

# Chapter 29

## Fertilization of Brown Algae: Flagellar Function in Phototaxis and Chemotaxis

Gang Fu, Nana Kinoshita, Chikako Nagasato, and Taizo Motomura

**Abstract** Sexual reproduction of brown algae includes isogamy, anisogamy, and oogamy. The mechanisms of fertilization events remain largely unknown despite the diverse reproduction patterns. It is thought that the flagella of brown algal reproduction cells play crucial roles in not only cellular motility but also signal transduction in the aquatic habitat. Flagella of brown algae are composed of 9+2 axonemes and several appendage structures, such as mastigonemes and a paraflagellar body, which have close associations with flagellar function. We observed flagellar activities during recognition and fusion of male and female gametes. We also investigated flagellar proteins involved in phototaxis of brown algal motile cells.

**Keywords** Brown algae • Chemotaxis • Fertilization • Flagella • Phototaxis

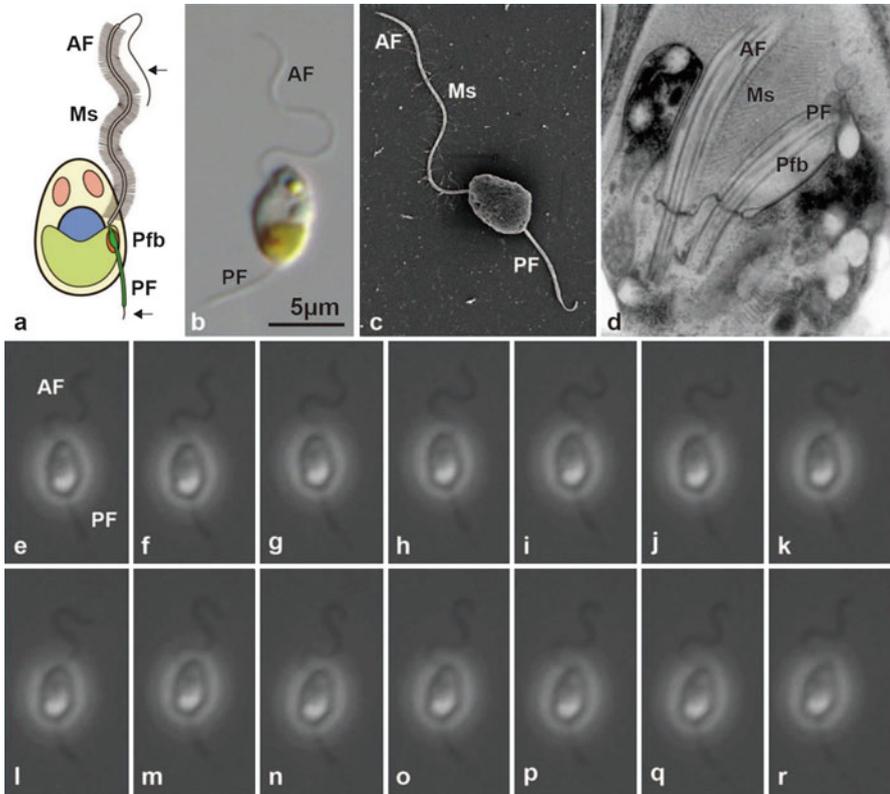
### 29.1 Flagellar Structure of Brown Algal Swimmers

Stramenopiles, including heterokonts, comprise an independent group of eukaryotes and are phylogenetically distinct from red and green algae, land plants, and animals (Baldauf 2003). One of the characteristic features of this group is that they have heterogeneous flagella, one of which is decorated with mastigonemes, tripartite fine hairs (van den Hoek et al. 1995). Brown algae, such as *Saccharina*, *Undaria*, and *Sargassum*, are a group of heterokonts and the only group in heterokonts having a complex multicellular organization.

