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Evolution and communication of subcellular compartments

An integrated approach

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Compartmentation is a fundamental feature of eukaryotic cells and the basis for metabolic complexity. We recently reported on the protein compartmentation in the moss *Physcomitrella patens*. This study utilized a combination of quantitative proteomics, comparative genomics, and single protein tagging and provided data on the postendosymbiotic evolution of plastids and mitochondria, on organellar communication, as well as on inter- and intracellular heterogeneity of organelles. We highlight potential organelle interaction hubs with specific protein content such as plastid stromules, and report on the plasticity of protein targeting to organelles.

Keywords: Chloroplasts, metabolic compartmentation, mitochondria, moss, organelle connectivity, quantitative proteomics, stromules

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Eukaryotic cells are highly compartmentalised. Each subcellular compartment constitutes a different context for biochemical reactions in terms of protein composition, substrate concentration, and connectivity to other organelles. These different compartments need to coordinate cellular functions in a smooth and effective way while conserving responsiveness to internal and external stimuli. In plants, 2 of these compartments, plastids and mitochondria, are of endosymbiotic origin.[1] From early eukaryotes the integration of endosymbionts has demanded the evolution of new protein functions, e.g., transporters for organellar protein import,[1] resulting in as many as 40% of eukaryote-specific proteins in mammalian mitochondria.[2] On the one hand, evolution has shaped endosymbionts into metabolically integrated cellular compartments, on the other hand chloroplasts and mitochondria retain their own transcriptional and translational machinery and can influence nuclear gene expression by retrograde signaling.[3-5]

Primarily, compartmentation of function largely depends on the allocation of proteins to organelles, although a crucial role of interorganellar communication and connectivity for organelle biogenesis, signaling, and dynamics is emerging (**Fig. 1A**).[7,10] However, the increasing amount of genome data often lacks experimental validation concerning the subcellular localization of proteins. In addition, the targeting of proteins and entire metabolic pathways can change during evolution because selection might favor a different metabolic compartmentalisation.[11] Hence, subcellular localization and its variability can hardly be detected by automatic genome annotation or localization prediction as both methods are biased toward well annotated, sometimes evolutionary distant, model species.

Recently, we generated a quantitative proteomics data set for chloroplasts and mitochondria of the non-vascular model plant *Physcomitrella patens* using full metabolic labeling.[8] This top-down study started from a relative comparison of protein abundance between plastid and mitochondrial extracts and integrated these data with phylogenetic and functional information from several databases like KEGG, PLAZA, Phytozome, and PlantCyc,[9,12-14] as well as a bioinformatic

