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MicroReview

Light matters: phototaxis and signal transduction in unicellular cyanobacteria

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Summary

Many photosynthetic microorganisms have evolved the ability to sense light quality and/or quantity and can steer themselves into optimal conditions within the environment. Phototaxis and gliding motility in unicellular cyanobacteria require type IV pili, which are multifunctional cell surface appendages. Screens for cells exhibiting aberrant motility uncovered several non-motile mutants as well as some that had lost positive phototaxis (consequently, they were negatively phototactic). Several negatively phototactic mutants mapped to the *tax*1 locus, which contains five chemotaxis-like genes. This locus includes a gene that encodes a putative photoreceptor (TaxD1) for positive phototaxis. A second chemotaxis-like cluster (*tax3* locus) appears to be involved in pilus biogenesis. The biosynthesis and regulation of type IV pilus-based motility as well as the communication between the pilus motor and photosensory molecules appear to be complex and tightly regulated. Furthermore, the discovery that cyclic AMP and novel gene products are necessary for phototaxis/motility suggests that there might be additional levels of communication and signal processing.

Introduction

The dramatic ability of certain microorganisms to move towards or away from a light source has been documented well over a hundred years ago (for a summary of several ingenious early experiments as well as definitions of various photomovements, see Nultsch, 1975). Despite several early attempts at detailed characterization of this fascinating phenomenon in both unicellular and filamentous cyanobacteria (Nultsch and Hader, 1979; Castenholz, 1982; Hader, 1987), the molecular and biochemical underpinnings of motility and photoperception have only recently been uncovered. This review will focus on recent studies of unicellular cyanobacteria that have improved our understanding of phototaxis, an oriented or directed movement with respect to light direction, which results in individual cells or the cell population moving into a more favourable position for optimal growth. Four related themes will be highlighted: (i) why it is critical for cyanobacteria to respond to fluctuating and suboptimal light levels in the environment; (ii) the known mechanisms of motility in cyanobacteria; (iii) the link between photoperception and motility; and (iv) the role of novel genes in the regulation of phototaxis and pilus biogenesis.

Recent research on motility has focused on *Synechocystis* sp. strain PCC6803 (hereafter *Synechocystis*), a unicellular cyanobacterium that is easy to manipulate genetically and for which the complete genome sequence is available. *Synechocystis* has long been considered a model organism for studying photosynthesis, and several of its biochemical and regulatory pathways are exquisitely sensitive to light levels (Kaneko and Tabata, 1997; Mullineaux, 2001). Now, there is potential to extend these studies to developmentally complex genera such as the filamentous, nitrogen-fixing *Nostoc* (*Anabaena*) *PCC7120*, and to the symbiont *Nostoc punctiforme*, which forms an association with the gymnosperm cycad *Macrozamia* sp., as well as to ecologically important marine cyanobacteria that have evolved to survive in nutrient-poor environments (Meeks and Elhai, 2002; Dufresne *et al*., 2003; Palenik *et al*., 2003). These and several other cyanobacterial species have completely sequenced genomes that can be accessed through the Joint Genome Institute Microbial Genomes website and the Cyanobase website. The combined use of comparative genomics and molecular biology should greatly increase our understanding of the molecular basis of motility and its associated signal transduction systems.

Fluctuating environments and the need for motility

One of the hallmarks of cyanobacteria is that they flourish in a wide variety of environments where key parameters

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for growth such as light, nutrients and temperature can be severely limiting or fluctuate rapidly. Light, in particular, can be a mixed blessing for an obligate photoautotroph. Photosynthesis usually saturates at ≈200 μmol photons m^{-2} s^{-1} and, at light intensities below this, growth rates are often reduced. However, excess light can also be extremely debilitating, and the excess energy absorbed by the cell needs to be dissipated efficiently or it will cause damage to various cell components. The effect of light on the growth potential of the cell is also impacted significantly by the nutrient status of the cell. In a nutrient-poor environment where the cell is unable to grow, the reducing equivalents generated from photosynthesis cannot be used productively and, indeed, light becomes damaging at even lower intensities than for a cell growing under nutrient-replete conditions (Grossman *et al*., 2001; Mullineaux, 2001). Filamentous cyanobacteria such as *Oscillatoria* sp. and *Spirulina* sp. respond to changes in diurnal light levels by positioning themselves within the mat to optimize metabolic and photosynthetic capacity. Typically, they migrate vertically down into the microbial mat (in hot springs and hypersaline environments) when light levels are too high (Garcia-Pichel *et al*., 1994; Ward and Castenholz, 2000). Many unicellular cyanobacteria also exist as members of complex communities of microorganisms or in microbial mats that are stabilized by a complex polysaccharide matrix within which bacteria might have limited movement (Ramsing *et al*., 2000; Stal, 2000; Fenchel, 2002).

