Cyanobacteria in motion

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Highlights

- Cyanobacteria use type IV pili to move directly towards a light source
- Spherical cyanobacteria are micro-lenses which focus light to sense light direction
- *Synechocystis* sp. PCC 6803 can integrate multiple stimuli to regulate its phototaxis response
- Phototaxis is relevant in phototrophic biofilms

Abstract

Cyanobacteria are able to move directly towards or away from a light source, a process called phototaxis. Recent studies have revealed that the spherical unicellular cyanobacterium *Synechocystis* sp. PCC 6803 exhibits a cell polarity in response to unidirectional illumination and that micro-optic properties of cyanobacterial cells are the basis of their directional light sensing. Further functional and physiological studies highlight a very complex control of cyanobacterial phototaxis by sensory proteins, histidine kinases and response regulators. Notably, PATAN domain response regulators appear to participate in directional control of phototaxis in the cyanobacterium *Synechocystis* sp. PCC 6803. In this review we explain the problem of directional light

sensing at the small scale of bacteria and discuss our current understanding of signal transduction in cyanobacterial phototaxis.

Introduction

Many prokaryotes have more complex lifestyles than would be suspected from observation under standard laboratory conditions, as the predominant form of bacterial and archaeal life in nature is not a planktonic culture. Prokaryotic cells can live in a single or multi-species biofilm, can become motile to reach better conditions or can enter a starvation program to survive under harsh conditions. Mechanisms which control these lifestyle decisions have been studied in great detail in some model bacteria. Since the advent of molecular techniques, cyanobacteria have been mainly studied as model systems for plant-like photosynthesis or carbon and nitrogen fixation. Although motility in cyanobacteria was discovered in the 19th century [1], with excellent further studies in the 1960-1970s [2,3] to reveal fundamental mechanisms of light-driven motility, our current understanding of phototaxis remains far from the detailed understanding of the chemotaxis system in flagellated bacteria. However, recent research has revealed how cyanobacteria sense the direction of light and which regulators might be involved in control of their phototactic behavior.

How do cyanobacteria move in response to a light signal?

Cyanobacteria do not assemble flagella. Instead they use different motility mechanisms including type IV pili and possibly also surface proteins and slime extrusion [4]. The unicellular coccoid cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*), a model organism for photosynthesis studies and biotechnological applications, has been shown to use type IV pili for twitching movement on surfaces similar to the motility described for *Pseudomonas* and *Myxococcus* [5]. Many filamentous cyanobacteria are also motile. It was hypothesized that a polysaccharide secretion system, the so-called junctional pore complex, extrudes slime directionally, thereby pushing the cells forward [6]. However, in the filamentous strain *Nostoc punctiforme*, the junctional pore complex of the differentiated motile filaments known as hormogonia was recently shown to be partially composed of type IV pilus structures, and polysaccharide secretion does not provide the directional motive force [7,8]. Instead, a type IV pilus-like nanomotor is suggested to drive motility in filamentous cyanobacteria, commensurate with unicellular strains. Cyanobacterial motility is partially controlled by light, and several genes that are important for phototaxis have been identified. These encode homologs of the chemotaxis (Che) signal transduction pathway, and photoreceptors implicated in directional light sensing [9,10].

Chemotactic bacteria typically show a biased random walk, with changes in the direction of flagella rotation leading to switching between a running mode and tumbling with random reorientation to a new direction [11]. For a biased movement within a chemical gradient, cells have to detect concentration changes. However, bacterial cells are too small for direct detection of spatial differences in the concentration of an

attractant or repellent. Instead they change the sensitivity of their chemoreceptors to external stimuli during movement, thereby measuring temporal changes in the concentration of a substance. This memory mechanism works close to the physical limits of sensing [12]. Phototaxis in *Synechocystis* and some filamentous cyanobacteria appears fundamentally different, because cells do not exhibit a biased random walk: rather, they can move directly towards a light source [2,13].

Model for regulation of phototaxis in cyanobacteria

We recently proposed a model whereby spherical *Synechocystis* cells exploit their micro-optic properties to sense light direction [13] (Fig. 1). Essentially the cell acts as a microscopic eyeball. Light impacting the front side is focused at the distal side of the cell. We showed that this light spot is at least 4 times brighter than the incoming light intensity. For discussion of the micro-optical properties of bacteria, see Box 1.

