Motility in Unicellular and Filamentous Cyanobacteria

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Abstract

Both unicellular and filamentous cyanobacteria exhibit diverse types of motility, including the ability to move on surfaces and to swim in liquids. Motile behaviour can be regulated such that cyanobacteria can respond to important environmental signals such as light or chemical gradients. In some cases, components of the motility machinery, such as Type IV pili that allow certain unicellular species to move on surfaces have been characterized. In others, as is the case for swimming in certain marine unicellular species, and gliding in filamentous genera, the motility apparatus has yet to be identified. We describe here recent advances that have led to the identification of multiple photoreceptors and novel proteins involved in motility. The complex signal transduction pathways governing motile behaviour and attempts to model this behaviour are discussed. We conclude with some of the major challenges that remain in our understanding of the complexities of motile behaviour.

Introduction

Ever since Leuwenhoek's observations of 'animalcules' under the microscope, the motility of microorganisms has fascinated microbiologists. Many prokaryotes have been observed to move either in liquid or on moist surfaces, and this phenomenon was variously described as 'swimming', 'twitching', 'swarming' or 'gliding'. Both unicellular and filamentous microorganisms exhibit motility and cells can move as individuals or in large coordinated groups. Furthermore, motile behaviour can be regulated which allows microorganisms to respond to various environmental signals such as light or to chemical gradients. In some cases, the motility apparatus required for movement and the signal transduction cascade that allows for a response to environmental cues are well understood, while in others they remain to be discovered. It is also apparent that motility is a complex and regulated phenomenon where more than one mechanism for movement can be used depending on the lifestyle and environmental conditions (Jarrell and McBride, 2008). For instance, some bacteria encode both flagella and Type IV pili (TFP), while others can produce extracellular materials that aid or are required for motility.

Unicellular and filamentous cyanobacteria as well as many photosynthetic microalgae exhibit motile behaviours, and their responses to light are particularly well studied (Gomelsky and Hoff, 2011; Hader and Lebert, 2009; Hoff *et al.*, 2009; Jekely, 2009). The rich history of the study of their motility (initiated by Treviranus in 1817 and continued by Engelmann in Germany), stretches well over a hundred years and has been covered in many excellent and detailed reviews (see section 2). Nevertheless, we feel it is important to keep this history in perspective, particularly for younger researchers, because many fundamental issues that were addressed are still relevant today. Thus, we start with a brief overview of the major mileposts that describe the various motility phenomena in unicellular and filamentous cyanobacteria. These careful experiments and resultant hypotheses set the stage for molecular and mechanistic approaches, although in this chapter, we primarily focus on recent advances. We conclude

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with a brief discussion of the challenges that lie ahead in advancing our understanding of the complexities of motile behaviour.

Mileposts in the history of motility in cyanobacteria

We have roughly divided the study of motility in cyanobacteria into three periods. Initially, the behaviour of motile cyanobacteria, bacteria and photosynthetic algae was observed using available microscopes. These careful studies and ingenious experiments by Treviranus, Engelmann and others, beginning from the early 1800s, documented the movement and light-responsiveness of several species of filamentous cyanobacteria and are summarized in excellent early reviews (Castenholz, 1982; Drews, 2005; Hader, 1987, 1988; Nultsch, 1975; Nultsch and Hader, 1979). In the second phase, starting in the early 1960s, several groups including those of Castenholz, Nultsch, Hader, and Clayton, among others, carried out numerous experiments that attempted to answer some of the fundamental questions regarding photoperception, signal transduction and mechanisms for motility, as well as the role of photosynthesis and metabolism in controlling motility responses. Most of these experiments were carried out with filamentous cyanobacteria (*Anabaena* sp., *Phormidium* sp. and *Oscillatoria* sp.), some of them isolated from hot springs (Castenholz, 1969), which exhibited rapid movements called 'gliding'. This motility was carefully described and it was recognized that gliding motility could look similar in different organisms yet the mechanisms that controlled these movements could be quite distinct (Castenholz, 1982; Diehn, 1976). The two models proposed to explain gliding in filamentous cyanobacteria, which are still being considered today, movement through mucilage secretion and movement through the production of surface waves, originated during this period. These phenomenological and environmentally relevant studies set the stage for the third or current phase in understanding motility, which relies strongly on molecular and biochemical tools to understand the mechanistic aspects of motility.

In the third phase, starting in the early 1990s, the availability of complete genome sequences, the development of molecular genetic methods, and the advent of new imaging techniques, have made possible a mechanistic and molecular understanding of motility. For instance, the use of mutants has revealed that surface proteins were required for the mysterious swimming behaviour of certain marine *Synechococcus* species. For freshwater unicellular cyanobacteria, several important advances have been made in describing the motor apparatus required for surface motility as well as the photo-perception responses that rely on multiple photoreceptors and complex signal transduction cascades. In addition, the modelling of motile cells in directional light has led to a better understanding of phototactic behaviour. In filamentous cyanobacteria, several models to explain gliding behaviour have been proposed and some are currently being tested. We anticipate that in the next few years these approaches will continue to advance our knowledge of surface-dependent and swimming motility in cyanobacteria. It should also provide insights into the ecological advantages that motility provides to photosynthetic organisms that cope with fluctuations in the environments, particularly light intensity and quality.

In this chapter, we focus on the advances made in the last two decades and identify the remaining challenges. Cyanobacteria have evolved a variety of means of movement, some of which share features with the types of motility exhibited by other bacteria. When sufficient similarities exist, we have used the accepted prokaryotic motility nomenclature (Jarrell and McBride, 2008). We will use **twitching motility** to refer to the surface-dependent motility of certain unicellular cyanobacteria that requires Type IV pili (or TFP); **swimming motility** to refer to the non-flagellar swimming exhibited by certain marine *Synechococcus* species; and **gliding motility** to refer to the surface-dependent movement of filamentous cyanobacteria for which no organelle has yet been identified.

Swimming motility in unicellular marine *Synechococcus* sp.

In 1985, Waterbury and colleagues reported that certain strains of marine *Synechococcus* were

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capable of swimming in liquids at speeds ranging from 5 to 25 μm/s (Waterbury *et al*., 1985). This discovery was remarkable for two reasons: first, unlike other cyanobacteria which require contact with a surface in order to move, these strains of *Synechococcus* moved in liquids, and second, they did so in the absence of flagella or any other readily visible organelle. Flagellated bacteria are propelled in liquids by the rotation of flagella and this imparts certain features to their motile behaviour (Jarrell and McBride, 2008). Like flagellated bacteria, swimming cells of *Synechococcus* rotate about their longitudinal axis as they translocate, are slowed down and ultimately immobilized by increasing viscosity, and cells that become attached by one pole to a glass surface rotate about their point of attachment (Waterbury *et al*., 1985; Willey, 1988). Hence, like flagellated bacteria, swimming *Synechococcus* generate both torque and thrust. However in spite of the use of a variety of approaches to detect and visualize flagella (Willey, 1988), reviewed by Brahamsha (Brahamsha, 1999), these were not seen, and the mechanism for swimming remains elusive.

Molecular approaches to swimming

The development of genetic tools for certain strains of marine *Synechococcus* has made it possible to address this problem at a molecular level (Brahamsha, 1996; McCarren and Brahamsha, 2005; McCarren *et al*., 2005). Early work combining biochemistry and reverse genetics revealed that an abundant 130 kDa cell-surface glycoprotein, SwmA, was required for the generation of thrust in *Synechococcus* sp. strain WH 8102 (hereafter Syn WH 8102) (Brahamsha, 1996). A survey of other motile strains of marine *Synechococcus* revealed that, unlike non-motile isolates, they too produced an abundant polypeptide of similar molecular weight (Brahamsha, 1996) and encoded genes highly similar to *swmA* (Brahamsha, unpublished). SwmA encodes the S-layer that covers the surface of strain WH 8102 (McCarren and Brahamsha, 2005) but how it is involved in swimming is not understood. Interestingly, SwmA shows some similarity, particularly in repeated putative Ca^{2+}-binding motifs, to oscillin, a glycoprotein which forms fibrils on the surface of the gliding cyanobacterium *Phormidium uncinatum* (Hoiczyk, 1998) (discussed below) and to other S-layer and Repeats-in-toxin (RTX) proteins (Brahamsha, 1996), including the surface protein HlyA (sll1951) identified in the freshwater unicellular cyanobacterium, *Synechocystis* sp. PCC 6803 (hereafter Syn PCC 6803) (Bhaya *et al*., 1999).

Transposon mutagenesis screens for motility mutants identified three loci in the genome of Syn WH 8102 that encode proteins required for swimming (McCarren and Brahamsha, 2005). Mutations in genes at two of these loci (chromosomal region 1, which includes *swmA* (SYNW0085), and chromosomal region 2), affect the production and the localization of SwmA (McCarren and Brahamsha, 2009). Some of these genes encode homologues of type I secretion systems, which have been shown to be involved in export of S-layer proteins in other organisms, and homologues to glycosyltransferases that might play a role in the glycosylation of SwmA. Chromosomal region 3 contains a very large gene (32 kb long, SYNW0953) which encodes the protein SwmB. SwmB (10,791 aa) is a 1.2 mDa cell-surface protein with several repetitive domains. In terms of its large size, repetitive architecture, and atypical amino acid usage (McCarren and Brahamsha, 2007), SwmB resembles a number of other giant cell-surface proteins (Reva and Tummler, 2008) such as adhesins, although none of these have been implicated in motility.

An examination of the genome of *Synechococcus* sp. strain WH 8102 corroborates that swimming in this species represents a unique type of motility (Palenik *et al*., 2003). None of the proteins typically required for flagellar-dependent motility (flagellar motor, flagellar filament proteins, and chemotaxis proteins) were encoded on the genome. Six open reading frames (ORFs) associated with the biogenesis and function of TFP (homologues of *pilB*, *C*, *D*, *Q*, and *T*) were encoded on the genome but they do not encode the full complement of proteins required for pilus assembly and function, and pilin subunit homologues could not be identified. Furthermore, pili have not been observed in Syn WH 8102, nor does Syn WH 8102 exhibit surface-associated twitching (McCarren and Brahamsha, 2005). In addition, we insertionally inactivated the *pilT* and *pilC* homologues in Syn WH 8102, and neither

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of these inactivations had an effect on swimming (McCarren and Brahamsha, unpublished).

Cell surface proteins and the envelope structure of swimming *Synechococcus* sp.

The envelope structures of two strains of motile *Synechococcus*, Syn WH 8102 (McCarren *et al.*, 2005) and Syn WH 8113 (Samuel *et al.*, 2001), have been examined by a variety of electron microscopy techniques, including conventional methods as well as cryo-fixation and freeze-fracture and etching. This has revealed a multilayered Gram-negative architecture characteristic of cyanobacteria. In addition, these motile strains have an S-layer forming a paracrystalline array external to the outer membrane that can be observed using cryofixation and freeze substitution methods (McCarren *et al.*, 2005). In addition, in one of the strains (Syn WH8113), 5-nm-wide and 150-nm-long fibrils or spicules appear to cover the surface of the cell (Samuel *et al.*, 2001); however, such spicules were not observed in Syn WH 8102 and hence may not be characteristic of all motile strains. Studies with mutants (McCarren and Brahamsha, 2007; McCarren *et al.*, 2005) revealed that in Syn WH 8102, the S-layer is composed of the SwmA protein, while the giant SwmB protein is arranged in a punctate manner on the cell surface. Mutants lacking SwmA exhibit a fibrillar layer covering the outer membrane; the nature of this fibrillar layer is not currently known. While these studies point to the cell surface as being important for this type of motility, the role SwmA or SwmB plays is not yet clear. Both are abundant cell-surface proteins and may constitute part of the motility apparatus or their role in swimming may be indirect. It is possible that in their absence, outer membrane integrity is affected in such a way that as-yet unidentified proteins required for motility cannot be localized correctly or cannot interact properly.