There are numerous reports of fast gliding motility in filamentous cyanobacteria, a few reports of gliding motility in unicellular cyanobacteria and a report of mysterious fast 'swimming' behaviour in a marine unicellular *Synechococcus* strain (Castenholz, 1982; Waterbury *et al*., 1985). Rates of gliding motility vary from 1 to 2 μm min^{-1} (during phototaxis in *Synechocystis*) to rates approaching 600 μm min^{-1} in the filamentous *Oscillatoria* sp. (Castenholz, 1982; Choi *et al*., 1999; Ng *et al*., 2003). Directed slime extrusion from junctional pores might be responsible for motility in some filamentous species, but the biochemical and molecular details of this process are as yet unknown, and it has been argued that the force generated might not be adequate to sustain movement (Castenholz, 1982; Hoiczyk, 2000; Wolgemuth *et al*., 2002).

Over billions of years of evolution, cyanobacteria have developed a host of sophisticated mechanisms to cope with environmental onslaughts. Whole-genome sequencing of several cyanobacterial species has revealed the presence of an impressive suite of histidine kinases and response regulators, which allow the cell to sense its environment and respond appropriately. *Synechocystis* has at least 80 genes coding for components of two-component signal transduction systems, while the filamentous cyanobacterium *Nostoc* sp. PCC7120 appears to have more than twice this number (Mizuno *et al*., 1996; Kaneko *et al*., 2001). Although we have an incomplete understanding of the environmental parameters that affect these sensory histidine kinases and the downstream signal cascades, one can envisage that it would be a significant advantage to be able to sense environmental fluctuations and respond by moving into a more optimal micromilieu.

The *Escherichia coli* paradigm for chemotaxis

The ability to move to a more favourable environment would require a minimum of three interacting components: (i) the organism must sense minor fluctuations in the environment; (ii) transduce this signal to a motility apparatus and (ii) respond or move in an appropriate direction. A well-studied paradigm for this temporally and spatially controlled process is chemotaxis in enteric bacteria. In the absence of a chemical gradient, cells move or 'tumble' in what has been described as a 'random walk'. However, in the presence of an attractant, cells decrease their tumbling frequency; this biases the random walk towards the attractant, and they swim in the direction of the attractant for longer time periods. There are four key players in the chemotaxis signal perception cascade. Methyl-accepting chemoreceptor (MCP) dimers or multimers bind attractants or repellents present in the periplasm. The binding of the ligand to the MCP causes a conformational change in the MCP dimer, allowing for interaction with the histidine kinase, CheA, through an adaptor protein, CheW. The interaction triggers a phosphorylation cascade that is initiated by autophosphorylation of CheA at a conserved histidine residue and the subsequent transfer of the phosphoryl group to CheY, the fourth member of the cascade. Phospho-CheY acts as a mobile regulator protein and interacts with flagellar motor proteins, where it induces a sudden switch in flagellar rotation direction, thus triggering a 'tumble' or change in swimming direction. Conversely, CheY in its non-phosphorylated state allows the flagellar motor to continue to rotate in the same direction so the cell swims smoothly and 'tumbles' less frequently. This abbreviated view of chemotaxis is provided as a context for the later sections on cyanobacterial phototaxis. The reader is directed to recent reviews that cover specific aspects of chemotaxis (Stock *et al*., 2000; Bourret and Stock, 2002; Webre *et al*., 2003).

Mechanisms of motility

Flagella-based motility of enteric bacteria in liquid is very well studied. However, flagellar-independent 'twitching motility', which allows for translocation of bacteria over moist surfaces, is likely to be an important characteristic

of unicellular planktonic and filamentous cyanobacteria and has only recently begun to receive attention.

Cyanobacterial cell appendages

Flagella have never been detected in cyanobacteria, but electron micrographs of many unicellular cyanobacterial species have revealed the presence of a thick mesh of appendages surrounding cells (Vaara, 1982). Careful inspection of the cell surface of *Synechocystis* (a coccoidal cell with a diameter of ≈1–2 μm) indicates that there are at least two pilus morphotypes. The cell is uniformly covered with a layer of thin, brush-like pili, which have an average diameter of 3–4 nm and a length of ≈1 μm. Cells also have thick flexible pili (average diameter 6–8 nm) that extend several cell lengths (≈ 4–5 μm) and often make connections between adjacent cells (Bhaya *et al*., 2000). It has not been established whether these thick pili are located only at specific positions at the cell surface; the number of pili per cell is also unknown.