The sharp focal point on the cell surface is most probably sensed by photoreceptors, which then transduce the signal to downstream regulators with homology to chemotaxis proteins. The link between these regulators and the motility apparatus remains uncertain, but it appears that localized signal transduction pathways cause the cells to move away from the focused light spot at the distal side, thereby moving towards the external light source. In *Synechocystis* there are at least six CheY-like response regulators fused to so-called PATAN domains [14]. Five of these were shown to be involved in regulation of motility or are part of a gene cluster encoding gene products with similarity to chemotaxis proteins. Three of these clusters include a known photoreceptor protein and are involved in directional control (Fig. 2). PixJ1 is a blue/green sensing cyanobacteriochrome fused to a methyl accepting chemotaxis protein (MCP) [15], PixD is a BLUF (blue light sensor using FAD) photoreceptor [16] and UirS is a UV-A light sensor [17]. What is the evidence that these proteins are involved in directional light sensing? Inactivation of the receptor protein always leads to a 180° change in the direction of movement, meaning that the cells have reversed the orientation of phototaxis but not lost their ability for directional light-sensing. Wild-type cells move towards low-intensity far-red, red, green and also white light, whereas UV light at about 50 µmol photons m^{-2} s^{-1} induces negative phototaxis. Consequently, *pixJ1* [18] and *pixD* [16] mutants move away from light qualities that induce positive phototaxis, whereas a *uirS* mutant moves towards a UV-light source [17,19]. Interestingly, overexpression of the PATAN domain response regulator LsiR (encoded in the *uir* gene cluster) leads to negative phototaxis also under red light [17]. This implies that the PATAN-domain regulators control local assembly of type IV pili in response to photoreceptor activation. The PATAN domain was named after the PatA protein, which is involved in spatial heterocyst organization in filamentous cyanobacteria [20]. PATAN domains are not confined to cyanobacteria and were shown to regulate gliding motility in *Myxococcus xanthus* [21]. In our model we suggest that these response regulators interact with the type IV pilus machinery in response to light, thereby inactivating or activating pilus assembly on one side of the cell. We hypothesize

that these response regulators act through a common target and constitute the fundamental pathway for phototactic orientation in *Synechocystis*. While the possible target remains unidentified, the ultimate outcome of locally-activated PATAN-response regulators is most likely the spatial organization of the motor ATPases. The pilus extension ATPase PilB1 is known to localize in a crescent shape at the leading edge of motile *Synechocystis* cells [22] and we suppose that the retraction ATPases PilT1/2 may also relocalize.

Our knowledge of the signal transduction components involved in cyanobacterial phototaxis comes largely from null mutant phenotypes. Loss of a particular photosensor or signal transduction component could affect phototaxis in at least two distinct ways, which are not easy to distinguish experimentally. The signal transduction component could be responsible for transmitting information for directional light sensing. Alternatively, it could be helping to control the expression of other components that are more directly involved in phototaxis. This leaves open the possibility that the known photosensors and their associated signal transducers might merely be involved in tuning the phototactic response through regulation of gene expression, as suggested by Sugimoto et al. [23]. Directional light signaling would then involve something else, possibly localized signals arising from the photosynthetic apparatus. In our view, the photoreceptors PixJ1, UirS and PixD remain the best candidates for directional light sensing, but to test their roles we need more information on their sub-cellular localization and interactions. A true directional light sensor should be rather evenly distributed around the cell perimeter, and it should initiate a post-translational signal transduction pathway that directly regulates the motility apparatus [13].

Complex decisions and cross-talk between light sensing and other environmental signals

Even from our limited knowledge of motility control in *Synechocystis,* it is clear that the cells make complex decisions, influenced by multiple sensory inputs on different timescales. For example, light-dependent synthesis of cyclic nucleotide second messengers plays an important role in tuning cyanobacterial phototaxis. In different cyanobacterial species, the intracellular cAMP content is regulated in a light-dependent manner [24–27]. Synthesis of cAMP by the adenylate cyclase Cya1 and binding by the cAMP receptor protein Sycrp1 are crucial for motility at the biofilm level in *Synechocystis* [28]. Although the exact mechanism is unknown it seems that Sycrp1 is a transcriptional activator of minor pilins and cell surface proteins that could control cell-cell interactions during phototaxis.

In many bacteria, the transition between motile and sessile lifestyles is regulated by the second messenger c-di-GMP. The hybrid photoreceptor Cph2 is a key signaling system that regulates this behavioral switch in *Synechocystis*. It comprises an N-terminal red/far-red interconverting phytochrome fused to a c-di-GMP degrading EAL domain

and a green- and blue-absorbing C-terminal cyanobacteriochrome linked to a GGDEF domain [29,30]. Excessive blue light illumination activates c-di-GMP synthesis by the GGDEF domain, leading to elevated c-di-GMP levels that inhibit motility through an unknown signal transduction pathway [30]. The co-occurrence of many cyanobacterial signaling proteins consisting of various combinations of phytochrome, GGDEF and EAL domains indicates that c-di-GMP constitutes an important signaling pathway in cyanobacteria to adapt to changing environmental factors, especially light quality [31].