Models for swimming in *Synechococcus* sp.

Models have been proposed for how marine *Synechococcus* might swim in the absence of flagella, and one of these, self-electrophoresis, in which cells establish and use an electrical field to propel themselves, has been ruled out experimentally (Pitta and Berg, 1995). Given the lack of any observable external organelles, models have focused on how the cell surface may generate thrust. In 1996, Ehlers *et al.* (1996) presented a model in which travelling surface waves generated by contractions and expansions of the outer membrane could create sufficient thrust to allow cells to swim at experimentally observed speeds. The rotation of the cell body during swimming could be explained by waves that are not axially symmetrical. Pitta and Berg showed that calcium was required for swimming and that inhibitors of voltage-gated calcium channels blocked motility, and proposed that calcium depolarization may serve to generate local swellings on the cell surface which could then be propagated along the surface to produce waves (Pitta *et al.*, 1997). Samuel *et al.* (2001) proposed that the spicules seen on the surface of Syn WH 8113 could be used to transmit the expansions and contractions to the surface and in effect function as oars to generate travelling waves in a manner analogous to that of ciliated eukaryotes. The absence of spicules in Syn WH 8102 implies that such structures are not required for motility in all swimming strains, and the Ehlers model (Ehlers *et al.*, 1996) does not require them.

Recently, another mechanism by which these surface waves could be generated has been proposed (Nan and Zusman, 2011; Ehlers and Oster, 2012). This model is based on one proposed for adventurous motility (A-motility), the gliding of individual cells of *Myxococcus xanthus*, in which motor proteins (MotA and MotB homologues) move along a continuous looped helical track composed of the cytoskeletal protein MreB (Nan and Zusman, 2011; Nan *et al.*, 2011). The motors carry cargo consisting of multiprotein complexes encoded by certain genes required for gliding in *M. xanthus*. These motor–cargo complexes distort the cell surface as they move along the track, generating surface waves that propel the cells. Ehlers and Oster (Ehlers and Oster, 2012) postulate the existence of such a helical track in *Synechococcus* and propose that rotation of the track itself, or of motor–cargo complexes moving along it could generate waves on the cell surface. In their model, the S-layer composed of SwmA plays a critical role. Because of its tilted geometry on the cell

surface, it creates asymmetry and amplifies the travelling waves in a manner to provide sufficient mechanical coupling to the fluid in which the cells are swimming (Fig. 11.1). This model explains the rotational behaviour of cells during swimming and also that of SwmA mutants, which cannot swim but can still rotate when attached to a surface.

There is at present no experimental evidence for the occurrence of waves or undulations on the cell surface of swimming *Synechococcus*. The waves are predicted to be of an amplitude not resolvable by conventional light microscopy, but techniques such as total internal reflection fluorescence microscopy which were used to view deformations of the cell surface in *Myxococcus* (Nan *et al*., 2011), as well as atomic force microscopy, should be applicable to *Synechococcus* and are currently being tested (Brahamsha, unpublished). While the presence of a helical cytoskeletal track has not been demonstrated in marine *Synechococcus*, the genome of Syn WH 8102 encodes an *mreB* homologue, and the small molecule A22, which depolymerizes the MreB cytoskeleton in other bacteria, rapidly inhibits swimming, implying

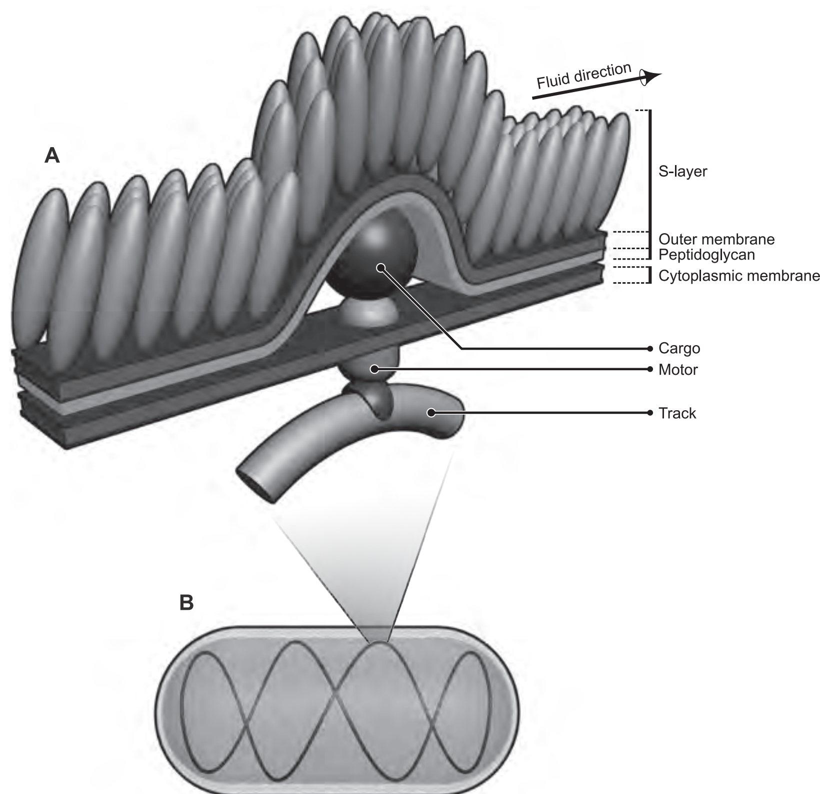

Figure 11.1 Surface wave model for non-flagellar swimming in marine *Synechococcus.* These drawings represent the model described in Nan *et al*. (2011) and Ehlers and Oster (2012). In this model, motor complexes carrying a cargo (yet-to-be identified motility proteins) move along a helical track and, as they do so, cause deformations of the cell surface, generating surface waves. In marine *Synechococcus* these waves are amplified by the tilt of the S-layer (≈60°) to provide sufficient mechanical coupling to the surrounding fluid. In (A) the motor–cargo complexes are moving in the same direction as the fluid, against the grain or tilt of the S-layer. Another version of the model proposes that such surface waves could also be generated by rotation of the helical track itself, driven by motors attached to the peptidoglycan layer. B. A cell of marine *Synechococcus* showing the helical track which is proposed to either serve as the track over which motor–cargo complexes move, or to rotate itself. While the presence of such a track has not been demonstrated in marine *Synechococcus*, the genome of Syn WH 8102 encodes an *mreB* homologue and the small molecule A22 which de-polymerizes the MreB cytoskeleton rapidly inhibits swimming, implying that an intact MreB cytoskeleton is required for swimming in *Synechococcus*.

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that an intact MreB cytoskeleton is required for swimming in *Synechococcus* (Brahamsha, in preparation) as it is for A-motility in *M. xanthus* (Mauriello *et al*., 2010). These results suggest that aspects of the helical rotor model proposed for *M. xanthus* may indeed be applicable to *Synechococcus*. Like other forms of swimming or gliding in prokaryotes that are powered by an ion motive force, the energy source for swimming in *Synechococcus* is the sodium motive force (Willey *et al*., 1987), implying that the motor converting chemical into mechanical energy is located in the cytoplasmic membrane.

Surface dependent motility in unicellular freshwater cyanobacteria

The phenomenon of surface dependent motility

Motility exhibited by certain unicellular freshwater cyanobacteria isolates was noted by groups at the Pasteur Institute (Rippka *et al*., 1979; Stanier *et al*., 1971) and elsewhere. It was observed that Syn PCC 6803 cells exhibited slow motility (typically 1–2 μm/min) on plates, but that this phenotype was often lost under standard laboratory conditions, possibly because it is not a characteristic that is under strong selection under normal maintenance conditions on plates (Choi *et al*., 1999). Indeed, Syn PCC 6803 isolates currently used in different laboratories often have either subtle or significant differences in their motile behaviour, which has led to some confusion in the literature and problems with data interpretation and consistency (Bhaya *et al*., 2000; Choi *et al*., 1999; Wilde *et al*., 2002; Yoshihara *et al*., 2000). Whole genome sequencing is becoming relatively easy and inexpensive, and recently a few isolates of Syn PCC 6803 have been re-sequenced and correlated with certain motility phenotypes (Kaneko *et al*., 1996; Kanesaki *et al*., 2012; Trautmann *et al*., 2012). In the future, it may be possible to identify exact changes at the gene/nucleotide level when isolates demonstrate phenotypic changes. For several years, the unicellular freshwater cyanobacterium Syn PCC 6803 has been extensively used for studies relating to photosynthesis and metabolism. Syn PCC 6803 has also become the preferred model system for the study of motility because of its consistent phototaxis phenotype, rapid doubling time, ease of genetic manipulation and ability to create targeted mutants.

Characteristics of motility and phototaxis in Syn PCC 6803

The simplest assay for surface-dependent motility is to place cells on a moist surface, such as low concentration agarose (0.4–0.8%); cells typically exhibit a slow surface dependent twitching behaviour (Bhaya *et al*., 1999; Burriesci and Bhaya, 2008). If cells are placed in a directional light source, phototactic motility is easily visualized under a dissecting microscope as cells moving in large groups that typically make finger-like projections towards the light source (~1 mm in 6 h, or ~3 cell lengths per minute) (Fig. 11.2). A second, more quantitative method, is to use time-lapse video microscopy to track the behaviour of single cells or groups of cells (Burriesci and Bhaya, 2008; Choi *et al*., 1999; Ng *et al*., 2003). These time-lapse movies can be analysed using commercial or custom cell-tracking software to track various parameters (e.g. velocity, direction) of individual cells under various conditions and to build models of phototactic behaviour (see later).

Surface-dependent Syn PCC 6803 motility (Bhaya, 2004; Bhaya *et al*., 1999; Yoshihara and Ikeuchi, 2004) shares characteristics with the behaviour of several pathogenic Gram-negative bacteria and the soil-dwelling bacterium *Myxococcus xanthus.* In these cases it has been associated with Type IV pili (TFP)-dependent motility, also known as twitching motility, because of the slow and 'jerky' movement of individual or groups of cells (Craig and Li, 2008; Mattick, 2002; Nan and Zusman, 2011; Pelicic, 2008). Choi *et al.* (1999) tracked the motion of individual Syn PCC 6803 cells suspended in media on glass slides and demonstrated that very low levels of white light (1 μmol/m^2/s) saturated the phototactic response, and that threshold fluence rates were as low as 0.002 μmol m^{-2} s^{-1}. They suggested that Syn PCC 6803 cells sensed light direction rather than light intensity and that ATP generated by oxidative phosphorylation was required for phototaxis, but that photosynthesis was not essential. A

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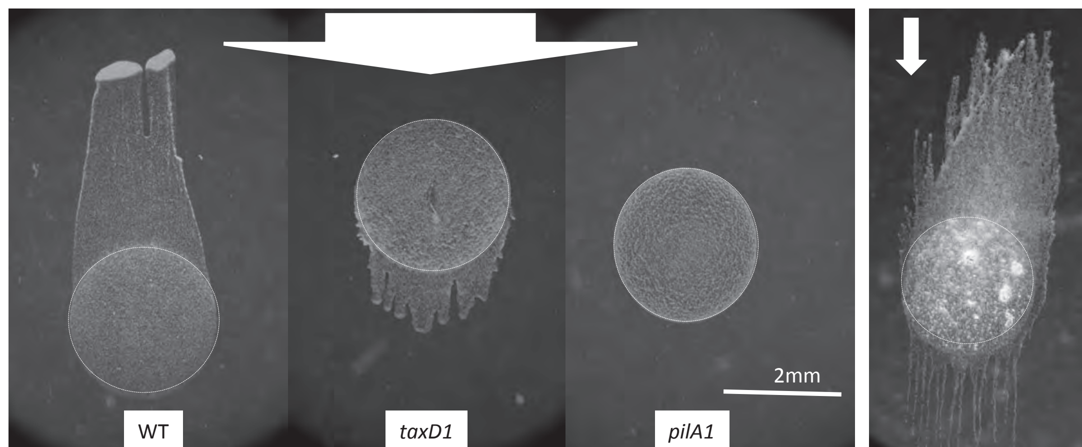

