) in guard cells and mesophyll cells. This simple but useful method allowed us to focus on 165 and 151 proteins out of 1153 proteins in guard cells and mesophyll cells, respectively. The results facilitated understanding of plant photosynthesis plasticity at the single cell-type level.

Keywords: *Mesembryanthemum crystallinum*; Label-free proteomics; Diel pattern analysis; Inverse protein change pattern; C3 to CAM transition

DESCRIPTION

Although isobaric chemical labeling proteomics approaches like TMT and iTRAQ have better performance in multiplexing, they are quite costly and require extra experimental steps such as in vitro N-hydroxysuccinimide chemical reaction. In this study, we applied a label-free quantitative proteomics method [1,2]. Guard cells respond to complex interplay of signals, and guard cell proteomics may reveal interesting protein changes underlying the responses [3]. Computational methods can be useful in normalizing label-free proteomics data and using relative quantification to determine proteins involved in the biological processes. Here we developed a method to select and cluster proteins that exhibit temporal inverse abundances during the ice plant photosynthetic transition from C3 to Crassulacean Acid Metabolism (CAM).

A workflow designed to analyze inverse protein abundance patterns (Figure 1) was applied to M. *crystallinum* guard cells and mesophyll cells during the C3 to CAM transition induced by salt stress [4]. First, the raw data were analyzed using Proteome Discoverer 2.3 and the peak area for each protein was used for quantification. To analyze the changes of diel protein abundance in the two cell-types during the transition, median normalization followed by z-score transformation was used as a normalization method. Secondly, proteins have Person Correlation Coefficient (PCC) score less than -0.8 were selected as inversely expressed proteins [5] in the analysis of temporal changes of proteins during the C3-CAM transition (control versus treatment) in guard cells and mesophyll cells. Out of a total of 1153 identified proteins, 165 and 151 proteins showed inversed protein change patterns in guard cells and mesophyll cells, respectively. Thirdly, clustering of k-means based on Euclidean distance for control

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group proteins, in which each time point acts as a dimension, was used as the clustering method. Lastly, each cluster was analyzed using Gene Ontology (GO) enrichment [6] so that the functional aspects of the changed proteins in each cell type could be analyzed.

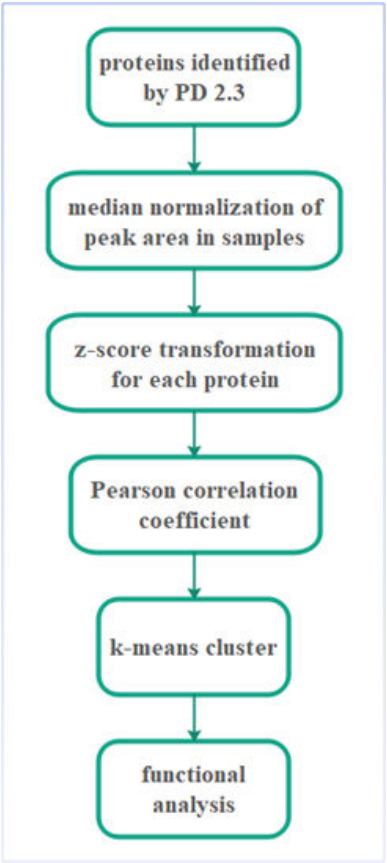

Figure 1: Workflow for the analysis of inverse protein abundance patterns in *M. crystallinum* guard cells and mesophyll cells during the C3 to CAM transition.

According to our proteomic result, the light response proteins in guard cells showed inversed abundance patterns during the transition, while those in mesophyll cells did not. This result is consistent with the inversed stomata movement behavior in the CAM plants [7]. Based on the protein changing patterns and the transition taking place before day 7 of treatment [8], we focused on cluster 3 of guard cell proteins and cluster 4 of mesophyll cell proteins. Another interesting finding from the enriched GO functions in two clusters is that ATP binding proteins and zinc ion binding proteins in two cell-types had very similar abundance changing patterns. This result indicates that there are common processes in the guard cells and mesophyll cells during the C3 to CAM transition.

LIMITATIONS

Our results highlight that the mechanisms underlying the shift from C3 to CAM in the two cell-types in M. *crystallinum* are different. A stringent cutoff of PCC was used to filter the proteins with the inversed patterns. However, some other potentially useful information may be sifted out. In addition, functional testing using reverse genetics in ice plant is challenging. Developing a model system (e.g., *Kalanchoe blossfeldiana* in Crassulaceae [9,10] for functional analysis of the candidate proteins in the C3 to CAM transition is needed.

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