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Proteomics Analysis of Heterogeneous Flagella in Brown Algae (Stramenopiles)

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Flagella are conserved organelles among eukaryotes and they are composed of many proteins, which are necessary for flagellar assembly, maintenance and function. Stramenopiles, which include brown algae, diatoms and oomycetes, possess two laterally inserted flagella. The anterior flagellum (AF) extends forward and bears tripartite mastigonemes, whilst the smooth posterior flagellum (PF) often has a paraflagellar body structure. These heterogeneous flagella have served as crucial structures in algal studies especially from a viewpoint of phylogeny. However, the protein compositions of the flagella are still largely unknown. Here we report a LC-MS/MS based proteomics analysis of brown algal flagella. In total, 495 flagellar proteins were identified. Functional annotation of the proteome data revealed that brown algal flagellar proteins were associated with cell motility, signal transduction and various metabolic activities. We separately isolated AF and PF and analyzed their protein compositions. This analysis led to the identification of several AF- and PF-specific proteins. Among the PF-specific proteins, we found a candidate novel blue light receptor protein involved in phototaxis, and named it HELMCHROME because of the steering function of PF. Immunological analysis revealed that this protein was localized along the whole length of the PF and concentrated in the paraflagellar body.

Key words: Blue light receptor; brown algae; creatine kinase; flagella; phototaxis; proteomics.

Introduction

Flagella or cilia are almost ubiquitous organelles in a diverse range of eukaryotic cells and substantial studies have revealed their versatile roles in cellular motility and signal perception (Cavalier-Smith 2002; Davenport and Yoder 2005). The structure of the flagellum is evolutionarily conserved (Carvalho-Santos et al. 2011; Mitchell 2007) and for a motile flagellum, its core structure is a "9+2" axoneme comprising nine outer doublet microtubules and central pair of microtubules. Various macromolecular components such as outer and inner dynein arms, radial spokes and central pair projections, are periodically organized along the microtubule-based axoneme and

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responsible for flagellar beating (Nicastro et al. 2005). The axoneme extends from the basal bodies beyond the cell surface and is covered by flagellar membrane, which is continuous with the plasma membrane but has different protein and lipid compositions relating to the sensory function of flagella (Pazour and Bloodgood 2008). In addition to the axoneme structure, an intraflagellar transport (IFT) system is also present in the compartment enclosed by the flagellar membrane and the outer doublet microtubules (Kozminski et al. 1993). The IFT consists of anterograde and retrograde protein complexes responsible for flagellar assembly and maintenance (Rosenbaum and Witman 2002). Given the functional diversity and structural complexity, it is not surprising that hundreds of proteins are required for the correct assembly, maintenance and functioning of flagella. As a result of a combination of bioinformatics, genomics and proteomics analyses of the flagella of model organisms, our understanding of the flagellar proteins has greatly advanced in the last decade (Gherman et al. 2006; Inglis et al. 2006).

The stramenopiles constitute a large independent group among eight eukaryotic lineages, and contain diverse organisms from unicellular parasitic flagellates to giant kelp, including five non-photosynthetic subgroups (such as oomycetes and labyrinthulomycetes) and eleven photosynthetic ones (such as brown algae and diatoms) (Baldauf 2008). Stramenopiles are characterized by possessing an anterior flagellum (AF) with tripartite mastigonemes in the motile stage (gametes and zoospores). An alternative name for the stramenopiles is the heterokonts, because most species within this group often have a shorter, smooth posterior flagellum (PF) in addition to the AF. Functionally, the AF generates propulsive force through waveform bending to power the cell forward swimming motility and the PF exhibits rapid lateral beating to steer the swimming direction (Geller and Müller 1981; Matsunaga et al. 2010).

The brown algae (Phaeophyceae) are a major group within the stramenopiles that are mostly found in marine habitats, and distributed worldwide. During a typical brown algal life cycle, the two heterogeneous flagella (AF and PF) are observed in the unicellular reproductive cells (gametes and zoospores). As one of the most important organelles in brown algae, a great deal of attention has been paid to flagella and their ultrastructural characters have been studied extensively (Henry and Cole 1982a, b; Maier 1997a, b; Manton and Clarke 1951; Manton et al. 1953). The flagella are also critical structures when considering phylogenetic relationships within the brown algae (Clayton 1989; O'Kelly 1989). However, the protein composition of brown algal flagella remains largely unknown, probably owing to the difficulty in isolating high quality brown algal flagella in sufficient quantity and, until recently, the lack of a brown algal genome database to underpin the flagellar protein analysis.

Although the genome sequence of the model brown alga *Ectocarpus siliculosus* has become available (Cock et al. 2010), proteomics analysis of its flagella is hampered by limitations in the amount of this organelle that can be isolated. In this study, we used plurizoids of *Colpomenia bullosa* collected from the field to carry out flagellar proteomics analysis. Light and electron microscopy showed that plurizoids of *Colpomenia* and *Ecto*carpus are structurally similar. Moreover, the two species both belong to the Ectocarpales according to traditional brown algal classification criteria (Guiry and Guiry 2014), and a multi-marker based phylogenetic analysis of 72 brown algal taxa revealed that *Colpomenia* is phylogenetically close to *Ectocarpus* (Silberfeld et al. 2010). These data encouraged us to use the genome sequence of *E. siliculosus* as a database to identify *C. bullosa* flagellar proteins. Here, we present the result of LC-MS/MS based proteomics analysis of isolated flagella from the latter species. We also identified several AF- and PF-specific proteins. A candidate novel blue light receptor protein responsible for phototaxis of brown algal swarmers is also discussed.

Results and Discussion

Heterogeneity and Isolation of Brown Algal Flagella

The flagellated cells of *C. bullosa* and *E. siliculosus* are around 5x7 μm in size and bear two heterogeneous flagella in different lengths (Fig. 1A). Under blue-violet (BV, 400-440 nm) irradiance, the PF emits green autofluorescence (Fig. 1B), which is thought to be associated with a flavin-like protein (Kawai 1988). Both flagella are laterally inserted into the cell body (Fig. 1C) and the basal part of the PF is closely associated with the eyespot (Fig. 1D). This swollen basal part of the PF, the paraflagellar body (Fig. 1A, B, D), is filled with crystalized materials and electron dense materials (Fig. 1E) (Fu et al. 2013). The AF is decorated with tripartite mastigonemes on the flagellar surface (Fig. 1F). The axonemes of both flagella display the typical "9+2" microtubular arrangement, however, the two flagella exhibit distinct beating patterns when cells