Figure 11.2 Phototactic behaviour of Syn PCC 6803, mutant cells and thermophilic *Synechococcus* sp. A directional phototaxis assay on 0.4% agarose plates was performed for 48h. Wild-type (WT) cells (left) show positive phototaxis with groups of cells moving out from the original drop, making finger-like projections towards the light (white arrow); a *taxD1* mutant (middle) exhibits negative phototaxis; a *pilA1* mutant (right) is non-motile. Size of the drop is ~2–3mm (marked with dotted white lines). Right panel: Thermophilic *Synechococcus* sp. isolated from microbial mats cells exhibiting both positive and negative phototaxis, in directional light. Note that most cells are positively phototactic with a smaller number of cells exhibiting motility away from light. A colour version of this figure is available in the plate section at the back of the book.

limited action spectrum indicated that phototaxis was maximal in red light and that UV-A (360 nm) caused negative phototaxis. In these experiments, phototaxis did not exhibit red/far red reversibility, which is expected of typical phytochrome-based behaviour. Ng *et al.* (2003) used a modified system to monitor motility of individual Syn PCC 6803 cells, which attached to, and moved along a glass surface. By using a number of different wavelengths and fluence rates, they established that positive phototaxis was a low fluence rate response, and that at higher fluence rates cells exhibited negative phototaxis, particularly in the blue light regions of the spectrum. This assay was also used to establish the light responses of mutants that lacked positive phototaxis (see later) (Ng *et al.*, 2003). More recently, these assays have been improved by the ability to use LEDs that have very specific wavelengths and bandwidths so that reproducibility and accuracy can be maintained (Burriesci and Bhaya, 2008).

Surface appendages in unicellular cyanobacteria

Several unicellular cyanobacterial species have been examined by electron microscopy revealing the presence of appendages around the cell, variously referred to as mucilage, filaments, fimbriae, sheaths, pili and spinae (Vaara, 1982). Although the role and biochemical nature of these diverse structures have yet to be completely described, it is likely that these extracellular layers play several important roles, including a role in motility (Pereira *et al.*, 2009). We focus on Syn PCC 6803 since molecular approaches to dissecting motility have led to a fairly detailed understanding of phototaxis and motility (see later).

Syn PCC 6803 cells are typically coccoidal in shape with a diameter of ~1 μm; cells examined by electron microscopy, using uranyl acetate based negative staining, indicated that there are at least two pilus morphotypes at the cell surface (Bhaya *et al.*, 1999, 2000). The cell is uniformly covered with a dense layer of very thin, brush-like pili, with an average diameter of 3–4 nm and a length of ~1 μm (Fig. 11.3). The biochemical characterization of these pili has not yet been attempted and it is possible that they are composed of polysaccharides or glycoproteins that constitute the extracellular matrix (Pereira *et al.*, 2009). Cells also have tufts of thick pili (average diameter 6–8 nm) that extend several cell lengths (~4–5 μm) and which have

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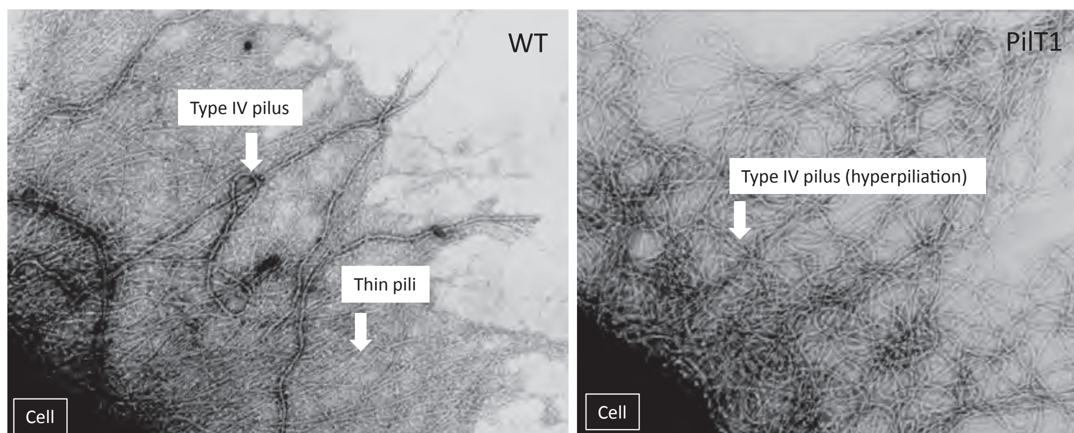

Figure 11.3 Transmission electron microscopy of Syn PCC 6803 WT and *pilT1* mutant cells. Cells were stained with 1% uranyl acetate. WT cells show both TFP and thin pili at the surface while the *pilT1* mutant is hyper-piliated with a large excess of TFP.

been identified as TFP (see below). Cells grown on soft agar surfaces often appear to be connected by these pili, when visualized by electron microscopy (Bhaya *et al.*, 2000). The exact number and location of these thick pili (i.e. TFP) in a cell are not yet known although in rod-shaped bacteria, such as *M. xanthus*, TFP are typically found at the poles (Hartzell and Youderian, 1995; Nan and Zusman, 2011; Sogaard-Andersen, 2004).

Analysis of Type IV pilus-dependent motility in Syn PCC 6803

A reverse genetic approach was attempted to identify the motility apparatus in Syn PCC 6803. Because of the well-documented presence of surface pili, the twitching-like motility behaviour of cells, and the fact that flagella have never been identified in cyanobacteria, certain candidate genes were targeted for inactivation (Bhaya *et al.*, 1999). A *pilA1* (sll1694) gene encoding a protein with some similarity to a pilin, the repeating subunit that comprises a pilus (Craig and Li, 2008; Mattick, 2002; Pelicic, 2008) was identified and insertionally inactivated. The *pilA1* mutant was completely non-motile (Fig. 11.2). This experiment provided the first direct evidence that surface-dependent motility in Syn PCC 6803 was likely to be mediated by TFP. Consistent with its non-motile phenotype, the *pilA1* mutant completely lacked the thick pili (i.e. TFP), although the normal complement of thin pili were still present (Bhaya *et al.*, 1999).

Although the requirement for the structural subunit of pili (i.e. pilins encoded by *pilA1*) for TFP-dependent motility was clearly established (Bhaya *et al.*, 1999), inspection of the Syn PCC 6803 genome showed the existence of at least five other *pilA*-like genes. This similarity was confined mostly to the N-terminus. Inactivation of these genes (*pilA2–pilA6*) had no obvious effect on motility (Bhaya *et al.*, 1999; Yoshihara *et al.*, 2000, 2001) and their role in motility is not clear, although minor pilins and pseudopilins have been identified in other organisms. Type IV pilus biogenesis is a complex process and requires several steps, including proteolytic processing and N-methylation of pre-pilin polypeptides, which is carried out by a novel bi-functional signal peptidase, PilD. Proteins with weak similarity to Type IV pilins (and which often share a conserved cleavage site) have been shown to function in extracellular protein secretion and in transformation competence (recent reviews include Proft and Baker, 2009; Burrows, 2012).

The critical role of TFP in surface-dependent motility of Syn PCC 6803 was further demonstrated by creating mutants in genes encoding components required for pilus biogenesis and assembly. As expected, mutants in *pilQ* (encoding the secretin pore), *pilD*, (encoding the membrane-bound signal peptidase) and *pilC*, *pilM*, *pilN* and

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pilO (encoding components of pilus biogenesis and assembly apparatus) were all non-motile and also lacked TFP at the cell surface (Bhaya *et al.*, 2000; Yoshihara *et al.*, 2001). Interestingly, several, but not all, of these pilus biogenesis mutants were also found to be defective in natural transformation (Fusseneger *et al.*, 1997; Long *et al.*, 2003; Nakasugi *et al.*, 2006; Yoshihara *et al.*, 2000).

Surface-dependent motility occurs when TFP are extended, and attach to a surface (non-specifically to a surface such as agarose or specifically to polysaccharide fibrils of neighbouring cells). Next, the TFP retract and consequently the cell body is pulled along; it has been calculated that TFP may exert a substantial force of up to ~100 pN, to mediate cell movement (Maier *et al.*, 2002; Merz *et al.*, 2000, Merz and Forest, 2002). TFP dependent motility is an energy (ATP) requiring process and the sequential retraction and extension of TFP requires the activity of two ATPases. These are the conserved motor proteins, PilB (for extension) and PilT (for retraction), located at the inner membrane (Okamoto and Ohmori, 2002). In contrast to some other microorganisms, Syn PCC 6803 has two *pilB*-like genes, *pilB1* and *pilB2* (Bhaya *et al.*, 2000). As expected, inactivation of *pilB1* results in a non-motile phenotype. However, *pilB2* mutants retain motility so the role of PilB2 is still to be elucidated (Yoshihara *et al.*, 2001).

Syn PCC 6803 also has two copies of a *pilT*-like gene (*pilT1* and *pilT2*), which would encode the PilT motor required for pilus retraction. Both the *pilT1* and *pilT2* genes were individually inactivated (Bhaya *et al.*, 2000) and surprisingly, exhibited very different phenotypes. The PilT1 and PilT2 proteins are similar, although PilT2 has an N-terminus extension of ~80 amino acids, rich in proline residues. The *pilT1* mutant is unable to retract its pili and as a consequence is non-motile. Since the *pilT1* cells continue to synthesize pilins which are extended at the cell surface as TFP, but are not retracted, this would be predicted to result in a hyper-piliated cell and this was convincingly demonstrated in electron micrographs of *pilT1* mutants (Fig. 11.3) (Bhaya *et al.*, 2000). Interestingly, *pilA1* mRNA levels increased eight-fold in the *pilT1* mutant, suggesting a link between pilin expression levels and pilus activity, although the details of how this regulation is mediated awaits further study. The *pilT2* mutant exhibited an unexpected phenotype: mutants moved *away* from a directional light source, while wild-type cells exhibited positive phototaxis. One possible interpretation of the 'negative phototaxis' phenotype is that PilT2 is required for positive phototaxis, directly or indirectly, so that an inactivation of this machinery unveils a negative phototaxis which is otherwise masked by the dominant positive phototaxis (Fig. 11.4). These results provided important evidence that phototaxis can be either towards or away from a light source (referred to as positive and negative phototaxis, respectively). Under most light conditions and wavelengths, tested so far, positive phototaxis is the dominant response, but there are exceptions, as described later. This phenotype allows one to screen for mutants with aberrant motility and identify other components involved in motility and photoperception (see later sections).

There are three reports that pilins in Syn PCC 6803 are glycosylated (containing xylose and fucose residues) or post-translationally modified (via acetylation or trimethylation of the C-terminal lysine of pilin) and that these have an effect on pilus biogenesis and/or function. Two proteins, a putative nucleotidyl transferase (encoded by sll0899) and a putative kinase (encoded by slr1443) may be required for this process (Kim *et al.*, 2004, 2009, 2011). The multiple and complex post-translational modifications and glycosylation of pilins in other organisms have also been reported to play an important role in infection, biofilm formation and adhesion (Aas *et al.*, 2006; Chamot-Rooke *et al.*, 2011; Marceau *et al.*, 1998).