Type IV pilus (TFP)-based motility

Slow surface-dependent motility (referred to as 'twitching' or 'gliding') over a variety of hydrated surfaces has been observed in many Gram-negative bacteria such as *Pseudomonas aeruginosa* and the myxobacterium *Myxococcus xanthus*. In these rod-shaped cells, the polarly located TFP are required for the movement of single cells as well as for the co-ordinated group movement of cell populations, known as 'social motility' in *M. xanthus* (Wall and Kaiser, 1999; Mattick, 2002). TFP are typically 5–7 nm in diameter, often several micrometres in length and are composed primarily of a single protein subunit termed pilin or PilA. Genes for pilin and pilus biogenesis have been identified in the genomes of many Gram-negative bacteria and, more recently, in archaea and on plasmids, raising the possibility of lateral gene transfer (Mattick, 2002; Averhoff and Friedrich, 2003; Gophna *et al*., 2003). Recent explorations of bacterial behaviour in the environment are producing increasing evidence that TFP are common and important multifunctional appendages on the surface of many bacteria. Apart from their role in surface-dependent motility, TFP are required for DNA transformation, host cell receptor adhesion in bacterial pathogens such as *P. aeruginosa*, *Neisseria meningitidis* and *Neisseria gonorrhoeae*, and have also been shown to be critical for biofilm formation. The reader is referred to reviews and papers that describe the multiple roles of TFP in biological processes, as this is not directly relevant to the current review (McBride, 2001; Mattick, 2002; Shi and Sun, 2002; Winther-Larsen and Koomey, 2002; Bardy *et al*., 2003; Harshey, 2003).

The role of type IV pili in motility/phototaxis

The first hint that surface appendages similar to TFP might play a role in cyanobacterial motility came from analysis of a *pilA1* mutant. In phototaxis assays carried out on soft agar motility plates, wild-type cells move towards a white light source whereas *pilA1* mutants are completely non-motile (Fig. 1). The *pilA1* gene encodes a putative pilin-like polypeptide with 21% identity (34% similarity) to pilin from *M. xanthus*, the major structural component of TFP. Pilins are typically synthesized as precursor polypeptides, and the mature N-terminus is the only highly conserved region between different PilA polypeptides (Mattick, 2002). Electron micrographs of the *pilA1* mutant demonstrated that they lacked the thick pilus morphotype, but had a normal complement of thin pili (Bhaya *et al*., 1999). This suggested that phototaxis and motility might be mediated by TFP-like appendages in S*ynechocystis*.

There are a total of six *pilA*-like genes on the *Synechocystis* genome, yet inactivation of all the other five *pilA*-like genes (*pilA2–pilA6*) had no obvious effect on motility (Yoshihara *et al*., 2000; 2001). It is not yet known whether these genes are expressed, but some of these genes might encode pilin-like proteins (or 'pseudopilins') that might be involved in other processes such as DNA transformation (Yoshihara *et al*., 2001) or type II secretion,

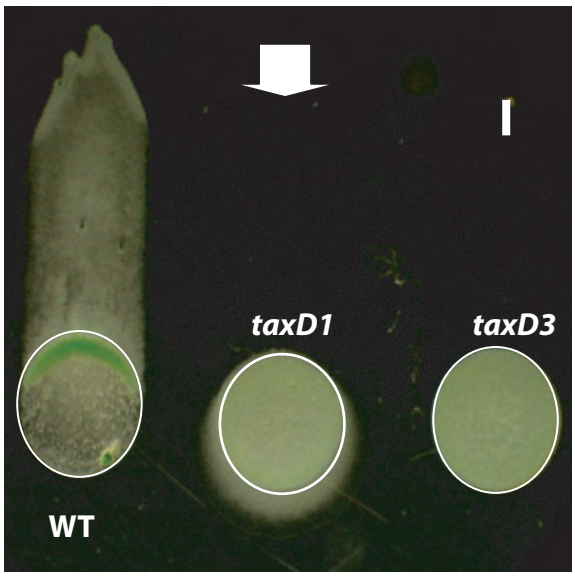

Fig. 1. Directional motility assay. Cells were spotted (indicated by white circle) on soft agar (0.4% agarose in BG-11 medium) and subjected to directional white light (arrow) for 16 h at a fluence of 25μmol m^{-2} s^{-1}. Typical examples of cells exhibiting positive phototaxis (wild type), negative phototaxis (*taxD1* mutants) or lack of motility (*taxD3* mutants) are shown. Motile wild-type cells have moved about 10 mm towards the light source; *taxD1* mutant cells have lost positive phototaxis and exhibit weak negative phototaxis. Bar represents 1.5 mm. Time lapse movies of positive phototaxis can be viewed at http://carnegiedpb.stanford.edu/research/research_bhaya.php.

as components of the TFP biogenesis machinery share similarity and functionality with polypeptides involved in type II secretion (Sauvonnet *et al*., 2000).

Further confirmation of the role of TFP in phototactic motility was obtained when it was shown that mutations in genes encoding the pilus biogenesis apparatus, such as *pilQ*, encoding the secretin pore, *pilD*, encoding the membrane-bound signal peptidase, and *pilC*, *pilM*, *pilN* and *pilO* (required for pilus biogenesis/assembly), were all non-motile and also lacked thick pili (i.e. TFP) at the cell surface (Bhaya *et al*., 2000; Yoshihara *et al*., 2001). Interestingly, *pilC* and *pilD* mutants that are defective in TFP biogenesis also lack thin pili at the surface. The structure and role of the thin pili in phototaxis remains completely unknown; yet, given the mesh-like nature of the thin pili, it is tempting to speculate that they might play a role in maintaining cell–cell contact, known to be important for surface-dependent motility. Several of these mutants were also defective in DNA transformation, indicating a strong link between TFP and DNA transformation (Yoshihara *et al*., 2000).

