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Review article

Functional proteomics of light-harvesting complex proteins under varying light-conditions in diatoms☆

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ABSTRACT

Comparative proteome analysis of subcellular compartments like thylakoid membranes and their associated supercomplexes can deliver important in-vivo information on the molecular basis of physiological functions which go far beyond to that what can be learnt from transcriptional-based gene expression studies. For instance, the finding that light intensity influences mainly the relative stoichiometry of subunits could be obtained only by high resolution proteome analysis. The high sensitivity of LC-ESI–MS/MS based proteome analysis allows the determination of proteins in very small subfractions along with their non-labeled semi quantitative analysis. This provides insights in the protein-protein interactions of supercomplexes that are the operative units in intact cells. Here, we have focused on functional proteome approaches for the identification of microalgal light-harvesting complex proteins in chloroplasts and the eyespot in general and in detail for those of diatoms that are exposed to varying light conditions

1. Introduction

Photoautrotrophic microorganisms (microalgae) are key contributors to global carbon fixation (Field et al., 1998) and comprise several taxonomic groups of very divergent phylogenetic origin. The phytoplankton in freshwater and marine environments includes the prokaryotic cyanobacteria as well as eukaryotic algae which can be divided in those originating from a primary endosymbiosis (green and red algae) and those derived from a secondary endosymbiosis (Keeling et al., 2005; Lane and Durnford, 2010). Within the second group two different lines can be distinguished: a “green line” forming the phylogenetic cluster of the Chlorarachniophytes and Euglenids, and the “red line” which was diversified amongst others into the Cryptophytes, Heterokonts including the diatoms and Haptophytes. During the past decades, whole genome sequences and/or EST libraries from numerous algae were released and provide an efficient basis to investigate in depth photosynthetic life on Earth (Tirichine and Bowler, 2011). Comparative molecular and physiological analysis of these organisms revealed that metabolic pathways associated to specific compartments along with their regulatory mechanisms diverged specifically in the “green” and the heterokont red lineage during algal evolution. This became obvious not only by comparative genome analysis (Armbrust et al., 2004; Bowler et al., 2008) but also on the level of ultrastructure and physiological peculiarities (Wilhelm et al., 2006). In contrast to higher plants, the thylakoids in diatoms are organized in bands consisting of three lamellae without the differentiation in grana and stroma. Therefore, the separation of both photosystems is not as strict as in higher plants, increasing the probability of energy exchange between both photosystems. Again deviating from green plastids, the absorption spectra of both photosystems are quite similar and the so-called state 1-state 2 transitions are lacking. This membrane organization is quite stable in response to the incident light conditions (for a recent review see Wilhelm et al., 2014).

The available genomes paved the way for performing proteomic approaches to gain a deeper understanding in the function and regulation of proteins and their complexes in algal cells. Since many microalgae can be grown axenically and relatively easily in high amounts within a short timeframe, enough pure biological material is available to establish purification procedures for subproteomes of algal cells and to identify the proteins that are present in these organelles by mass

Abbreviations: AUREO, aureochrome; BL, blue light; BN-PAGE, blue native polyacrylamide gel electrophoresis; FCP, fucoxanthin-chlorophyll protein; IEX, ion exchange chromatography; LC-ESI–MS, liquid chromatography-electrospray ionization-mass spectrometry; LHC, light-harvesting complex; NPQ, non-photochemical quenching; PS, photosystem; RL, red light

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spectrometry based approaches. Thereby, one of the first algal model organisms being used for functional proteomics was the unicellular biflagellate green alga *Chlamydomonas reinhardtii*. Proteins of several specific subproteomes were identified in *C. reinhardtii*, including those of the flagella, mitochondria, the chloroplast, the eyespot and its phosphoproteome as well as the redox signaling network (reviewed in Rolland et al., 2009; Terashima et al., 2011). In general, untargeted proteomic approaches of special organelles such as the eyespot or mitochondria also helps to shine light into developmental processes (Schmidt et al., 2006; Plohnke et al., 2014; Eitzinger et al., 2015).

The development of an efficient process for light harvesting to drive photosynthesis was a special challenge in evolution. This is the reason why at least ten chlorophylls and about 80–100 xanthophylls have been invented during evolution in algae to optimize light capture in the aquatic environment (Takaishi, 2011). The selection parameters driving light harvesting systems evolution had been the molar absorption coefficient (with chlorophyll a having the highest of all known organic molecules in the visible light spectrum) and the wavelength of maximum absorption. Here the “green gap” between the Soret and the red band absorption was a target for optimization by developing green light absorbing xanthophylls (e.g. fucoxanthin) or phycobiliproteins which exist only in cyanobacteria, red algae and Cryptophytes. All pigments are bound to proteins in the antenna complexes of the two photosystems, called light-harvesting complex (LHC) proteins. Due to the specific molecular interaction of the pigment with special binding domains in the different light harvesting proteins, the high diversity of pigments developed in co-evolution with the LHC proteins. Much effort was taken to unravel the composition of the LHCs in different algal systems and to compare them to higher plants. One of the first proteome studies to investigate light harvesting proteins in depth was conducted by Stauber et al. (2003) in *C. reinhardtii* using a combination of two-dimensional gel electrophoresis for the separation of these proteins followed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI–MS/MS) of the tryptically generated peptides. The obtained data as well as additional proteomic data from chloroplast proteins of. *C. reinhardtii* (Terashima et al., 2011) showed exemplarily that the light harvesting system of green algae and vascular plants have some major differences, e.g. *Arabidopsis thaliana* has six Lhca gene products (Lhca1-6 belonging to photosystem I (PSI)), whereas *C. reinhardtii* has nine Lhca gene products that were all identified at the protein level (Stauber et al., 2003). LHC proteins are not only found in the chloroplast thylakoid membranes in *C. reinhardtii*, but are also present in those of its eyespot, a primitive visual system located at the edge of the chloroplast that allows the cell to detect the direction and intensity of light. The eyespot contains two layers of carotenoid-rich globuli that are extended by thylakoid membranes (Schmidt et al., 2006; Eitzinger et al., 2015); it was purified by flotation on sucrose gradients. Numerous LHCs of PSI and II are situated in the eyespot thylakoids membranes as identified in the eyespot subproteomes by LC-ESI–MS/MS of the corresponding peptides (Schmidt et al., 2006; Eitzinger et al., 2015).