Action spectra for phototaxis

The fact that Syn PCC 6803 cells exhibit both negative and positive phototaxis (Figs. 11.2 and 11.4) implies that the cell has (i) the ability to sense light direction, intensity and/or quality via photoreceptors, (ii) a signal transduction mechanism that allows the motility apparatus to respond to these light cues, and (iii) the capacity to make decisions about directional movement which may also depend on the metabolic or photosynthetic status of the cell (Mullineaux, 2001). Several of these features have been addressed successfully in the last few years using a variety of approaches (for

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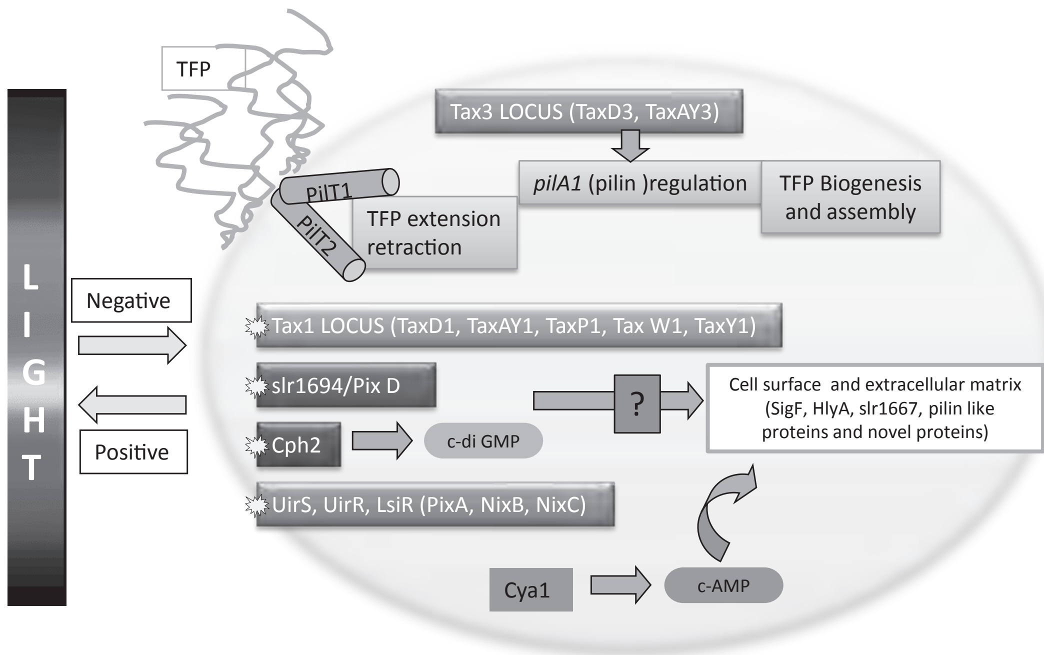

Figure 11.4 Model of positive and negative phototaxis in Syn PCC 6803. The major identified photoreceptors (shown with stars) are indicated (see Table 11.1 for more details). The Tax3 locus, which is required for TFP biogenesis and assembly, is also shown. PilT1 and PilT2 are required for TFP activity. The exact roles of cAMP and di-cGMP in regulating the cell surface and various other proteins are not known, although it is known that many of these proteins play a role on motility. A colour version of this figure is available in the plate section at the back of the book.

reviews see Bhaya, 2004; Yoshihara and Ikeuchi, 2004).

The action spectra for phototaxis have been measured for some cyanobacteria and can be quite complex and vary significantly between species. The action spectrum for phototaxis often overlaps with the absorption spectra of the dominant pigments in the cell, phycobilins and chlorophyll. However, inhibitors of photosynthesis do not have a major effect on phototactic orientation, or on gliding speed. Thus energy derived from photosynthesis may not be directly required for phototaxis, although addition of glucose increases motility (Bhaya *et al.*, 2006; Choi *et al.*, 1999; Nultsch, 1975). As mentioned earlier, Syn PCC 6803 cells have a very low threshold fluence rate (0.001 μmol/m^2/s) required to elicit phototaxis, and the response saturates at ~1 μmol/m^2/s (Choi *et al.*, 1999; Ng *et al.*, 2003).

When motility assays were carried out in a large spectrograph (in which both light wavelength and fluence rates can be precisely controlled), the thermophilic unicellular cyanobacterium *Thermosynechococcus elongatus* (or *Synechococcus elongatus*) exhibited markedly different action spectra that were dependent on fluence rates. At low fluence rates, red light was the only major elicitor of positive phototaxis, while at higher fluence rates (10 μmol/m^2/s^1) several action peaks (at 530, 570, 640 and 680 nm) were observed, and at even higher fluence rates, the red action peaks (at 640 and 680 nm) disappeared and far-red action peaks (at 720 and 740 nm) appeared. Consistent with this result, cells illuminated simultaneously with red and far-red wavelengths showed a drastic reduction in phototaxis, which the authors suggested might indicate the possible involvement of phytochrome (Kondou *et al.*, 2001). In Syn PCC 6803, a similar action spectrum was also reported although there were some striking differences such as the strong negative phototaxis in UV-A light (360 nm) (Choi *et al.*, 1999; Ng *et al.*, 2003).

Thermophilic *Synechococcus* isolates from microbial mats at Yellowstone National Park

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exhibited both negative and positive phototaxis. This complex dual behaviour might reflect strain-specific preferences for different light conditions (Ramsing *et al.*, 1997) (Fig. 11.2). Interestingly, *Synechococcus* isolates from these microbial mats which have recently been sequenced encode several components in common with Syn PCC 6803, including genes required for Type IV pilus biogenesis and photoreceptors that include TaxD1 and phytochrome-like photoreceptors (Bhaya *et al.*, 2007; Ulijasz *et al.*, 2009). In summary, these results lead to the conclusion that multiple photoreceptors with different spectral characteristics are likely to be involved in phototactic responses (see next section). Cyanobacteria encode a very large number of potential photoreceptors, including phytochromes, cryptochromes and blue-light photoreceptors, among others, making identification of their roles in phototaxis both revealing and challenging (Auldridge and Forest, 2011; Ikeuchi and Ishizuka, 2008; Rockwell and Lagarias, 2010).

Photoreceptors and signal transduction in phototaxis

One of the powerful ways to identify potential photoreceptors and other unknown components of the signal transduction cascade required for phototaxis is to screen for mutants that exhibit aberrant phototactic responses (Fig. 11.2). A transposon-tagged library of mutants was generated and almost 300 individual mutants with an aberration in motility were identified in the initial screen of approximately 10,000 individual colonies (Bhaya *et al.*, 2001a). Identification of the lesions in these mutants revealed that many novel proteins were involved in phototaxis. In addition, a number of chemotaxis-like operons encoding proteins involved in pilus biogenesis, signal transduction components and photoreceptors were also identified (see later). The majority (~90%) of mutants were either non-motile or demonstrated a reduced motility phenotype, but approximately ten per cent of the mutants had lost positive phototaxis (which is the phenotype of wild-type cells) and instead exhibited only negative phototaxis in white light. This particular class of mutants was predicted to have lesions in photoreceptors or in components of the signal transduction cascade (Bhaya *et al.*, 2001b) and were explored further.

One of the first putative photoreceptors to be identified via the transposon mutagenesis was TaxD1 (SyPixJ1, sll0041; see Fig. 11.2) in a mutant that exhibited negative phototaxis in white light (Bhaya *et al.*, 2001a). This protein was also independently identified by Yoshihara *et al.* using a reverse genetic approach (PisJ1, later renamed PixJ1) (Bhaya *et al.*, 2001b; Yoshihara *et al.*, 2000, 2002). The TaxD1 protein is encoded by *taxD1*, which is in the *Tax1* locus that has at least six genes [*taxP1*, *taxY1*, *taxW1*, *taxD1*, *taxD1′* (a truncated version of *taxD1*) and *taxAY1*]. It was noted that genes in this locus had similarity to the chemotaxis genes of *E. coli*, and mutants in all of these genes produced a negatively phototactic phenotype, suggesting that this entire locus (perhaps an operon) is required for positive phototaxis.

The TaxD1 protein is a novel protein that has some similarity to methyl-accepting chemoreceptors of *E. coli*. It has a well-conserved C-terminal methyl-accepting (MA) domain, with an adjacent HAMP domain (a domain present in histidine kinases, adenyl cyclases, methyl-accepting proteins and phosphatases) and, in addition, two GAF domains at the N-terminus. GAF domains are part of a superfamily of proteins that includes the PAS/PAC and LOV domains that bind diverse small molecules such as tetrapyrroles, flavins, flavinoids and nucleotide cofactors (Crosson *et al.*, 2003; Herrou and Crosson, 2011; Vreede *et al.*, 2003). These typically occur as tandem repeats, with a bewildering combination of domains often in a single protein; this is especially common in some filamentous cyanobacteria (Auldridge and Forest, 2011; Ikeuchi and Ishizuka, 2008; Montgomery and Lagarias, 2002; Pepper, 1998; Rockwell and Lagarias, 2010) and in most cases their physiological role is unknown.

TaxD1 homologues as well as several other proteins that contain GAF domains have been identified in cyanobacterial genomes and represent a very large class of relatively simple photosensory proteins that are related to the well-studied plant phytochromes. This class of putative photosensing GAF-containing proteins have recently been termed 'cyanobacteriochromes' (or CBCRs) and some have been shown to bind a linear tetrapyrrole (either phycocyanobilin or phycoviolobilin)

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(Ikeuchi and Ishizuka, 2008). Here we will focus on the biochemical and spectroscopic characterization of the chromophore that binds to the GAF domains in TaxD1/SyPixJ1. When His-tagged SyPixJ1 was expressed in Syn PCC 6803 and isolated from detergent-solubilized membranes it contained a covalently bound linear tetrapyrrole (Yoshihara *et al.*, 2004). Quite unexpectedly, this version of the protein showed a reversible photoconversion between a blue absorbing form (Pb, ~430–435 nm peak) and a green light absorbing form (Pg, ~535 nm) when irradiated with blue or green light, respectively. Surprisingly, it did not exhibit absorption in the red or far-red area of the spectrum, which is typical of phytochromes. It was also established, using site directed mutagenesis, that the chromophore was bound to the second GAF domain at a conserved Cys-His region (Yoshihara *et al.*, 2004, 2006).

The identification of this unusual photoconverting behaviour of GAF domains has been followed by several recent studies analysing the photoconversion of GAF domains from other cyanobacteria. These include the TePixJ_GAF (Ishizuka *et al.*, 2007) and the cyanobacteriochrome Tlr0924 from the thermophilic unicellular cyanobacterium *T. elongatus* (Rockwell *et al.*, 2008), as well as AnPixJ from *Anabaena* sp. PCC7120, which likely binds phycocyanobilin and exhibits a red/green photoconversion (Fukushima *et al.*, 2011; Narikawa *et al.*, 2008). The identification and properties of CBCRs is an area of active research and the reader is referred to recent reviews (Auldridge and Forest, 2011; Ikeuchi and Ishizuka, 2008; Rockwell and Lagarias, 2010).

It is worth noting that cyanobacteria synthesize a very large number of photo-pigments, including chlorophyll, haems and phycobilins, flavins and carotenoids, many of which play an important role in photosynthesis. Thus, there is great scope for binding of different chromophores in proteins with multiple GAF domains. Furthermore, it has been shown that based on the binding of these chromophores and the pocket to which they are attached, their absorption characteristics can be dramatically different. In the context of photomovements, this is a particularly critical feature, since it suggests that cyanobacteria may have evolved the capacity to exquisitely respond to light qualities as they vary from dawn to midday to dusk. In a broader context, it has been shown that cyanobacteria also use light signals and chromophore-binding proteins (e.g. RcaE) for complex adaptive processes such as chromatic adaptation (Kehoe, 2010; Kehoe and Grossman, 1996) and circadian rhythms (e.g. SyCikA) (Ivleva *et al.*, 2006; Schmitz *et al.*, 2000).

