Abstract

Role of the plastid envelope membrane in integrating the plastid into cellular metabolic networks

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Plastids are the defining organelle of the Archaeplastida which include all land plants. They can differentiate into several subtypes in a tissue dependent manner, for example, brightly colored chromoplasts in bell pepper fruits, green chloroplasts in leaves, or colorless proplastids in meristems, and each subtype is adapted to the cell it resides in. All plastid types of land plants are separated from the cytosol by two membranes, the inner envelope and the outer envelope membrane, and metabolites and signals have to cross these envelope membranes to connect to the remainder of the cell. This work is focused on integrating the plastids into the metabolic network of their respective cells; the adaptations of envelope membranes of different plastid subtypes were analyzed by comparative proteomics.

Initially, envelope proteomics of plants without a sequenced genome or transcriptome was established with the garden pea *Pisum sativum* as the new model. A novel sequencing technology, pyrosequencing, was used to create a transcriptome database which is suitable for proteomics applications. Data generated with pea leaf chloroplasts served as the template to which other differentiated plastid types were compared both qualitatively and semi-quantitatively.

The qualitative comparison of chloroplast and proplastid envelopes revealed specific adaptations of the proplastid envelope to its role in meristematic cells: Proplastids serve as cellular factories for amino acids, fatty acids and nucleotides for the proliferating cells. The transport protein complement is geared to import precursor metabolites and export products from the heterotrophic plastid. Chloroplast envelopes of pea were also compared to those of maize mesophyll cells in a semi-quantitative manner. Maize plants employ a specific subtype of photosynthesis called C4 photosynthesis which causes immense metabolite fluxes across the chloroplast envelope. The comparison revealed quantitative changes in the envelope protein composition indicating that the flux is accommodated by increased amounts of transport protein. It also revealed new candidate transport proteins for metabolite fluxes of C4 chloroplasts. One of these candidate transport proteins was characterized in more detail in the model plant *Arabidopsis thaliana* and it likely is a monocarboxylate transporter employed in photorespiration in C3 plants. Finally, proteome samples of the plastid associated membranes, the inner and the outer envelope of chloroplast and endomembrane system were investigated. GFP fusion protein analyses of candidate outer envelope residents demonstrated that the outer envelope is a dynamic system capable of producing extensions of the envelope membrane as well as vesicles. Plastid associated membranes are hypothesized to be part of the autophagy system for chloroplasts since they contain stromal proteins in addition to envelope and endomembrane system residents. The hypothesis is supported by GFP fusion protein analysis.