The modulation of *Synechocystis* phototaxis by ethylene provides an example of a chemical sensory input into motility control. Many cyanobacteria live in biofilms together with other microorganisms or even enter into symbiotic interactions with fungi, protists or plants. This coexistence means that cyanobacterial cells are exposed to signals released by different organisms and recent research indicates that certain chemical cues also influence phototaxis. Ethylene is a phytohormone that is synthesized in response to various biotic and abiotic stresses and plays various roles in plant development [32]. But ethylene is also synthesized by some microorganisms and therefore can act as an universal signal in plant-bacterial communities [33]. In addition, ethylene is also produced photochemically from organics in aquatic environments. Recently it was shown that externally applied ethylene accelerates motility in *Synechocystis* phototaxis [34]. Ethylene is sensed by the same receptor protein UirS that senses UV-A light (Fig. 2). The protein is therefore also called SynETR1 (for *Synechocystis* Ethylene response 1) [35]. It consists of an N-terminal ethylene-binding domain followed by an UV-A absorbing cyanobacteriochrome domain and finally a C-terminal histidine kinase domain. Upon UV-A irradiation, autophosphorylation of the histidine kinase of UirS is enhanced and phosphate is transferred to the response regulator UirR. Phosphorylated UirR binds to the promoter of the downstream transcriptional unit consisting of the putative sRNA gene *csiR1* and the PATAN domain response regulator LsiR and activates transcription [36]. Ethylene seems to inactivate UirS/SynETR1, thereby promoting positive phototaxis, because mutants lacking the ethylene binding domain show accelerated movement.

Future studies are likely to reveal still further complexity in sensory information processing for control of motility in *Synechocystis.* For example, the phototactic photoreceptor PixJ1, shows strong similarity to the MCP domain of chemoreceptors and is encoded in the *tax1* operon together with associated signal transduction components [37]. But the *Synechocystis* genome also includes the *tax2* and *tax3* operons, which are similar to *tax1* except that the equivalents of PixJ1 are MCP-like proteins that lack the photoreceptor domain [38]. The stimuli detected by the proteins encoded in the *tax2* and *tax3* operons are unknown, but they are likely to feed further chemical or mechanical signals into the control of motility. The way in which *Synechocystis* integrates information from so many sources to regulate its motility will be an exciting topic for future research.

Biological role of cyanobacterial phototaxis

Many studies on *Synechocystis* have used a non-motile mutant background, perhaps giving the impression that this is a planktonic organism. However, from the gene content and behavior of *Synechocystis* it is plausible that, in its natural environment, this bacterium readily develops biofilm communities [39]. In photosynthetic mats, phototactic motility is important for optimizing the photosynthetic performance of filamentous cyanobacteria [40]. In mixed photosynthetic mats also containing organotrophs, motility can lead to diel migrations and a stratification of these communities. In such biofilms, which are only a few millimeters thick, there is no need for fast migration of cells. Instead, the movement needs to be robust and able to operate in the viscous exopolysaccharide matrix of a biofilm. Type IV pili are indeed a major factor for biofilm formation of many heterotrophic bacteria like *Pseudomonas*. In addition, the positive phototaxis response of *Oscillatoria* filaments in mats is extremely precise and relatively fast [40]. Within 20 minutes, filaments were able to move into a light area spotted onto the mat. When the light was turned off, the filaments quickly moved back into the mat. There is little quantitative knowledge of light penetration into phototrophic biofilms and the ways that it may be influenced by cellular organisation, and very little understanding of the dynamics of mat organisation. Our recent work [13] has established that bacterial cells are very effective optical structures, capable of focusing light and also trapping it by total internal reflection. We think that this insight will be crucial to the understanding of the structure, dynamic organisation and function of photosynthetic biofilms.

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BOX

Micro-optic properties of spherical cyanobacterial cells and their influence on phototactic behaviour

Directional light perception by *Synechocystis* depends on the cell focusing a sharp image at the edge of the cell furthest from the light source [13] (Fig. 1). The optical properties of a cell depend on its size and shape, its refractive index, and the refractive index of the surrounding medium. *Synechocystis* cells are roughly spherical and around 3 μm in diameter. Objects at these scales of just a few times the wavelength of light have optical properties that cannot be predicted from simple ray diagrams. Uniform microspheres of similar size to *Synechocystis* cells are able to focus light beyond the optical diffraction limit. This phenomenon is called a photonic nanojet, characterized as

a narrow, high-intensity light beam emerging from the shadow-side surface of an illuminated dielectric microcylinder or microsphere [41]. Microlenses are currently being tested for super-resolution nanoscopy [42]. It has been observed that the generation of focused light beams by *Synechocystis* cells strongly resemble photonic nanojets [13]. These phenomena were observed by photolithography for cells on a dry surface, and also by fluorescence microscopy for cells on the wet agar surface used for phototaxis assays. They could be predicted by finite difference time domain (FDTD) simulations using the Maxwell equations, considering air as the medium and modelling the cell as a sphere with a uniform refractive index of 1.4. This value is reasonable, as optofluidic imaging of cells of other bacteria indicates refractive indices in the range of 1.37-1.42 [43].