Much less is known about the physical location of the photoreceptors, signal transduction components and the TFP. However, it has been demonstrated that the TaxD1 homologue in the rod-shaped *T. elongatus* is localized at the poles (Kondou *et al.*, 2002). In *E. coli*, the majority of chemoreceptor complexes are clustered in large arrays at the poles of the cell, and the flagella are peritrichously arranged (Gestwicki *et al.*, 2000; Maddock and Shapiro, 1993; Webre *et al.*, 2003). Although we know that a chemotaxis-like operon is required for positive phototaxis in Syn PCC 6803, many fundamental questions remain about how the light signal is perceived by an individual cell and the signal transduction pathway progresses from the TaxD1 photoreceptor, via mobile regulators and histidine kinases, to the TFP apparatus.

Other chemotaxis-like loci required for motility and phototaxis

The *tax3* locus was also initially identified by transposon mutagenesis (Bhaya *et al.*, 2001a) and by reverse genetics (Chung *et al.*, 2001; Yoshihara *et al.*, 2002). Mutants in the Tax3 locus exhibit a non-motile phenotype. Furthermore, electron micrographs of mutants of the putative receptor protein (TaxD3) and the cognate hybrid histidine kinase (TaxAY3) indicated that they lacked TFP (Bhaya *et al.*, 2001a). The N-terminus of TaxD3, which might contain a sensing or interacting domain, has a tetratricopeptide (TPR) domain, which is often associated with protein–protein interactions. Further biochemical approaches will be required to establish why mutants at this locus block pilus biogenesis. In addition to the *tax1* locus and the *tax3* locus there is a *tax2* locus (Bhaya, 2004), which encodes all the components of a chemotaxis signal transduction cascade, but it does not appear to have a strong effect on motility (Yoshihara *et al.*, 2001).

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Interestingly, all three histidine kinases in the Tax loci (TaxAY1, TaxAY2 and TaxAY3) are hybrid sensor histidine kinases, with a fused CheY-like regulator domain at the C-terminus (in fact TaxAY3 has *two* tandemly arranged CheY domains at the C-terminus) (Ashby and Houmard, 2006; Mizuno and Matsubara, 2003). TaxAY1 and TaxAY2 are classified as Class II kinases with canonical histidine phosphotransfer (HPt) domains at the N-terminus but TaxAY3 does not have a well-conserved HPt domain (Dutta *et al.*, 1999; Stock, 1999). Hybrid histidine kinases and Class II kinases have not been examined as carefully as other simple histidine kinases, but it has been suggested that the presence of multiple HPt domains and attached CheY-like domains might allow for complex phosphorelay signals whereby several inputs can be integrated into the signalling pathway (Jung *et al.*, 2012; Mizuno and Matsubara, 2003; Wuichet *et al.*, 2010). In fact, other proteins that carry a Hpt domain have been implicated in motility (Yoshihara *et al.*, 2000). In *E. coli*, where the signal transduction cascade for chemotaxis has been very well–studied, it appears that information from the multifunctional chemoreceptors is integrated through the single histidine kinase/regulator pair (CheA/CheY). So far, it has not been established whether the three Tax loci in Syn PCC 6803 act independently or if there is any cross-talk between them. Recent work from several research groups suggests that chemoreceptors are in large macromolecular complexes often at the poles of a cell and that this is important for signal cross-talk and amplification (Hazelbauer *et al.*, 2008).

Multiple photoreceptors are involved in phototaxis

Although TaxD1 (SypixJ1) is the best characterized of the photoreceptors in terms of the chromophores, other photoreceptors have also been implicated in regulating phototaxis. These include the blue light photoreceptor, BLUF (PixD or slr1694), the UV-A sensing photoreceptor (UirS, PixA) and the phytochrome-like multi domain Cph2, which are described below (Fig. 11.4 and Table 11.1). The fact that multiple photoreceptors have now been shown to play a role in phototaxis, suggests that cyanobacteria must be exquisitely responsive to their light environment and we have only just begun to appreciate the complexity and interplay between these signal transduction pathways.

BLUF domain photoreceptors

There are at least three classes of blue-light receptors that contain the flavin chromophore. These include the phototrophins (which contain a LOV domain), the cryptochromes and the recently discovered BLUF domain proteins (Gomelsky and Klug, 2002; Herrou and Crosson, 2011; Losi and Gartner, 2012). Cyanobacteria encode BLUF proteins and it has been demonstrated that the photocycle of BLUF proteins is similar to that of other BLUF proteins such as AppA, that show a UV-visible absorption spectrum, and a fast return from the excited to the ground state (Masuda *et al.*, 2004), strongly suggesting that it can act as a reversible photoreceptor. In the case of Syn PCC 6803, it was shown that a mutation in BLUF (PixD or Slr1694) blocked positive phototaxis in red light but surprisingly, neither the wild-type nor the mutant showed any motility towards blue light (460 nm) (Kita *et al.*, 2005; Okajima *et al.*, 2005). Furthermore, it has been shown that Slr1694 is present as an oligomer in the cell and that it likely forms a hetero-oligomer with a response regulator, Slr1963 (PixE), with a stoichiometry of PixD10-PixE5 (Yuan *et al.*, 2006, 2011). Very recently, it has been suggested that this protein–protein interaction is required for signal transduction and phototactic behaviour. The disassembly kinetics of this hetero-oligomer is light-intensity dependent, raising the intriguing possibility that this may be a mechanism for sensing light intensity. Although further follow-up research is required, the possibility that photomovements will be sensitive to both light intensity and light quality is quite plausible. How changes in PixC-D oligomerization are transduced to changes in phototactic behaviours is still to be established.

Cph2 and the role of cyclic di-GMP in phototaxis

The Cph2 (Sll0821) photoreceptor was initially implicated as inhibiting motility of Syn PCC 6803 towards blue light, since WT cells do not move

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Table 11.1 <Please supply legend>

Locus tag (size in nt)	Synonyms	Photoreceptor class	Mutant phenotype	Function	Domains (positions)	Chromo-phores	Domain organization	References
sll0041 (3003)	*taxD1*, *pixJ1*, *pisJ1*, *SypixJ1*, *cheD*, *tsr*	Cyanobacteriocrome (Phytochrome superfamily)	Negative phototaxis (WL, UV, BL, GL, RL)	Reqired for positive phototaxis	Phycocyanobilin	Blue/Green receptor Pb, λmax = 435 nm Pg λmax = 535 nm	GAF GAF MCP	Bhaya (2004), Yoshihara *et al*. (2004)
slr1694 (453)	*pixD*, *SyPixD*, *synbluf*, *bluf*	BLUF	Non motile (BL)	Reqired for positive phototaxis	Flavin	Blue receptor	BLUF	Okajima *et al*. (2005)
slr0473 (2247)	*cph1*, *hik35*, *phy*	Phytochrome	Positive phototaxis	Not Known	Phycocyanobilin	Red/Far red receptor Pr, λmax = 668 nm Pfr, λmax = 718 nm	PAS GAF HisKA HATPase	Hughes *et al*. (1997), Lamparter *et al*. (2001)
sll0821 (3831)	*cph2*	Phytochrome	Positive phototaxis (UV, BL, WL)	Inhibits phototaxis towards blue light (role in c-diGMP signaling)	Phycoerythrobilin Phycocyanobilin	N-terminal module Pf, λmax = 644 nm Pfr, λmax = 695 nm C-terminal module (GAF: 939-1090) Pb, λmax = 400 nm Pg, λmax = 525 nm	GAF GAF GGDEF EAL GAF GGDEF	Fiedler *et al*. (2005), Anders *et al*. (2010), Savakis *et al*. (2012)
slr1212 (2535)	*uirS*, *ETR1*	Phytochrome	Positive phototaxis (UV, BL, GL, RL)	Promotes negative phototaxis	Phycocyanobilin	UV receptor Pv, λmax = 382 nm Pg, λmax = 534 nm	PAC PAS PAC PAS PAC GAF HATPase	Song *et al*. (2011), Narikawa (2012)

towards blue light, but a *cph2* mutant exhibited motility towards blue light (Fiedler *et al.*, 2005) and towards UV-A (Moon *et al.*, 2011). This photoreceptor has a complex structure and contains six domains (GAF1-GAF2-GGDEF-EAL-GAF3-GGDEF) (Table 11.1). The first two GAF domains bind phycocyanobilin and behave as a P_r/P_{fr}-photosensory module (Anders *et al.*, 2011) while GAF3 binds a tetrapyrrole chromophore that can absorb blue light (Park *et al.*, 2000; Wu and Lagarias, 2000). Thus Cph2 has been called a 'composite phytochrome' with a Cph2-type phytochrome module at the N-terminus and a CBCR module at the C-terminus. In addition to these photoreceptor domains, Cph2 also contains GGDEF domains and EAL domains. GGDEF domains catalyse the synthesis of cyclic-di-GMP from two GTP molecules, while proteins with an EAL domain can catalyse cleavage of cyclic-di-GMP to linear 5′-pGpG. However, the N-terminal GGDEF1 domain is unlikely to have active diguanylate cyclase activity because it bears mutations in residues essential for catalytic activity (Savakis *et al.*, 2012). GGDEF and EAL domains are often found on the same polypeptide, and there are cases in which these domains contain mutations in their active site, although these mutants still show residual and/or regulatory activity (Cao *et al.*, 2010; Christen *et al.*, 2006; Hengge, 2009).

From studies using a number of specific mutants and complementing various background mutations under different light conditions, it was suggested that the terminal CBCR-GGDEF was critical for the blue light phototactic behaviour of Syn PCC 6803 cells (Savakis *et al.*, 2012). Furthermore, Cph2 may play an important role in sensing light across a broad range (in the green/blue/UVA regions of the spectrum). Changes in the light inputs are likely transduced to the GGDEF-EAL domains to finally result in a change in internal cyclic-di-GMP levels. Cyclic-di-GMP appears to be produced in a light-dependent manner by the C-terminal GGDEF2 domain and these high levels of cyclic-di-GMP inhibit motility in Syn PCC 6803. At this point it is not clear exactly how changes in cyclic-di-GMP levels are translated into behaviour of the motility apparatus (Savakis *et al.*, 2012). Notably, homologues of the hybrid photosensor Cph2 identified in Syn PCC 6803 are not universally present in cyanobacteria and so far have only been found in the unicellular *Cyanothece* sp. (PCC 7424 and PCC 7822) and in the filamentous *Lyngbya* sp. PCC 8106, raising the possibility that other novel photoreceptors may be present in other cyanobacteria.

A photoreceptor involved in UV-A/violet-regulated phototaxis

A locus containing four genes has recently been independently identified by two groups (Narikawa *et al.*, 2011; Song *et al.*, 2011) as being required for regulation of phototaxis. This region encodes a putative photoreceptor (UirS/PixA, Slr1212), and two putative response regulator proteins (UirR/NixB, Slr1213) and (LsiR/NixC, Slr1214) and the less well-characterized protein (Slr1215). Narikawa *et al.* introduced mutations into different background strains (either in cells that were constitutively positively phototactic (PCC-P) or negatively phototactic (PCC-N). They found that in the PCC-P background the *slr1212* mutant was negatively phototactic, while *slr1213* and *slr1214* mutants did not have an obvious altered phenotype. On the other hand, these same mutations when placed in the PCC-N background showed different effects, with *slr1212* exhibiting no obvious altered phenotype, while the *slr1213* and *slr1214* mutants appeared to reverse the negatively phototactic behaviour. Thus it was suggested that the opposing effects of PixA and NixBC imply that this pathway is involved in the switching between positive and negative phototaxis. Although the exact signal transduction pathway and mechanism by which this phototaxis switching behaviour is controlled remains to be determined, it is clear that multiple photoreceptors are involved in the phototactic behaviour of single-celled cyanobacteria, particularly in the UV/Blue/violet region of the spectrum.