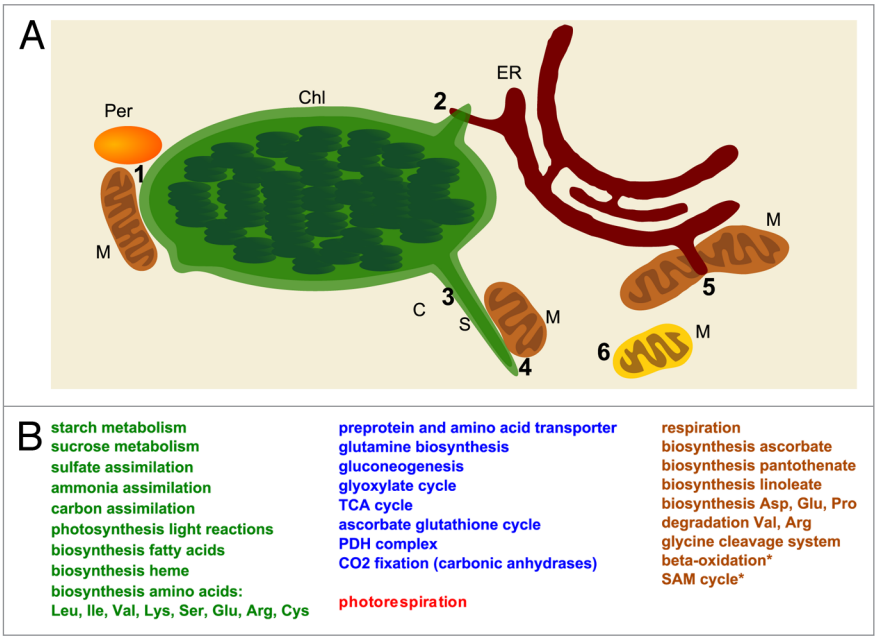

Figure 1. Organelles are often closely associated. (**A**) The nature and function of putative contact sites is mostly unclear. Peroxisomes (Per) are often in proximity to chloroplasts (Chl) and mitochondria (M), which all contribute to the photorespiratory pathway (1). Specific interfaces may exist for stromules (stroma-filled tubules of plastids) and ER (2), stromules (S) and the cytosol (C) (3), and stromules and mitochondria (M) (4). The biochemical continuity of ER and plastids has been proven for several nonpolar metabolites in the plastid envelope.[6] ER-mitochondria contact sites promote mitochondrial fission in animals[7] but are not yet described for plants (5). Mitochondria show intracellular heterogeneity (6). (**B**) Simplified overview of the bioinformatic analysis of metabolic pathway compartmentation in *Physcomitrella patens*.[8] Metabolic pathways were allocated to organelles by multivariate analysis integrating MossCyc[9] pathways. Green: pathways in plastid cluster; brown: pathways in mitochondrial cluster, *were shown to be localized to peroxisomes and cytosol by single protein validation;[8] red: peroxisomal cluster; blue: pathways which had an intermediary position in the analysis and require the communication between plastids and mitochondria or share functionalities.

analysis of ortholog groups.[15] Thus, we provided the first high-throughput analysis of pathway compartmentation (**Fig. 1B**) in a moss, which is separated from flowering plants by 500 million years and from green algae by 300 million years of evolution.[16] Subsequently, we addressed the sub- and neo-functionalisation of protein isoforms in expanded gene families by linking the quantitative proteome data to phylogenies, thus characterizing the relative abundance of isoforms within the same organelle as well as the allocation of isoforms between organelles. This is a novel approach to map gene family diversification, employed to an organism with high metabolic redundancy like *P. patens*,[17] which has undergone a genome duplication 30–60 million years ago and has retained many paralogs afterwards.[18]

Moreover, the analysis of the relative protein abundance in mitochondria and chloroplasts revealed candidate proteins for dual targeting and organellar interaction hubs. As by dual or multiple targeting the same nuclear-encoded protein might be involved in distinct metabolic pathways in different organelles, metabolic complexity is often increased without an increase in gene number.[19] This mechanism might be important for the coordination of organellar functions in metabolic pathways distributed in several compartments.[19,20] The relative protein abundance determined by our metabolic labeling approach provided information about the extent of dual targeting, which was confirmed by fusion to a fluorescent reporter. Proteins with multiple subcellular localizations can show a preference toward one, as seen for the dual-targeted transporter PpPRAT3.1.[8]

Taking advantage of the high rate of homologous recombination in *P. patens* ,[21] we used targeted knock-in of a reporter into the endogenous genomic locus of candidate genes. Thus, a fusion protein was expressed from the native genomic context, enabling the analysis of spatio-temporal variability in protein targeting. The targeting in a specific cell depends on conditions such as developmental stage and tissue. Further, our data revealed heterogeneity of the mitochondrial population inside a single cell, as monitored by the abundance of the caseinolytic protease proteolytic subunit (ClpP). Thus, patterns of organelle functionality are organized on several levels: gene expression, protein targeting, and the status of single organelles.

In addition to the "one way street" of protein import, the communication between organelles can also occur directly by physical contact sites or even biochemical continuity.[6,7] To analyze subcellular proteomes usually a combination of centrifugation steps with increasing velocity (differential centrifugation) and purification on density gradients is used.[22,23] A certain amount of contaminants of other cellular fractions may co-purify during this procedure, depending on the organism and the tissue. While in yeast the ER is the most prominent contaminant of mitochondrial fractions,[24] in plants the proteins of peroxisomes and of the photosynthetic apparatus are major contaminants.[25] We assessed the importance of co-purification in proteomics experiments on the single protein level for several proteins with conspicuous quantitative proteomics data. While some were identified as contaminants, other proteins were found at interaction sites between organelles or in specific suborganellar localizations, such as plastid stromules. Our data suggest a specific protein content for these stroma-filled tubules that emanate from plastids and can in turn serve as a starting point to unravel stromule function. Different parts of the organelle surface might contribute distinctly to transport processes, such as between the chloroplast and the cytosol, or define contact sites to other compartments such as the ER (**Fig. 1A**). The underlying principles will likely generate asymmetric distribution of effectors inside the

compartments and further strengthen a relationship between form and function in organelles. Proteins such as the newly discovered MELL1 (*mitochondria-ER-localized LEA-related LysM domain protein1*) appear of particular interest since their abundance influences organelle morphology.[8]

For plants in particular the allocation of proteins and thereby functions as well as the cooperativeness between organelles is highly flexible, depending on developmental programs and on external stimuli. Top-down studies can serve as a starting point for the characterization of organelle biology on multiple levels, especially when quantitative data are available, addressing the evolution of metabolic compartmentation and organellar communication. The moss *Physcomitrella patens* offers a unique platform for follow-up studies as the plasticity of macro- and microcompartmentation can be tracked on the single protein level by utilizing gene targeting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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