Motility motors PilT and PilB

It is suggested that the polar TFP move the cell body along a surface by a reiterative process of pilus extension, adhesion and retraction. Cells attach to a surface after pilus extension; subsequently, when TFP retract, the cell body is pulled along, somewhat analogous to a climber using a grappling hook to move along a rock surface (Merz and Forest, 2002). Recently, it was shown that, when the TFP retract, they generate substantial force, upwards of 100 pN, and directly mediate cell movement (Merz *et al*., 2000; Maier *et al*., 2002). Pili might attach non-specifically to solid surfaces or, in some cases, to polysaccharide fibrils extending from the surface of neighbouring cells, as in *M. xanthus* (Li *et al*., 2003). Thus, when *M. xanthus* cells glide along in groups, they appear to be in rafts with cells arranged end to end. Two highly conserved motor proteins, PilB and PilT, are required for the extension and retraction of TFP respectively. Both PilT and PilB have conserved ATPase domains (Walker box A and B) and are likely to be located on the inner membrane (Okamoto and Ohmori, 2002).

Interestingly, *Synechocystis* has two *pilT*- and two *pilB*-like genes; inactivation of the *pilB1* gene results in a non-motile phenotype, as expected, whereas *pilB2* mutants retain motility (Yoshihara *et al*., 2001). The products of the two *pilT*-like genes, PilT1 and PilT2, are 40% identical to each other at the amino acid level; PilT2 is distinguished by a proline-rich N-terminus extension. Inactivation of these genes leads to distinct phenotypes (Bhaya *et al*., 2000). The *pilT1* mutant is non-motile and strongly hyperpiliated, consistent with the role of PilT in TFP retraction. *pilA1* mRNA levels are also increased eightfold in the *pilT1* mutant (but not in the *pilT2* mutant), suggesting that pilin expression might be under the control of a feedback loop that has still to be defined. Interestingly, *pilT2* mutant cells are still motile but move away from a directional light source, although this negative phototaxis is not as pronounced as the positive phototaxis of wild-type cells. This might imply that PilT2 somehow confers directionality to the motility apparatus (and is thus required for positive phototaxis) whereas PilT1 is necessary for all forms of motility. Apparently, under most light conditions examined so far, positive phototaxis is the dominant response; only when this response is ablated does one observe negative phototaxis in cells.

Photoperception and its link to motility

The fact that *Synechocystis* can exhibit both negative and positive phototaxis (Fig. 1) implies that the particular light environment can play a critical role in how and where the cell moves. Thus, the action spectra for phototaxis might provide information about possible photoreceptors for both positive and negative phototaxis.

Action spectra and energy requirements for phototaxis

In *Synechocystis*, the threshold fluence rate required to elicit phototaxis is as low as 0.001 μmol m^{-2} s^{-1}, and the response saturates at about 1 μmol m^{-2} s^{-1} and cells appear to move in response to light direction rather than to a gradient in the fluence rate (Choi *et al*., 1999; Ng *et al*., 2003). Action spectra for phototaxis are quite complex and vary significantly, depending on both the cyanobacterial species being examined as well as the fluence rate of the light being used. In most cases, the action spectrum for phototaxis overlaps with the absorption spectra of phycobilins and chlorophyll, suggesting that phycobilin and/or chlorophyll-like pigments might play a role in photoperception. Notably, inhibitors of photosynthesis do not appear to have a profound effect on phototactic orientation or on gliding speed, suggesting that energy derived from photosynthesis is not directly required for phototaxis (Nultsch, 1975; Choi *et al*., 1999). An equal-quantum action spectrum for phototaxis in *Thermosynechococcus elongatus*, a thermophilic unicellular cyanobacterium, showed several obvious action peaks at 530, 570, 640 and 680 nm at a fluence rate of 10 μmol m^{-2} s^{-1} but, at higher fluence rates, the red action peaks (at 640 and 680 nm) disappeared and far-red action peaks (at 720 and 740 nm) appeared. Cells illuminated simultaneously with red and far-red light showed a drastic reduction in phototaxis, indicating the possible involvement of phytochrome (Kondou *et al*., 2002). Similar action spectra were also reported for *Synechocystis*, although there