Song *et al.* (2011) also used a reverse genetics approach that targeted this cluster of genes for inactivation and further study involving biochemical and expression studies. They reach similar conclusions to suggest that the UirR/UiS along with the PatA-like LsiR are part of a UV-A activated signalling system, which is required for negative phototaxis. Their model proposes that UirS is bound to membranes, where it responds

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to UV-A and thereby releases UirR. UirR, in turn, recognizes the LsiR promoter. Consistent with this model, constitutive expression of LsiR induces negative phototaxis under conditions where cells would exhibit positive phototaxis. Finally, because there is evidence that LsiR may be induced by other general stress responses, Song *et al.* (2011) speculate that it may be part of a system that allows the cell to integrate light signals from multiple photosensors. This allows the cell to respond to light signals in a dynamic way.

The role of cyclic AMP (cAMP) and surface architecture in phototaxis

The genome of Syn PCC 6803 encodes an adenylyl cyclase (Cya1) that synthesizes cAMP, and a cAMP receptor protein (Sycrp1) (Masuda and Ono, 2004; Yoshimura *et al.*, 2000). This suggests that cAMP may play a role as a second messenger, as occurs in many organisms such as *E. coli* and pathogenic bacteria such as *Mycobacterium* sp. (Kalia *et al.*, 2012; McDonough and Rodriguez, 2012). Ohmori and co-workers inactivated these genes in Syn PCC 6803 and observed that the corresponding mutant strains were non-motile and formed tight, dome-shaped colonies and that cAMP levels in the *cya1* mutant were 5% of normal levels. (Terauchi and Ohmori, 1999; Yoshimura *et al.*, 2002b). Blue light (450 nm) markedly increased cAMP levels within a few minutes, while red (630 nm) and far red (720 nm) did not (Ohmori and Okamoto, 2004; Terauchi and Ohmori, 1999). Cya1 was also independently identified in a transposon mutagenesis screen to identify motility mutants (Bhaya *et al.*, 2001b). Careful analysis of the motility of the *cya1* mutant demonstrated that they had an impaired motility on surfaces, which could be effectively rescued by externally added cAMP (Bhaya *et al.*, 2006) (Fig. 11.5).

The connection between blue light, cAMP levels and motility has been documented by Ohmori and co-workers in both unicellular and filamentous cyanobacteria (Ohmori and Okamoto, 2004). They found that the cellular cAMP level in the filamentous cyanobacterium, *Anabaena* sp. PCC7120, decreased in red light and increased in far red light (unlike the strong blue light-mediated effect on cAMP and motility in Syn PCC 6803). They also demonstrated that cAMP had multiple effects in the filamentous gliding cyanobacterium *Spirulina platensis*. It stimulated respiration and gliding motility and cells rapidly formed mats when cAMP (at concentrations of 1 μM) was added to a cell suspension (Ohmori *et al.*, 1993). *S. platensis* cells have been reported to excrete

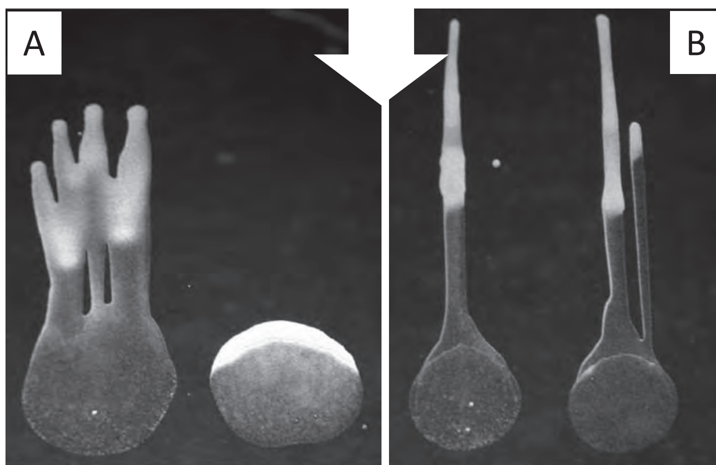

Figure 11.5 Effect of cAMP on phototaxis of Syn PCC 6803 and *cya1* mutant cells. WT (left), *cya1* mutant (right), were spotted on 0.4% agarose plates in the absence (A) or in the presence (B) of 0.1 mM cAMP. In the absence of added cAMP, *cya1* cells showed limited motility (A) but when cAMP is added to the plates, they exhibit typical finger-like projections, like WT cells. The arrow shows the direction of the light. Modified from Bhaya, 2006. A colour version of this figure is available in the plate section at the back of the book.

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cAMP into their surrounding medium and it is possible that cAMP-mediated motility changes may be important in the environment and possibly be part of a quorum sensing phenomenon, although these hypotheses have yet to be tested.

A transcriptomic analysis of the *sycrp1* mutant of *Synechocystis* identified a small set of down-regulated genes including *slr1667* (encoding CccS) and *slr1668* (CccP) and five other genes arranged in a putative operon (*slr2015* to *slr2019*) (Yoshimura *et al*., 2002a). Sycrp1 binds to a region upstream of *slr1667* that contains a typical *E. coli* CRP-binding consensus sequence (5′-TGTGA-N_6-TCACA-3′) (Yoshimura *et al*., 2002a). Transposon mutagenesis followed by identification of non-motile mutants also independently identified the gene cluster *slr2015–slr2019* (Bhaya *et al*., 2001b). Recently it has been suggested that CccS and CccP are responsible for the architecture of the cell surface. Many of the pleiotropic effects that were observed, such as release of yellow pigments, lack of TFP and release of proteins into the medium strongly resemble the phenotype of the SigF mutant, which was the first mutant linking TFP and cell–surface architecture to motility (Bhaya *et al*., 1999).

In summary, these results suggest that cAMP is an important player in the signal transduction for motility. It is not clear if cAMP controls motility through a pathway mediated by Sycrp1, and/or through regulation of genes such as *slr1667/slr1668* and the *slr2015* gene cluster. It is known that twitching motility in *P. aeruginosa* is controlled by the CRP homologue (Vfr) protein, which can bind both cAMP and cGMP (Beatson *et al*., 2002), and photo-sensing in the protist *Euglena* is mediated by a photoactivable adenylyl cyclase (Ntefidou *et al*., 2003). Furthermore, the connection between activation of cAMP-controlled pathways leading to photomovements, in response to either blue or red light, is further evidence that phototaxis is a complex pathway in which many light inputs working via secondary messengers and other pathways regulate motility.

Novel proteins in search of a role in phototaxis: tetratricopeptide repeat (TPR) domain proteins and pentapeptide repeat proteins

In addition to the gene products and pathways described above, a genetic approach has revealed that there are even more components of the phototaxis pathways in Syn PCC 6803 that need to be understood (Bhaya *et al*., 2001b). A number of mutants were identified in which genes encoding coiled-coil domain- or TPR domain-containing proteins were disrupted (Bhaya *et al*., 2001b). TPR domains (PFAM identifier PF00515) are ubiquitous in both eukaryotes and prokaryotes and are one of several repeat motifs that can act as scaffolds to facilitate the formation of multi–protein complexes involved in numerous cellular processes ranging from transcription to protein translocation and degradation (Dandrea and Regan, 2003; Lamb *et al*., 1995; Sun *et al*., 2011). Three non-motile mutants, created by transposon tagging, had inactivated genes (*sll0183*, *sll0414* and *sll0301*) encoding proteins that contained a tandem pentapeptide repeat domain (Bhaya *et al*., 2001b). This domain (PFAM identifier PF00805) is found in many organisms but is most common in cyanobacteria (Bateman *et al*., 1998; Liu and Wolk, 2011).

Two putative serine-threonine kinase mutants, inactivated in genes designated *spkA* and *spkB*, were also shown to lack motility (Bhaya *et al*., 2001b; Kamei *et al*., 2001, 2003). Very little is known about how these kinases function and it was originally assumed that serine threonine kinases were functional exclusively in eukaryotes. However, they have now been discovered in many bacteria where they might function in complex signalling pathways. In addition, a chaperone-like protein (Sll0058), the SigF sigma factor and other proteins (HlyA, Sll1951) have also been implicated in phototaxis (Bhaya *et al*., 2001b). These findings underscore that we still have a lot to learn about the complex pathways that cells use to sense light and move.

Modelling photoresponses

When wild-type Syn PCC 6803 cells are exposed to light, cells begin to move, although not necessarily in the direction of light. They instead form

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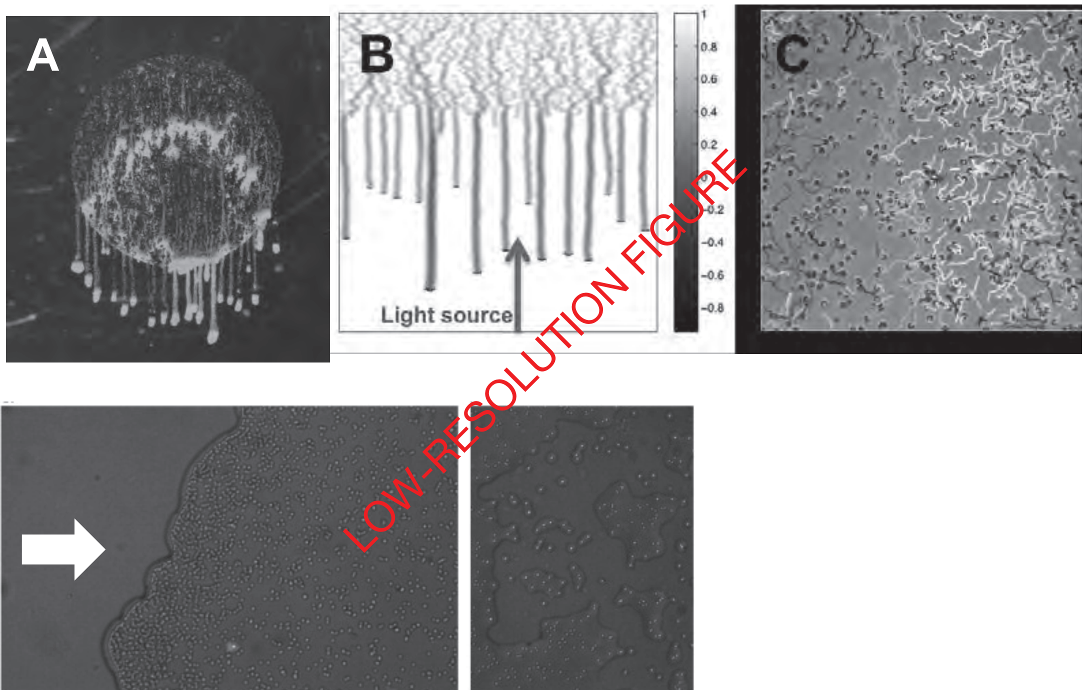

LOW-RESOLUTION FIGURE

Figure 11.6 Model of phototactic and group behaviour. Top panel. (A) Phototactic behaviour of Syn PCC 6803 showing typical finger-like projections of groups of cells. (B) Simulation of phototactic behaviour using modelling. Note how this simulates the behaviour of cells as shown in (A). (C) Tracking behaviour of single cells. Each cell track is shown in a different colour. Lower panel. Left: A group of Syn PCC 6803 cells in an extracellular matrix as they move towards the light, Right: when small groups of cells are moving they also are surrounded by a distinct extracellular matrix, of unknown composition (modified from Galante *et al*., 2012). A colour version of this figure is available in the plate section at the back of the book.

small aggregations and eventually cells move towards the light. At the front of a spot of cells placed on an agarose surface, cells align along the boundary of the spot before forming the characteristic finger-like projections or swarms of cells (Fig. 11.6). However, when the motion of individual cells is tracked, it is clear that they display a quasi-random motion, although they exhibit a weak positive biased movement towards the light source (Burriesci and Bhaya, 2008; Galante *et al*., 2012). We have also observed that as cells move along the surface they appear to release extracellular material (unpublished observations; Fig. 11.6), but the role and identity of this material is not yet known. It is possible to model these local interactions in Syn PCC 6803 and this may help us to predict the behaviour of mutant cells and understand how cells can sense light direction as well as the presence and behaviour of neighbouring cells. Some of the models that have been proposed imply that the typical behaviour of motile cells requires that these cells lay down extracellular material or extracellular polysaccharides as they move and this enhances motility (Burriesci and Bhaya, 2008; Galante *et al*., 2012; Ursell *et al*., 2013).