In the life cycle of many brown algae, sporophyte and gametophyte generations exist independently, and sexual reproduction (male and female gametes, or sperm

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**Fig. 29.1** Two heterogeneous flagella of brown algal motile cells. (a) Schematic representation of a motile cell. AF anterior flagellum, Ms mastigonemes, PF posterior flagellum, Pfb paraflagellar body. Arrows show the acronema, tip of the AF, and PF. (b) DIC image of a male gamete of *Scytosiphon lomentaria*. (c) SEM image of a male gamete of *S. lomentaria*. Note that mastigonemes are only on the AF. (d) TEM image of the AF and PF of *Ectocarpus siliculosus*. Note that Ms is on the AF and Pfb is on the PF. (e–r) High-speed video images (600 frame/s) of free swimming of a *S. lomentaria* male gamete

and egg) and asexual reproduction (zoospore or tetraspore after meiosis) connect the two generations. Swimmers, namely flagellated motile cells (gametes, sperm, and zoospores), have two heterogeneous flagella (Fig. 29.1a–d), a long anterior flagellum (AF) decorated with mastigonemes and a short posterior flagellum (PF) with a basal swollen part called the paraflagellar body, which is composed of crystallized materials and electron-dense materials (Green et al. 1989; Andersen 2004; Fu et al. 2012). The acronema, which is composed of two central microtubules of axonemes, makes the tip of each flagellum. The AF and PF are laterally inserted into the cell body in two opposite directions corresponding to the swimming orientation (O’Kelly 1989). Swimming force of motile cells of brown algae is produced by the AF, not the PF (Fig. 29.1e–r), because motile sperm of the brown alga *Dictyota* only have an

AF (Manton 1959), and it is thought that mastigonemes on the AF would be related to tractive force (Jahn et al. 1964). It has remained unclear how mastigonemes regularly attach only to the AF during flagellar elongation (Fu et al. 2012). The PF of brown algal motile cells functions as steering against signals of light (phototaxis) and chemicals (chemotaxis) (Geller and Müller 1981; Matsunaga et al. 2010).

## 29.2 Phototaxis of Brown Algae and a Putative Photoreceptor Protein

Phototaxis is widespread among motile cells of the major eukaryotic lineages, and a similar mechanism by which phototactic reorientation is complemented is shared despite the great diversity of cell types. When considering fertilization of algae living in an aquatic environment, several processes, including phototaxis, chemotaxis, and recognition, would be critical. Swimmers including gametes and zoospores of algae show a strong response to direction of light from the sun. For example, male and female gametes of the green alga *Monostroma angicava*, which have two equal-length flagella, swim toward the seawater surface after liberation from gametangia, showing positive phototaxis (Togashi et al. 1999). On the other hand, just after fertilization, motile zygotes having four flagella derived from male and female gametes show negative phototaxis and swim toward the bottom of the sea. The change of phototaxis before and after fertilization is necessary for continuous support of successful fertilization, because accumulation of unfertilized male and female gametes at the surface of the sea ensures easy fusion of both gametes. Zygotes settle on boulders on the bottom and develop into the next generation by negative phototaxis (Togashi et al. 1999; Togashi and Cox 2004). In the case of brown algae, for example, isogamous brown algae *Scytosiphon lomentaria*, *Colpomenia bullosa*, and *Ectocarpus siliculosus*, freshly liberated male and female gametes show strong negative phototaxis, and female gametes settle on the substratum sooner than do male gametes and secrete a sexual pheromone that attracts male gametes. Therefore, in the case of algal fertilization in an aquatic environment, phototaxis reaction is important for dense accumulation of gametes.