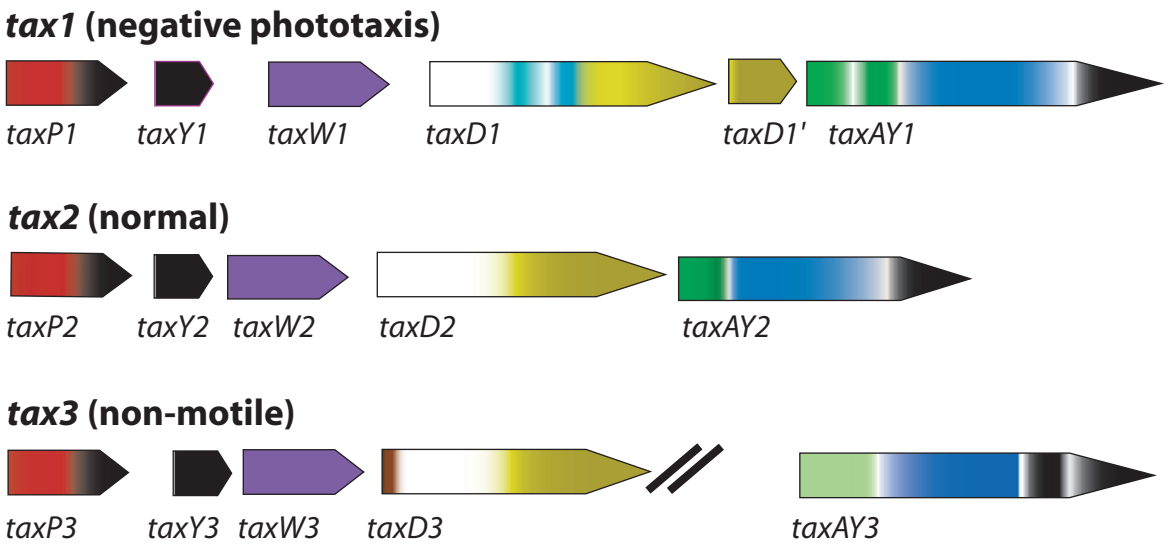

Fig. 2. The three *tax* loci (*tax1*, *tax2* and *tax3*) of *Synechocystis* showing gene arrangements. The phenotype of the mutants in each of these loci is shown in parentheses. Genes shown are *taxP1* (sll0038), *taxP2* (sll1291), *taxP3* (slr1041) (red); *taxY1* (sll0039), *taxY2* (sll1292), *taxY3* (slr1042) (black); and *taxW1* (sll0040), *taxW2* (sll1293), *taxW3* (slr1043) (purple). The genes encoding putative receptor proteins *taxD1* (sll0041), *taxD2* (sll1294), and *taxD3* (slr1044) and *taxD1′* (sll0042) are shown with the conserved C-terminus signalling domain (yellow); the two GAF domains (light blue) of *taxD1* and the TPR domain (brown) of *taxD3*. The genes for the multidomain histidine kinases [*taxAY1* (sll0043), *taxAY2* (sll1296) and *taxAY3* (slr0322)] are shown with the common kinase domain (dark blue), HPt domains (green) and the N-terminus domain of *taxAY3*, which has low similarity to the HPt domains of *taxAY1* and *taxAY2* and is shown in light green. The 3′ ends of *taxAY1*, *taxAY2*, *taxAY3*, *taxP1*, *taxP2* and *taxP3* genes encode potential CheY-like domains (black). The double lines in the *tax3* locus between *taxD3* and *taxAY3* indicate that these genes are not physically contiguous on the genome. Cyanobase gene identifiers are shown in parentheses after each gene name.

were some striking differences such as the strong negative phototaxis exhibited by *Synechocystis* illuminated with UV-A light (360 nm), whereas negative phototaxis was never observed in *T. elongatus* (Choi *et al*., 1999; Ng *et al*., 2003). Interestingly, thermophilic *Synechococcus* isolates from microbial mats exhibit both negative and positive phototaxis, and it is suggested that this might reflect strain-specific preferences for different light conditions, although the photoreceptors for these movements have not been identified (Ramsing *et al*., 1997). In summary, these results suggest that multiple photoreceptors are involved in phototactic responses, and that these photoreceptors may have different spectral properties, depending on the light quality/intensity to which the organism has adapted.

Screens for aberrant motility mutants

To identify potential photoreceptors and components of the signal transduction system, one can screen for mutants that exhibit aberrant phototactic responses (Fig. 1). Results from such a screen of *Synechocystis* yielded mostly non-motile mutants (Bhaya *et al*., 2001a; Chung *et al*., 2001). However, about 10% of the mutants exhibited constitutive negative phototaxis; the loss of the positive phototaxis response in these mutants suggested that they might have lesions in the photoreceptor for positive phototaxis or in components of the signal transduction cascade (Bhaya *et al*., 2001b). The lesions in several of the negatively phototactic mutants mapped to a locus that contained a cluster of genes arranged in a putative operon (Fig. 2). The genes in this locus have similarity to the well-studied chemotaxis genes of *E. coli* (described above). The *tax1* locus (named for its similarity to the *che* genes, yet controlling phototaxis rather than chemotaxis) contains at least six genes [*taxP1*, *taxY1*, *taxW1*, *taxD1*, *taxD1′* (a truncated version of *taxD1*) and *taxAY1*] (Bhaya *et al*., 2001b; Yoshihara *et al*., 2000; 2002).