Certain filamentous cyanobacteria, such as *Phormidium uncinatum*, use photophobic responses to position themselves in areas of optimal light intensity (reviewed in Castenholz, 1982; Nultsch and Hader, 1988; Hader, 1988). In response to sudden spatial or temporal changes in the fluence rate, the filaments stop gliding and following a pause, reverse direction. In step-down responses (decrease in light intensity) the filaments thus move away from areas of darkness, while in step-up responses they avoid light that is too bright. Only a few cells at either end of a filament need to be exposed to the sudden change in light intensity in order for these responses to

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be elicited (Hader and Burkart, 1982). In step-down responses, the photosynthetic pigments are believed to be the photoreceptors and a model for signal transduction has been proposed in which changes in photosynthetic electron transport result in membrane depolarization. On the basis of ionophore and radiolabel uptake experiments, it has been proposed that this depolarization is amplified by Ca^{2+} fluxes, ultimately resulting in a difference in electrical potential between the ends of the filament, triggering a reversal (Hader, 1988). How the signal is transmitted to, and ultimately controls the motility machinery, remains to be determined. Less is known about the step up response where the action spectrum appears to be different than that for step down, showing a peak in the blue region of the spectrum (Hader, 1988). A recent computational model predicts that photophobic responses serve to optimize the distribution of a population with respect to a light field (Tamulonis, 2011) and hence are likely to play an important role in the environment. However, biochemical or *in silico* identification of potential photoreceptors and the regulation of signal transduction pathways in filamentous cyanobacteria still require further investigation.

Modelling photoresponses is a relatively new field and its importance lies in being able to predict motile behaviour of single cells or filaments or of motile communities and groups (Galante *et al*., 2012; Tamulonis *et al*., 2011; Ursell *et al*., 2013). As more data about the behaviour of single cells and groups (both wild-type and mutant) from different species become available, it is possible that models of photoresponses will become more robust and predict behaviour patterns allowing us to understand the behaviour of motile organisms in the environment.

Gliding in filamentous cyanobacteria

Gliding motility is the active translocation of bacterial cells on a solid surface along their longitudinal axis. It is distinct from other types of surface locomotion such as twitching and swarming in that neither type IV pili, nor flagella are involved (McBride, 2001). Gliding is employed by several phylogenetically distinct groups of bacteria, including the *Chloroflexaceae*, the myxobacteria, members of the *Bacteroidetes*, and certain filamentous cyanobacteria. The mechanism is not well understood because neither structures nor organelles that could account for movement have been readily visible. Indeed it is probable that there is more than one mechanism underlying this type of movement (Jarrell and McBride, 2008; Mauriello *et al*., 2010).

Characteristics of cyanobacterial gliding motility

Filamentous cyanobacteria of the order *Oscillatoriales* as well as some members of the *Nostocales* are capable of gliding and, unlike genera that are only transiently motile though the differentiation of hormogonia (discussed below), these generally glide at all times (Castenholz, 1982, 1989a,b). In all such gliders, movement requires contact with a surface and is associated with the production of extracellular polysaccharide mucilage or 'slime' that is left as a sheath or trail behind the gliding filament (Hoiczyk, 2000). Gliding speeds can range from 1 to 11 μm/s, with members of the *Nostocales* generally exhibiting slower gliding than that of the trichomes of the *Oscillatoriales* (Castenholz, 1989a). Members of the *Oscillatoriales* glide as straight filaments and rotate about their longitudinal axis as they move in a direction parallel to it (Hoiczyk, 2000). The direction of rotation can trace either a right-handed or left-handed helix and is a species-specific characteristic. Gliding filaments can also exhibit rapid reversals (Castenholz, 1982). Gliding members of the *Nostocales*, such as certain *Anabaena* species, do not rotate as they glide (Castenholz, 1989a), and some can move laterally as bent or U-shaped filaments (Hoiczyk and Baumeister, 1998; Nultsch *et al*., 1979). These behaviours have intrigued researchers for decades and have led to several examinations of the ultrastructure of gliding cells with the intent of visualizing a motility apparatus. On the basis of these studies, which we describe below, two models have been proposed to account for this movement: surface waves and slime extrusion.

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Surface wave model of gliding motility

Early work by Halfen and Castenholz (1970, 1971) with *Oscillatoria princeps* revealed the presence of an array of 6- to9 nm-wide fibrils arranged in a helical fashion around the trichome and which appeared to be located between the peptidoglycan layer and the outer membrane. The right-handed helical arrangement of the fibrils corresponded with the direction of rotation of the trichomes, and its 60° pitch also matched that of a helix traced by a point on a trichome as it moved. They proposed a model whereby contraction of these fibrils would produce moving waves that could push against the sheath or substrate and propel cells in the opposite direction. The helical arrangement of the fibrils would account for the rotation of the trichome during gliding and indeed the orientation of the array in left- and right-hand rotating strains of *Oscillatoria* corresponded to the direction of rotation (Halfen and Castenholz, 1971).

More recently, using cryofixation techniques which preserve ultrastructure better than conventional chemical fixation, Hoiczyk and Baumeister described arrays of 10- to 12-nm-wide fibrils external to the outer membrane of *Oscillatoria*, *Lyngbya*, and *Phormidium* species (Hoiczyk and Baumeister, 1995). The orientation of the fibrillar arrays corresponded to the direction of rotation of the filament during gliding. Whether these fibrils are the ones described by Halfen and Castenholz (Halfen and Castenholz, 1971) is difficult to determine, as the strains of *Oscillatoria* used were not the same, and the fixation and staining techniques used for electron microscopy differed. The surface fibrils from *Phormidium* were purified and shown to be composed of oscillin, a single 646 amino acid Ca^{2+} binding glycoprotein. The oscillin is arranged in a helical fashion above an S-layer, and Hoiczyk and Baumeister (1997) have proposed that it plays a passive role in gliding, serving as a screw thread guiding the rotation of the filament during mucilage secretion (see below) (Fig. 11.7). Proton motive force is suggested to power gliding in *Phormidium* (Glagoleva *et al.*, 1980), which also supports a passive role for the oscillin array that is located above the S-layer, and is not close to the source of energy generation.

Fibrils in the cell envelope appear to be characteristic of the *Oscillatoriales*. David Adams' group has also shown the presence of a fibrillar array located in the cell envelopes of several *Oscillatoria* strains (Adams *et al.*, 1999; Adams, 2001; Read *et al.*, 2007). These fibrils are larger (25–30 nm wide) than the ones described by Halfen and Castenholz (1971) and Hoiczyk and Baumeister (1995, 1997) and, on the basis of the corrugated appearance of the outer membrane in thin sections, are proposed to be located between the outer membrane and the peptidoglycan. The authors propose a model similar to Halfen and Castenholz's in which they speculate that rhythmical undulations originating in the fibrillar array could then be transmitted through the outer membrane and serve to push exuded mucilage with sufficient force to propel the cell (Read *et al.*, 2007). A mathematical analysis of such model (Siddiqui *et al.*, 2001) predicts that sufficient force could be generated by such a mechanism to account for the observed gliding speeds of a number of gliding bacteria. The mechanism by which the undulations would be produced is unknown and at present there is no experimental evidence to support this idea. Physical deformation is difficult to detect in microorganisms and the amplitude of surface waves may be below the resolution limit of the light microscope, but even in *Oscillatoria princeps*, which has a trichome diameter of 35 μm, no surface deformations or undulations have been observed (Castenholz, 1982; Halfen and Castenholz, 1970). The role of cell envelope fibrils in gliding remains to be determined, and questions remain. For instance, are the fibrils seen in the various strains related by homology and/or do they serve the same function? Nevertheless, given the fact that some of these have been purified (Adams *et al.*, 1999; Hoiczyk and Baumeister, 1997) and the genes encoding one of them, oscillin, is known (Hoiczyk and Baumeister, 1997), it should be possible to localize them unambiguously using antibody staining techniques, as well as to generate knockouts using reverse genetics in tractable strains. In addition, it is possible that there may be other ways of generating surface waves in cyanobacteria. One way may be through the rotation of helical cytoskeletal elements as has been proposed for *M. xanthus* (Nan *et al.*, 2011).

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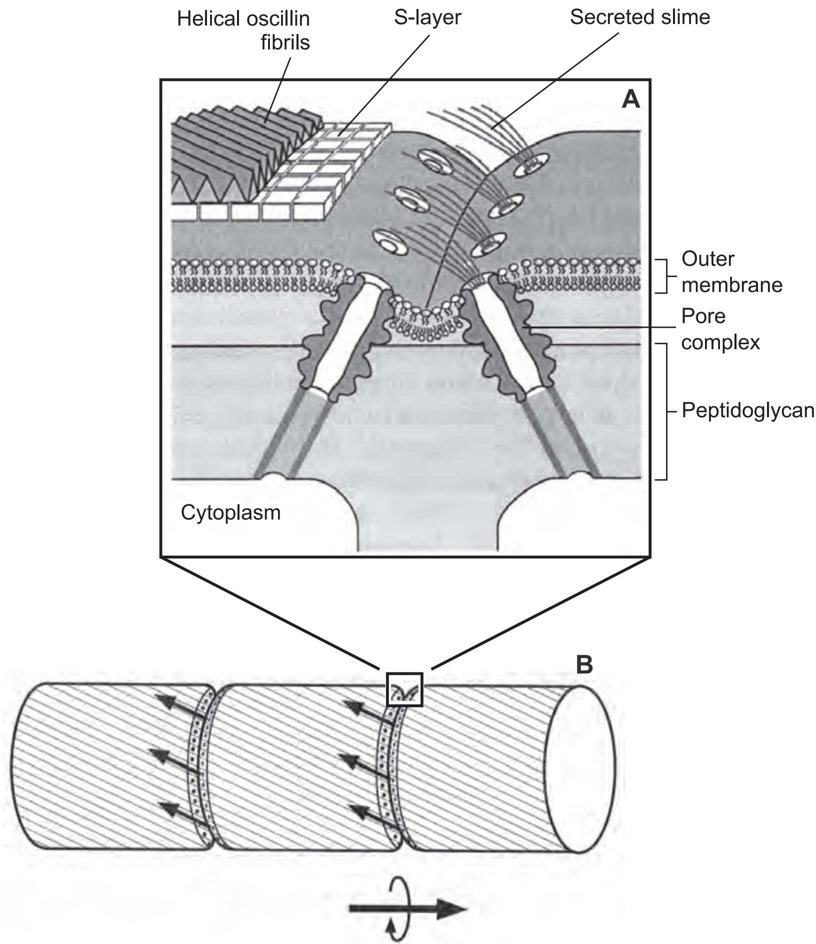

Figure 11.7 The slime extrusion model for gliding in filamentous cyanobacteria. (A) Model of the cell envelope of *P. uncinatum* showing the surface location of the oscillin fibrils, the pores located at the cell junction through which mucilage is secreted, and individual junctional pore complexes which traverse the outer membrane and peptidoglycan layers and extend into the cytoplasm. (B) Portion of a trichome of *P. uncinatum* showing mucilage being secreted at the junctional pores (small arrows) and being guided by the helically arranged oscillin fibrils. Secretion and flow of the slime that comes in contact with both the surface of the trichome and the substrate surface cause the cell to move in the opposite direction (large arrow). The cell body rotates as a consequence of the helical arrangement of the oscillin fibrils. (A) is reprinted from (Hoiczyk, 1998) with permission from Elsevier, and (B) is reprinted from (Hoiczyk and Baumeister, 1997) with permission from John Wiley and Sons.