The function of proteins is often altered by post-translational modifications. Reversible phosphorylation is one of the most prominent alterations on a protein. This holds also true for pigment-protein complexes. The analysis of the phosphoproteome of photosynthetic membranes revealed that the major environmentally dependent changes in phosphorylation are clustered at the interface between the PSII core and its LHC antennae (LHCII) (Turkina et al., 2006). In addition to this, a global phosphoproteome analysis in *C. reinhardtii* showed complex phosphorylation events in the chloroplast thylakoid membrane (Wang et al., 2014). Also in the eyespot fraction, the PSI reaction center subunit VI and members of the LHC proteins (LhcbM8, LhcbM4, LhcbM6, belonging to LHCII) were identified as phosphorylated proteins (Wagner et al., 2008). Recently, it was shown that another post-translational modification, namely methylation at arginine and lysine residues is not only relevant in the nucleus with regard to histones, but occurs also in the chloroplast of higher plants. A protein lysine and arginine methylation network was uncovered in *Arabidopsis* chloroplasts, showing methylation of subunits of PSI (PsaE-2) and II (PsbO-2) (Alban et al., 2014). Also, in eyespot fractions of *C. reinhardtii*, several methylated proteins were identified, including proteins of thylakoid membranes such as the ATP synthase subunits alpha and beta or Lhca7. Of interest is also methylation of the eyespot-specific protein SOUL3 that influences the size and position of the eyespot and of EYE2, a protein important for its development (Schulze et al., 2013; Eitzinger et al., 2015).

In recent years, more and more genomes from photosynthetic microalgae that originated from a secondary endosymbiotic event became available and allowed proteome analysis of pigment-protein complexes in several groups of the lineages containing secondary plastids. These so-called complex plastids are mostly characterized by four surrounding membranes whose outer membrane pair is continuous with the nuclear envelope. In some of these complex plastids the up-taken eukaryotic nucleus was reduced to a so called nucleomorph as it is present in the Chlorarachniophyte alga *Bigelowiella natans* or in Cryptophytes. In *B. natans*, a member of the green lineage, proteome analysis of isolated plastid-nucleomorph complexes was performed. It resulted in the identification of 324 proteins, from whom 32 were predicted to be involved in photosynthesis (Hopkins et al., 2012). Twelve are LHC and PSI and II proteins. Phylogenetic analyses revealed that many of the proteins identified are of apparent green algal ancestry, which is consistent with the evolutionary origin of the plastid and nucleomorph in Chlorarachniophytes. Beside the Chlorachniophytes and Cryptophytes, the nucleomorph was finally eliminated in algae with complex secondary plastids and thus cannot be found, for example, in the widely distributed diatoms. In Synchromophytes that also miss a nucleomorph, the plastids form large complexes, where several plastids are embraced by a double membrane being continuous with the nuclear envelope (Horn et al., 2007).

Most proteomics approaches in secondary endosymbiont algae with regard to the analysis of photosynthetic complexes and their subunit composition were indeed done in diatoms, members of the red lineage. Thereby, a few species were under focus, such as the pennate *Phaeodactylum tricornutum* or the centric *Thalassiosira pseudonana*. These analyses led to a detailed picture especially of the organization of LHCs, as outlined below in chapter 2. Moreover, proteomic analysis of the photosynthetic membranes of the heterokontic alga *Nannochloropsis gaditana* showed that the PSI supercomplex contains the PSI core complex and five peripheral antenna proteins (LHCr4-8) (Alboresi et al., 2017). Two of them are bound in a conserved position, as in PSI in higher plants, whereas three additional antennae are associated with the core on the other side.

A very unusual situation in the polypeptide composition of the pigment-protein complexes was found in the alveolate alga *Chromera velia,* the only phototropic representative of apicomplexan parasites. Here, two different types of antennae systems were identified; one with high similarity to the fucoxanthin-chlorophyll protein (FCP) complexes from diatoms whose protein structure also indicates its origin from Xanthophytes (Tichy et al., 2013). The second LHC system is closely related to the PSI–Lhcr system, the PSI specific antenna of red algae. Therefore, *C. velia* is an example that lateral gene transfer between phylogenetically distant groups is not only possible with genes encoding biosynthetic pathways of pigments (Frommolt et al., 2008), but also with genes encoding the pigment-binding proteins. Interestingly, high-light acclimated cells of *C. velia* showed that one out of three known Lhcr proteins was associated in higher concentration with PSI in high-light exposed cells, whereas in medium-light exposed cells, it was enriched in the *C. velia* LHC fraction (Mann et al., 2014). This leads to the conclusion that the acclimation of *C. velia* to high-light illumination shows features that are comparable to those of diatoms, while other characteristics more closely resemble those of higher plants and green algae.