In phototaxis reaction of motile cells, a specific photoreceptor senses the light source of restricted wavelength and transduces the signals to downstream molecular modules, which will eventually alter the flagellar beating activity with changing the swimming direction (Jekely 2009). However, different organisms have evolved exclusive strategies in response to light stimuli, for example, employing diverse photoreceptors and the corresponding downstream signaling. In the green alga *Chlamydomonas reinhardtii*, two light-gated cation-channel proteins, channelrhodopsin-1 (ChR1) and ChR2, were identified as photoreceptors regulating phototaxis through depolarizing photoelectric currents (Sineshchekov et al. 2002; Berthold et al. 2008). Similar to other types of rhodopsins, both proteins are 7-TM membrane proteins and bind retinal as a chromophore. An immunofluorescence assay indicated that ChR1 was localized near the eyespot, which is part of the chloroplast

(Suzuki et al. 2003). In the unicellular flagellate *Euglena gracilis*, photoactivated adenylyl cyclase (PAC), a flavoprotein, was shown to be the blue light receptor, which binds flavin adenine dinucleotide (FAD) as a chromophore and localizes to the paraflagellar body (Iseki et al. 2002; Ntefidou et al. 2003).

As another independent eukaryotic group, it has been well known that the PF of swimmers of brown algae has the capability to emit green autofluorescence when excited with blue light. Based on the results of spectral analysis, the substance that caused the green autofluorescence was identified as flavin (Müller et al. 1987; Kawai 1988). Flavins in the PF are widely distributed among chlorophyll *c*-containing algal species having an eyespot or paraflagellar body (Kawai and Inouye 1989; Kawai 1992). Close associations between the autofluorescence substance and presence of an eyespot or paraflagellar body, as well as results of spectral action studies on phototaxis of brown algal swimmers (Kawai et al. 1990, 1991), have suggested that the blue light receptor is a flavoprotein and is likely localized in the PF. However, the photoreceptor protein involved in phototaxis of brown algae has not yet been identified, although a fluorescent flagellar protein homologous to Old Yellow enzyme was found in isolated flagella of *S. lomentaria* (Fujita et al. 2005), which seems to play roles in general redox reactions rather than light-sensing activities.

In our recent flagellar proteomics studies based on the whole-genome sequence of the model brown alga *E. siliculosus* (Cock et al. 2010), a putative blue-light receptor protein was found in the PF of brown algal swimmers and might have a close relationship to phototaxis. Flagella were isolated from swimmers of several brown algal species, including *C. bullosa* and *S. lomentaria*, by vortexing in flagellar isolation buffer (30 mM HEPES, 5 mM MgSO<sub>4</sub>, 5 mM EGTA, 25 mM KCl, 1 M Sorbitol, pH 7.0). Flagellar proteins were further digested by trypsin and subjected to LC-MS/MS analysis, which yielded about 600 proteins of brown algal flagella. Among PF-specific proteins that were identified by proteomics analysis, an RGS/LOV domain-containing protein was found to be a potential photoreceptor. This protein contained 1,522 amino-acid residues and the predicted molecular weight was 168 kDa. In contrast to known photoreceptors, the protein has a unique domain architecture of two RGS (regulator of G-protein signaling) domains and four LOV (light, oxygen, and voltage sensing) domains. It is well known that the LOV domain is a ubiquitous molecular module capable of binding FMN (flavin mononucleotide) as a chromophore in diverse photoreceptors (Crosson et al. 2003; Losi and Gärtner 2012; Suetsugu and Wada 2013). The RGS domain has a key activity in accelerating GTP hydrolysis by the G $\alpha$  subunit; therefore, it is likely that heterotrimeric G proteins may be involved in the downstream signaling of blue-light sensing, which eventually modifies the beating pattern of the PF of swimmers.

Although the interactions between inner- and interproteins during phototaxis are far from understood, an antibody against the RGS/LOV domain-containing protein revealed that this protein is widely distributed in brown algal species. An immunofluorescence assay confirmed that this protein is localized throughout the PF with a stronger intensity at the paraflagellar body, corresponding to the distribution of green autofluorescence when observed under blue light. In addition, immunoelectron microscopy analysis revealed that the subcellular localization of this protein is in the compartment between the flagellar membrane and axoneme.

