197c Bioinformatics and Proteomics Approaches Towards Defining the Proteome of Chloroplast Envelope Membranes

Andreas P.M. Weber, Susanne Hoffmann-Benning, and Andrea Bräutigam

Chloroplasts are the metabolic factory of plant cells. They are the sites of carbon, nitrogen, and sulfur assimilation, and of many important biochemical pathways, such as amino acid, fatty acid, vitamin, isoprenoid, and hormone biosyntheses. The metabolism of chloroplasts is heavily intertwined with that of other cellular compartments. Metabolite exchange between plastid and cytosol is a central aspect of plant biology that was essential for establishing the endosymbiosis between a photosynthetic cyanobacterium and a heterotrophic host cell. Together with the establishment of a protein import apparatus, it was critical for the evolution of modern eukaryotic photosynthetic organisms. Metabolic precursors need to enter the chloroplast stroma and end products need to be exported from the chloroplast. However, chloroplasts are surrounded by a two lipid bilayer membranes, the so-called chloroplast envelope membrane. This prevents unspecific diffusion of polar molecules; the controlled exchange of metabolites between plastids and cytosol hence depends on the activity of transport proteins. Whereas the outer envelope is permeable to molecules up to 10 kD, the inner membrane represents the actual permeability barrier between the chloroplast and the cytosol. Transport of metabolites across the chloroplast envelope is catalyzed by metabolite transporters residing in the inner envelope membrane. Although these transporters are critical for efficient traffic of solutes across the chloroplast membrane, to date only less than 15 of these transporters have been functionally characterized. The majority of these known plastid envelope solute transporters have been identified by biochemical purification and peptide sequencing. This approach is of limited use for less abundant proteins and for proteins of plastid subtypes that are difficult to isolate in preparative amounts. Hence, the majority of plastid envelope membrane transporters are not yet identified at the molecular level. The availability of fully sequenced plant genomes, the progress in bioinformatics to predict membrane transporters localized in plastids, and the development of highly sensitive proteomics techniques open new avenues toward identifying additional, to date unknown, plastid envelope membrane transporters. We will present the recent results of bioinformatics and proteomics approaches towards identifying chloroplast envelope membrane transporters obtained in our and other labs. Special emphasis will be given to the difficulties associated with analyzing hydrophobic membrane proteins.