A novel photoreceptor for phototaxis

The C-terminus of TaxD1 (PixJ1) shows strong similarity to the conserved C-terminus signalling domain (PFAM identifier PF00015) of enteric MCPs, although the N-terminus domain contains two GAF domains (PFAM identifier PF01590). GAF domains are part of a large superfamily of proteins that include the PAS/PAC and LOV domains, which bind diverse ligands including small molecules such as tetrapyrroles, flavins, flavinoids and nucleotide cofactors (Crosson *et al*., 2003; Vreede *et al*., 2003). The GAF and PAS domains typically occur in multiple copies in the extended phytochrome family, which are major red/far-red light photoreceptors in plants and control numerous light-regulated activities such as flowering,

seed germination and circadian rhythms (Pepper, 1998; Montgomery and Lagarias, 2002). This raises the exciting possibility of TaxD1 being the first identified photoreceptor for cyanobacterial phototaxis, although the exact nature of the ligands that bind the GAF domains is not yet known. There are several additional lines of indirect evidence that corroborate the role of TaxD1 as a photoreceptor for positive phototaxis. Action spectra for phototaxis performed in *Synechocystis* and in the thermophilic cyanobacterium *T. elongatus* indicate that positive phototaxis is a red light-controlled phenomenon (although there are significant action peaks in other regions of the spectra), and movement of cells appears to be inhibited if red light-illuminated cells are simultaneously irradiated with far-red light (Kondou *et al*., 2002; Ng *et al*., 2003). These findings are strongly reminiscent of the classic red/far-red reversible phenomenon that is a hallmark of phytochrome-mediated effects in vascular plants. Furthermore, bona fide phytochrome-like proteins have been identified in cyanobacteria, and there is evidence that they might play a role in light-mediated processes including phototaxis and chromatic adaptation (Kehoe and Grossman, 1996; Hughes and Lamparter, 1999; Wilde *et al*., 2002; Iwasaki and Kondo, 2003; Terauchi *et al*., 2004).

It has been shown recently that the TaxD1 homologue in *T. elongatus* is localized at the poles in this rod-shaped cyanobacterium, suggesting that it might be involved in sensing light direction (Kondou *et al*., 2002). In *E. coli*, most of the chemoreceptor complexes are clustered at the poles (the 'nose' or 'nanobrain') of the cell, while the flagella are arranged peritrichously (Maddock and Shapiro, 1993; Gestwicki *et al*., 2000; Webre *et al*., 2003). So far, the physical proximity of TFP and receptor systems has not been demonstrated in any system. The arrangement of these multiprotein complexes also raises important questions regarding the role of the other components of the *tax1* locus. The role of CheY as the mobile regulator of flagellar movement is critical and well understood in enteric bacteria, but whether TaxY1 plays a similar role, allowing for signal transduction between the histidine kinase TaxAY1 and the pilus motor proteins, is one of the major unknowns of pilus-mediated motility.

A comparison of the tax *loci*

In *Synechocystis*, there are two other loci (*tax2* and *tax3*) that contain *tax*-like genes in addition to the *tax1* locus (Fig. 2). A comparison of these loci shows that there is strong similarity at both the level of gene arrangement as well as the similarity of the encoded polypeptides, perhaps indicative of relatively recent duplication events (Bhaya *et al*., 2001b; Wuichet and Zhulin, 2003). All three histidine kinases (TaxAY1, TaxAY2 and TaxAY3) can be classified as hybrid sensor histidine kinases as they have a fused CheY-like regulator domain at the C-terminus (TaxAY3 has two tandemly arranged CheY domains at the C-terminus). TaxAY1 and TaxAY2 are class II kinases with canonical histidine phosphotransfer (HPt) domains at the N-terminus (TaxAY1 appears to have two tandemly arranged HPt domains) whereas TaxAY3 does not have a well-conserved HPt domain. There is also evidence that a polypeptide (Cyanobase identifier slr0073) that consists of a single HPt domain might play a role in motility as inactivation of this gene causes a non-motile phenotype (Yoshihara *et al*., 2000). Hybrid kinases and class II kinases are not as well characterized as other classic histidine kinases, but HPt domains and attached CheY-like domains might be the hallmark of multistep His $\rightarrow$ Asp phosphorelay signals in which several inputs can be integrated into the signalling pathway (Mizuno and Matsubara, 2003). In *E. coli*, the signal from all chemoreceptors is apparently integrated through CheA and CheY. However, in *Synechocystis*, the three *tax* loci each contain all the components of a chemotaxis signal transduction cascade (i.e. signalling chemoreceptor, histidine kinase, an adaptor and a mobile regulator), except for the *tax3* locus in which the partner histidine kinase is not in physical proximity (Fig. 2). It remains to be established whether there is cross-talk or interaction between these pathways, or whether they all act independently. It is also intriguing that, at the start of all three *tax* loci, there is a highly conserved gene (*taxP1*, *taxP2* and *taxP3* respectively) that appears to be part of a small gene family and has a conserved CheY-like domain at the C-terminus (Fig. 2). Whether this putative regulatory element plays any role in the function and regulation of the *tax* loci is still unknown.