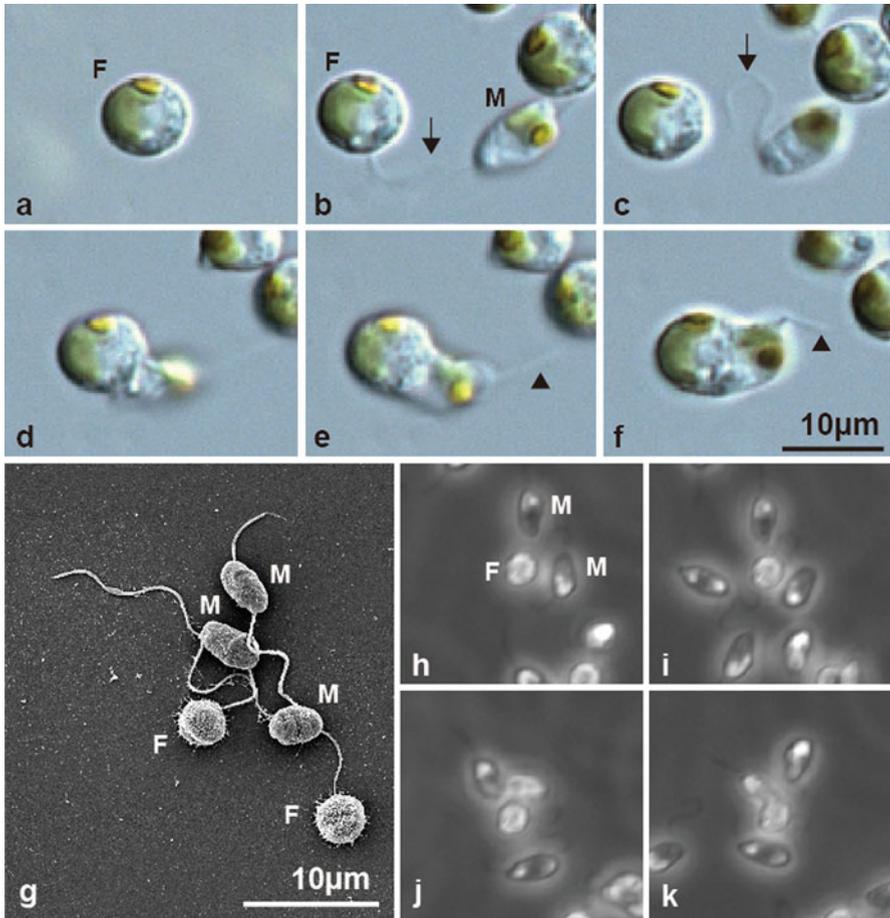
### 29.3 Chemotaxis of Brown Algae

Regarding sexual pheromones in brown algae, since the first discovery of the pheromone “ectocarpen” in *E. siliculosus* by the German phycologist Dieter G. Müller (Müller 1967, 1968; Müller et al. 1971), eleven sexual pheromones, including lamoxirene, fucoserraten, and hormosirene, have been reported (Maier and Müller 1986; Boland 1995). These brown algal pheromones are volatile, lipophilic, and fragrant and have a low molecular mass with unsaturated C<sub>8</sub> and C<sub>11</sub> hydrocarbons with biogenetically related structures. Male gametes actively surround the sexual pheromone that female gametes secrete soon after their settlement on the substratum (Fig. 29.2). Motile female gametes never gather around a settled female one. Therefore, it is clear that the pheromone receptor must naturally exist in the male gamete, not in the female one. In the case of isogamous brown algae, such as *E. siliculosus* and *S. lomentaria*, male gametes freely swim in a straight or slightly curved track with maximum velocity in seawater without a pheromone (Fig. 29.1e–r), whereas the PF of male gametes has occasional beats and strong lateral bias with the signal of a pheromone (Fig. 29.2h–k) and, as a result, male gametes swim in a characteristic U-turn (Maier and Müller 1986). Those authors reported chemo-thigmo-klinokinesis, which means that the pheromone has two effects for attracting male gametes around settled female gametes: (1) reducing male gamete velocity by a thigmotactic response and (2) increasing beating frequency of the PF of male gametes in proportion to the pheromone concentration. Unfortunately, the pheromone receptor in male gametes has not yet been identified.