Slime extrusion model of gliding motility

Gliding is always accompanied by the secretion of mucilage or slime. Staining of cells with India ink shows that the slime is translocated in bands or ribbons in a helical fashion, with a pitch of 60° (Halfen and Castenholz, 1971; Hoiczyk, 1998) and a handedness that corresponds to the orientation of the oscillin surface fibrils. The rate of elongation of the slime threads corresponds to that of movement and the threads emanate at cross walls where junctional pores are located (Hoiczyk and Baumeister, 1998) (Fig. 11.7). Such pore-like structures have been observed at cell junctions in a number of filamentous cyanobacteria and are arranged at an angle, forming a ring around the trichome at the septa between cells, and appear to extend from the cell membrane through the peptidoglycan layer (Halfen and Castenholz, 1971; Hoiczyk and Baumeister, 1995; Lamont, 1969;

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Pankratz and Bowen, 1963). The junctional pores have been suggested to be the sites of mucilage secretion (Hoiczyk and Baumeister, 1998; Pankratz and Bowen, 1963). In the *Oscillatoriales*, the junctional pores appear to be arranged circumferentially. In contrast, in *Anabaena variabilis* strain B1403-4b, which does not rotate while gliding but moves laterally, the junctional pores are not circumferential but rather are arranged laterally. In addition, in this strain, slime secretion is only visualized on one side of the filament, the one away from the direction of movement (Hoiczyk and Baumeister, 1998).

Organelles consistent in diameter and shape with the pores seen in whole cells were isolated from *Phormidium uncinatum* and image reconstruction analysis revealed a 32 nm long tube, 14 nm wide in the centre and 8 nm wide at each open end (Hoiczyk and Baumeister, 1998). In some instances, an additional 13-nm-long straight tube was found attached at one end and these complexes were proposed to span the entire cell envelope. These observations led Hoiczyk and Baumeister to propose that these structures represented junctional pore complexes and that these were secretion organelles composing the molecular motors responsible for gliding in cyanobacteria (Hoiczyk and Baumeister, 1998) (Fig. 11.7).

How then could slime secretion through pore complexes power motility? Pore-like complexes have also been seen in *Myxococcus xanthus*, another slime-producing gliding bacterium not closely related to cyanobacteria (Wolgemuth *et al.*, 2002). Wolgemuth and co-workers (Wolgemuth *et al.*, 2002) have proposed a model by which propulsive thrust is generated by the secretion of slime. They propose that the slime, a polyelectrolyte gel synthesized at the cytoplasmic membrane, enters the junctional pore, or 'nozzle', in a dehydrated form. As the slime is extruded through the nozzle, it is hydrated by water and it greatly expands. It is this expansion that forces the slime out of the nozzle and this produces thrust. A mathematical model, which takes into account the dimensions and geometry of the nozzle and assumes the slime behaves as a polyelectrolyte gel, predicts that the force exerted by the swelling is sufficient to propel the cells at the rates at which they have been observed to glide. This is in contrast to the model proposed by Siddiqui *et al.* (2001) in which the slime is propelled by the peristaltic waves produced by the fibrillar array.

This model is supported by several observations: the location of the pores corresponds to the sites of extrusion of slime threads; mutants that do not secrete polysaccharide (but which are pleiotropic as they also do not produce oscillin or the S-layer) are non-motile (Hoiczyk and Baumeister, 1997); the rate of extrusion of the slime threads matches that of gliding; and the direction of movement is opposite that of slime deposition. Of course, the latter two phenomena could be consequences of gliding, rather than being causative. Nevertheless, this is a testable model and future work should include establishing the chemical composition of the slime, identifying genes required for its biosynthesis and characterization of the junctional pore complex at the molecular level. Since the junctional pore complex can be isolated from other envelope components (Hoiczyk and Baumeister, 1998) it should be possible, assuming it is composed of protein, to identify its component polypeptides and hence the genes encoding them.

Hormogonia and motility

Hormogonia are short filaments that differentiate in certain heterocyst-forming and non-heterocyst-forming filamentous cyanobacteria from larger trichomes (Tandeau de Marsac, 1994; Herdman and Rippka, 1988). In the *Nostocales* (taxonomic subsection IV) and *Stigonematales* (taxonomic subsection V (Boone and Castenholz, 2001)) certain members of the genera *Nostoc*, *Fremyella*, and *Fischerella* (also called *Mastigocladus*), have vegetative trichomes that are not motile. Rather, under certain conditions which can include changes in nutrient and salt concentrations, light quality, and symbiotic associations (Meeks *et al.*, 2002), the vegetative filaments undergo fragmentation and differentiation to produce hormogonia, which are shorter gliding filaments consisting of smaller cells arising from cell division without concurrent growth. Hormogonia are thought to function in dispersal and as the infective units in plant symbiotic associations and have been shown to be chemotactic towards plant exudates (Knight and Adams, 1996; Nilsson *et al.*, 2006). Motility is

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transient and following a period of time (48–72 h) (Meeks *et al.*, 2002), the hormogonia cease to glide, differentiate heterocysts and re-enter the vegetative cycle.

Hormogonia glide at speeds that range from 0.7 to 3 μm/s (Duggan *et al.*, 2007; Hernandez-Muniz and Stevens, 1987) and can also glide through viscous environments at speeds of 0.5 μm/s (Robinson *et al.*, 2007). Movement can be, but is not always, accompanied by rotation of the filament about its longitudinal axis (Hernandez-Muniz and Stevens, 1987). As hormogonia glide, they deposit 'slime' trails (Hernandez-Muniz and Stevens, 1987; Robinson *et al.*, 2007), and extrusion of material from junctional areas has been observed by atomic force microscopy in *Mastigocladus laminosus* (Robinson *et al.*, 2007), although the rate of extrusion has not been reported for hormogonia nor correlated to the speed of gliding as was done for *Phormidium* and *Anabaena cylindrica* (Hoiczyk and Baumeister, 1998; Walsby, 1968).

In addition to the reduction in cell size, differentiation of motile hormogonia is accompanied by the appearance of pores in the peptidoglycan layer at junctions between cells (Damerval *et al.*, 1991; Robinson *et al.*, 2007). Robinson *et al.* (2007) report that hormogonia of *M. laminosus* can have up to three concentric rings of pores or 'nozzles' near the septa, separating cells and they postulate that these are the sites of slime secretion. Also, unlike the vegetative filament, hormogonia are peritrichously piliated (Dick and Stewart, 1980; Damerval *et al.*, 1991; Duggan *et al.*, 2007). Hence hormogonial gliding shares morphological characteristics of both filamentous gliding and TFP-mediated motility.

Molecular approaches to hormogonia gliding

The ability to genetically manipulate *Nostoc punctiforme* has made it possible to address aspects of its physiology and behaviour at the molecular level (Cohen *et al.*, 1994). The genome of *N. punctiforme* encodes fifteen proteins with similarity to proteins required for TFP-dependent motility including *pilT, D, A, B, C, M, N, O, Q*. Insertional inactivation of the *pilT* and *pilD* homologues altered surface piliation and the *pilT* mutant was non-motile, implying that motility in hormogonia of this species may require type IV pili (Duggan *et al.*, 2007). Transcriptional profiling during hormogonia differentiation in *N. punctiforme* revealed that several genes showing similarity to those encoding known motility proteins are up-regulated (Campbell *et al.*, 2007). These include five genes encoding TFP components, as well the *hmp* locus, which contains genes encoding proteins with similarity to proteins comprising a chemotaxis-like signal transduction system. In addition, a cluster of genes that encode putative polysaccharide synthesis and type II secretion proteins, the *hps* locus, is up-regulated in hormogonia (Campbell *et al.*, 2007; Risser and Meeks, 2013). Mutations in the *hmp* locus genes result in mutants that differentiate non-motile hormogonia that also do not secrete polysaccharide (Risser and Meeks, 2013). The *hps* locus encodes glycosyl transferases, pseudopilins, and proteins containing prepilin peptidase domains, and mutations in these genes affect motility and polysaccharide secretion (Risser and Meeks, 2013). These authors propose that this locus may encode the components of the junctional pore complex. Homologues of these genes are present in the genomes of all the filamentous cyanobacteria sequenced to date and appear to be confined to these organisms suggesting a common mechanism for motility (Risser and Meeks, 2013). The availability of a genetic system and a cast of candidate genes encoding both proposed structures and polysaccharide biosynthetic pathways should permit rapid progress on these issues.

Conclusions

In this chapter we have focused on recent advances made in the field over the last 20 years. Many important discoveries have been made in our understanding of the mechanistic aspects and regulation of motility, such as the identification of novel proteins involved in motility and the identification of photoreceptors and complex signal transduction pathways. These results underscore the complexity of motile behaviours in unicellular and filamentous cyanobacteria and point the way for future research. Some of the challenging questions that remain are mentioned below.

For unicellular cyanobacteria such as Syn PCC

6803, the motility apparatus consisting of TFP has largely been uncovered, and work is now centred on understanding the complex signal transduction and regulatory cascades governing phototaxis and motile behaviour. For instance, how do cells sense light intensity and direction and how does phototactic behaviour of individual cells and those that move as coordinated groups differ? What is the role of novel proteins and small molecules such as cAMP and cyclic-di-GMP in controlling motility? How are signals from photosynthesis and other metabolic pathways involved in controlling the motile behaviour of cyanobacteria?

For swimming and gliding cyanobacteria, the motility machinery has yet to be uncovered. What is the motility apparatus for swimming? How do the surface proteins SwmA and SwmB function in swimming? Do they constitute part of the motility apparatus or do they play a secondary structural role? How is the sodium-motive force transduced to the motor? Can we detect surface waves? In gliding cyanobacteria, what are the components of the junctional pore complex? Is the complex required for gliding? Is secretion of mucilage required for gliding? How is the proton-motive force utilized by the motility apparatus in gliding? What is the role of TFP in hormogonia gliding?

Ultimately, the goal is to understand the ecological implications of motility. What is the importance and role of motility in the natural environment? Does phototactic behaviour impart an advantage to motile cells when they are in light that is potentially damaging e.g. UV or high light? Unlike other motile unicellular cyanobacteria such as *Synechocystis*, marine swimming *Synechococcus* are not phototactic (Willey and Waterbury, 1989). Rather, they are chemotactic towards nitrogenous compounds at environmentally relevant concentrations. Does this provide them with a competitive advantage in the oligotrophic environments in which they are found where N is a limiting nutrient (Willey and Waterbury, 1989)? What receptors and signal transduction pathways might they employ? What signal transduction pathways do chemotactic filamentous cyanobacteria and hormogonia employ? In this review, we have attempted to highlight important advances and raise other unanswered questions. Given the advances in the genetic tractability of a variety of cyanobacteria, we are confident that rapid progress will be made in answering at least some of these questions.

Acknowledgements

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