Recent genome sequencing projects have revealed that bacteria often contain a bewildering variety of chemotaxis-like genes. For instance, the photosynthetic bacterium *Rhodobacter sphaeroides* has 13 chemoreceptors as well as several *cheA*, *cheY* and *cheW* genes; inactivation of specific genes does not result in an obvious defect, and the inputs sensed by chemoreceptors remain largely unknown for most bacteria other than *E. coli* (Armitage, 1999; Porter and Armitage, 2002). Thus, one of the attractive features of *Synechocystis* is its relatively small number of chemotaxis-like genes and their critical connection to TFP to regulate phototactic responses. In an organism such as *Synechocystis* where there are multiple kinases/receptors and potential interacting partners, the assembly process might be interlinked and is a rich, untapped source of information about the formation of multiprotein complexes and the maintenance of interactions among complexes. Indeed, we are just beginning to understand more about the circuitry, assembly and role of clustering of the chemoreceptor complexes (Bourret and Stock, 2002; Shapiro *et al*., 2002; Webre *et al*., 2003).

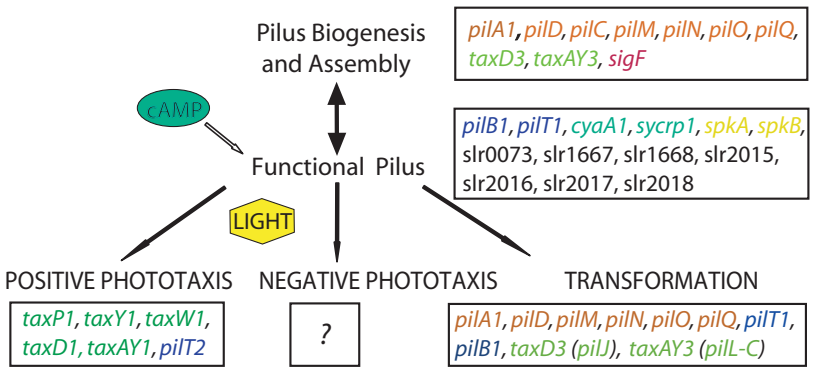

Fig. 3. Interaction between pilus biogenesis/assembly, phototaxis and DNA transformation. The genes involved in these processes are in the boxes alongside. Genes that may be related by function or are in the same locus are shown in the same colour (genes that have only a Cyanobase identifier are shown in black). Pilus biogenesis and function have been depicted as separate processes because there are proteins required for the formation of a functional pilus that are not part of pilus structure, nor are they required for biogenesis. Yet, these processes might be linked (as shown by the bidirectional arrow). The possible role of cAMP in the formation of a functional pilus, and of light in both positive and negative phototaxis, is shown. So far, no genes have been identified that specifically affect negative phototaxis. See text for further details and Yoshihara *et al.* (2001) for genes required for transformation.

Novel players in phototaxis and signal transduction

Analysis of motility mutants identified lesions in several novel genes, indicating that phototaxis and pilus biogenesis might be complex and tightly regulated processes, and some of these are discussed below (Fig. 3).

tax3 *locus*

Transposon mutagenesis identified a chemoreceptor-like protein (encoded by *taxD3/ctr1/pilJ*) and a CheA-like histidine kinase (encoded by *taxAY3*) that were critical for cell motility. Importantly, electron micrographs of both *taxAY3* and *taxD3* mutants showed a complete loss of TFP, although thin pili were still present at normal levels (Bhaya *et al.*, 2001b; Chung *et al.*, 2001; Yoshihara *et al.*, 2002). The similarity in the phenotypes of the *taxD3* and *taxAY3* mutants suggests that they might function in the same signal transduction pathway, although they are not adjacent to each other on the genome, and interactions between TaxAY3 and TaxD3 have not been verified experimentally (Fig. 2) (Bhaya *et al.*, 2001b; Yoshihara *et al.*, 2002). The N-terminus of TaxD3 does not have an obvious well-conserved sensing domain, but does have a tetratricopeptide (TPR) domain at the N-terminus. TPR domains are present in proteins that are part of multiprotein complexes (see below). The *tax2* locus, which contains all the components of a chemotaxis-like system, does not appear to be involved in motility (Yoshihara *et al.*, 2001).

Tetratricopeptide repeat (TPR) domain proteins and pentapeptide repeat proteins

Several mutants aberrant for motility were found to have lesions in genes encoding potential coiled-coil proteins or containing TPR domains, raising the possibility that pilus apparatus and the photosensing apparatus might be located in a multiprotein complex (Bhaya *et al.*, 2001a). TPR domains (PFAM identifier PF00515) are found in diverse organisms and are involved in a variety of protein–protein interactions. In many instances, several TPR domain-containing proteins aggregate to form multiprotein complexes, and it has been suggested that the TPR motif might represent an ancient protein–protein interaction module that has been recruited by different proteins and adapted for specific functions (Blatch and Lassle, 1999).