A male gamete attracted to a female gamete by the sexual pheromone shows a characteristic behavior in brown algal fertilization, first making contact with the surface of the female gamete by using the tip of the long AF (Fig. 29.2a–f) (Müller 1966), followed by fusion of both bodies. Therefore, initial recognition and contact between male and female gametes is carried out by using the AF of the male gamete. As already mentioned, the AF of gametes and zoospores of brown algae characteristically bears mastigonemes, which may be involved in the contact between male and female gametes. High-speed video and high-resolution scanning electron microscopy (SEM) reveal the the fusion process of both gametes (Fig. 29.2g–k).

### 29.4 Cytoplasmic Inheritance of Organelles

Finally, we briefly introduce the cytoplasmic inheritance of mitochondria, chloroplasts, and centrioles during zygote development of brown algae. Three types of sexual reproduction—*isogamy*, *anisogamy*, and *oogamy*—can be observed in brown algae, similar to green algae. Cytoplasmic inheritance of mitochondria, chloroplasts, and centrioles is restrictively regulated in each pattern of sexual reproduction (Motomura et al. 2010). In *oogamy*, mitochondria and chloroplasts of sperm are selectively digested in the lysosome after fertilization (Motomura 1990). In the case of *isogamy*, chloroplasts are biparentally inherited, whereas mitochondria



**Fig. 29.2** Isogamous fertilization of *Scytosiphon lomentaria*. (a–f) Process of fertilization between male (*M*) and female (*F*) gametes. Female gametes settle on the substratum and then release a pheromone, by which male gametes are attracted. The AF of the male gamete (arrow in b, c) attaches to the cell surface of the female gamete with the flagellar tip. Cell fusion starts, and then the PF (arrowheads in e, f) of the male gamete is withdrawn. The zygote becomes a spherical shape within a few minutes. (g) SEM image. Three male gametes gather around two female gametes. (h–k) High-speed video images. Note that the PF of male gamete bends

(or mitochondrial DNA) derived from the female gamete only remain during zygote development (Nagasato and Motomura 2002; Peters et al. 2004; Kato et al. 2006; Kimura et al. 2010). Similar to the paternal inheritance of centrioles in animal fertilization (Schatten 1994), centrioles in zygotes are definitely derived from the male gamete regardless of the sexual reproduction pattern (Nagasato 2005). In isogamous *S. lomentaria*, degeneration of the maternal centrioles was found to start 1 h after fertilization with degradation of triplet MTs from the distal end, and in a 2-h-old zygote, there was no trace of the maternal centrioles ultrastructurally (Nagasato and Motomura 2004).

## 29.5 Perspectives

The flagellar structure is the most important character for defining the stramenopile (Heterokontae) in the eukaryote groups. Brown algal swimmers have a long AF bearing fine hairs, mastigonemes, and a short PF having the basal swelling, the parabasal body. With these morphological differences, their behaviors in gamete swimming are also characteristic. During the fertilization process, these heterogeneous flagella play crucial roles in phototaxis, chemotaxis, and gamete recognition. Our proteomics analysis on flagella of the brown algae identified first about 600 flagellar proteins, and AF-specific and PF-specific proteins were found. A candidate protein of the new blue-light receptor, RGS/LOV protein, working in phototaxis of gametes, could be also detected in PF-specific proteins. These molecular approaches will expand a new insight for understanding the function of flagella of male and female gametes of the brown algae during fertilization, including the pheromone receptor that may exist in flagella of male gametes and the molecular nature of the flagellar tip of male gametes for the first attachment to the surface of female gametes.

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