Three non-motile mutants (Cyanobase identifier sll0183, sll0414 and sll0301) mapped to novel proteins that had only one domain in common, the tandem pentapeptide repeat domain (Bhaya *et al.*, 2001a). It has been noted that, although this domain (PFAM identifier PF00805) is found in diverse groups of bacteria and plants, it is most common in cyanobacteria (Bateman *et al.*, 1998). Based on the prediction that the tandem repeats might form a right-handed beta-helical structure, it is suggested that pentapeptide repeat proteins might have a targeting or structural function rather than enzymatic activity. It will be interesting to decipher the role of these proteins in pilus assembly and motility. In this context, it is interesting to note that a mutant in the alternative sigma factor (SigF) caused a non-motile phenotype but also caused other pleiotropic effects such as the loss of thin pili and release of specific cell surface-associated proteins and pigments into the medium (Bhaya *et al.*, 1999). It is possible that, in the *sigF* mutant, the cell wall or periplasmic components are incorrectly/incompletely assembled and, as a consequence, pilus biogenesis is adversely affected.

cAMP is a player in motility

Inactivation of *cya1*, encoding an adenylyl cyclase, or *sycrp1*, encoding a putative cAMP receptor protein, resulted in a non-motile phenotype. However, both

mutants still retained TFP, suggesting that neither pilus biogenesis or structure had been grossly affected (Terauchi and Ohmori, 1999; Yoshimura *et al*., 2002a,b). Externally added cAMP restored motility to *cya1* mutants. In microarray experiments performed with the *sycrp1* mutant of *Synechocystis*, a small set of genes showed a strong reduction in expression, which included the two genes slr1667 and slr1668 and five other genes arranged in a putative operon (Cyanobase identifier slr2015 to slr2019). Mutants in the slr2015 gene cluster were identified independently as non-motile mutants (Bhaya *et al*., 2001a). Of these genes, most appear to be unique (slr1668 has some similarity to a type1 pilus chaperone, whereas slr2019 is similar to an ABC transporter), and further investigation into their role in pilus biogenesis and function will be very revealing. Sycrp1 binds to a region upstream of slr1667 that contains an *E. coli* CRP-binding consensus sequence (5′-TGTGA-N6-TCACA-3′) (Yoshimura *et al*., 2002a). Taken together, these results might suggest that cAMP is an important player in the signal transduction for motility (Fig. 3). So far, it is not clear whether cAMP interacts with the gene products of the Tax loci or whether it controls motility through an independent pathway mediated by Sycrp1 and/or through regulation of genes such as slr1667/slr1668 and the slr2015 gene cluster. Interestingly, twitching motility in *P. aeruginosa* has been shown recently to be controlled by the Vfr protein, which is a CRP homologue that can bind both cAMP and cGMP (Beatson *et al*., 2002), and photosensing in the protist *Euglena* is mediated by a photoactivable adenylyl cyclase (Ntefidou *et al*., 2003). Although it is unclear whether there are any significant parallels between these systems and signal transduction in cyanobacteria, it does raise some interesting ideas about common elements between prokaryotic and eukaryotic signalling pathways and connects both motility and light-mediated processes to cAMP levels.

The possible role of eukaryotic-like serine-threonine kinases

Recently, two putative serine-threonine kinase mutants, designated *spkA* and *spkB*, have been shown to lack motility completely (Bhaya *et al*., 2001a; Kamei *et al*., 2001; 2003). Serine-threonine kinases were initially thought to be present exclusively in eukaryotes, but are now known to be found in many bacteria where they might function in complex signalling pathways. These findings underscore the notion that prokaryotic signalling pathways may be more complex than originally envisaged.

Concluding remarks

TFP-mediated motility and signal transduction in cyanobacteria have been studied for only the last few years, and many interesting advances are likely to be made in the near future (Armitage and Hellingwerf, 2003). In particular, the questions that are of general interest and can be addressed to advantage in cyanobacteria are: How is signal transduction linked to the pilus apparatus to cause directional movement? In enteric bacteria the chemotaxis sensing and transducing system interacts with the flagellar motor via CheY. Whether this paradigm holds for the interaction between the Tax components and the TFP is unknown and is an issue of fundamental importance. A related question of equal importance is: How are different signals integrated into the phototaxis response? So far, we have deciphered the role of TFP and gene products of the *tax* loci in positive phototactic responses. Yet, we do not know how the cell discerns the optimal light conditions for photosynthesis and transduces this information to the motility apparatus; nor do we know what triggers negative phototaxis. Cyanobacteria may also move in response to nutrient gradients or other signals in the environment and, so far, these parameters have not been explored. This might be particularly important to study as cyanobacteria participate in complex systems such as microbial mats and bacterial biofilms where interaction, communication and the close proximity of various bacteria are critical for the development of the entire system.

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As space constraints severely limited the number of original papers I was able to cite, I have included several recent review articles that cover a particular area, and I apologize to authors whose work has not been cited directly. I would like to thank Arthur Grossman and members of the laboratory for their careful comments on the manuscript. This work was supported by NSF funding (MCB 0110544).

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