It has been questioned whether this light focusing could work effectively for cells fully immersed in water with a refractive index of 1.33 [44]. Indeed, simple ray diagrams would suggest that water immersion would reduce the sharpness of the focus. However, studies on micro-optical effects within eukaryotic cells show that very small differences in refractive index between different parts of the cell can have dramatic effects on light path through the cell. For example, small refractive index contrasts within retinal glia cells (1.415 for heterochromatin, 1.385 for euchromatin and 1.36 for the surrounding tissue) allow the nuclei to act as converging lenses [45,46]. It has been suggested that mitochondria act as optical waveguides, based on refractive indices of 1.43-1.5 for the mitochondria and 1.35 for the surrounding cytoplasm [47].

The surrounding medium that is physiologically relevant for phototaxis is uncertain. It is highly likely that cells are surrounded by extracellular polymeric substances (EPS) which could have a refractive index different from water. Interestingly, EPS, which are thought to act as a lubricant during surface-based movement, also enhance phototactic orientation [48] and it is possible that EPS could enhance light focusing capabilities by changing the refractive index near the cell surface. Moreover, it is obviously an over-simplification to regard cells as microspheres with uniform refractive index. Light interacting with a *Synechocystis* cell must pass through the aqueous environment, then the extracellular matrix, the crystalline S-layer, cell membranes, thylakoid membranes, the cytoplasm and the nucleoid. There may well be strong refractive index contrasts between these closely-packed layers. The implications for the optical properties of the cell remain to be explored.

Figures

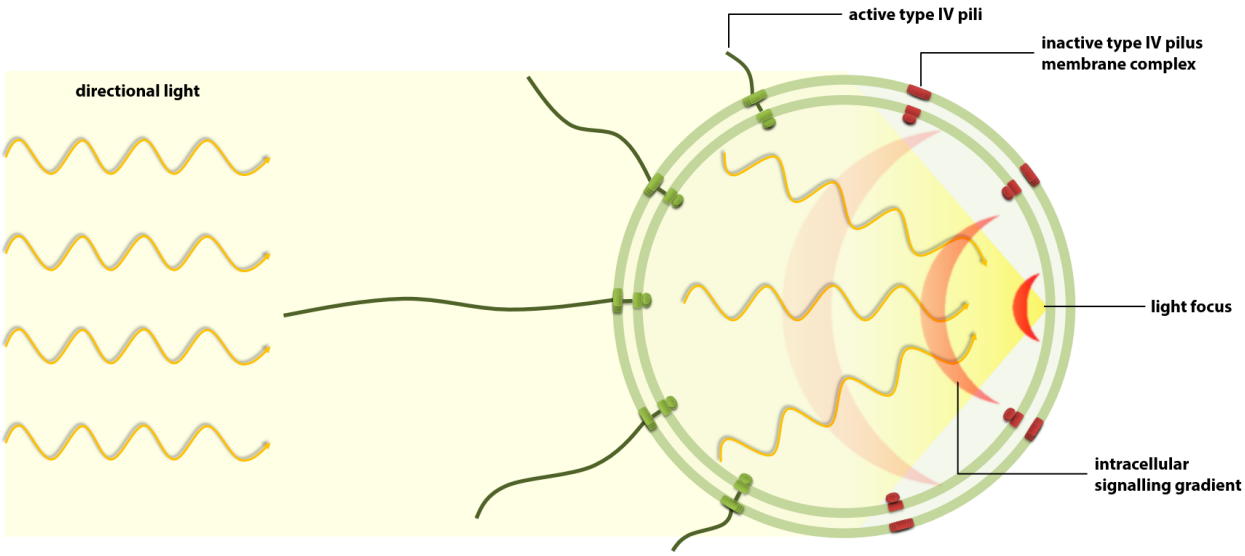

Figure 1. Model for directional light sensing in the spherical unicellular cyanobacterium *Synechocystis,* which has cells about 3 μm in diameter. Unidirectional light is focused at the distal edge of the cell, where it is perceived by photoreceptors. For positive phototaxis, localized signal transduction in the vicinity of the focused light spot inactivates the Type IV pilus apparatus. The pili that remain active are at the side of the cell facing the light source, leading to movement towards the light [13].

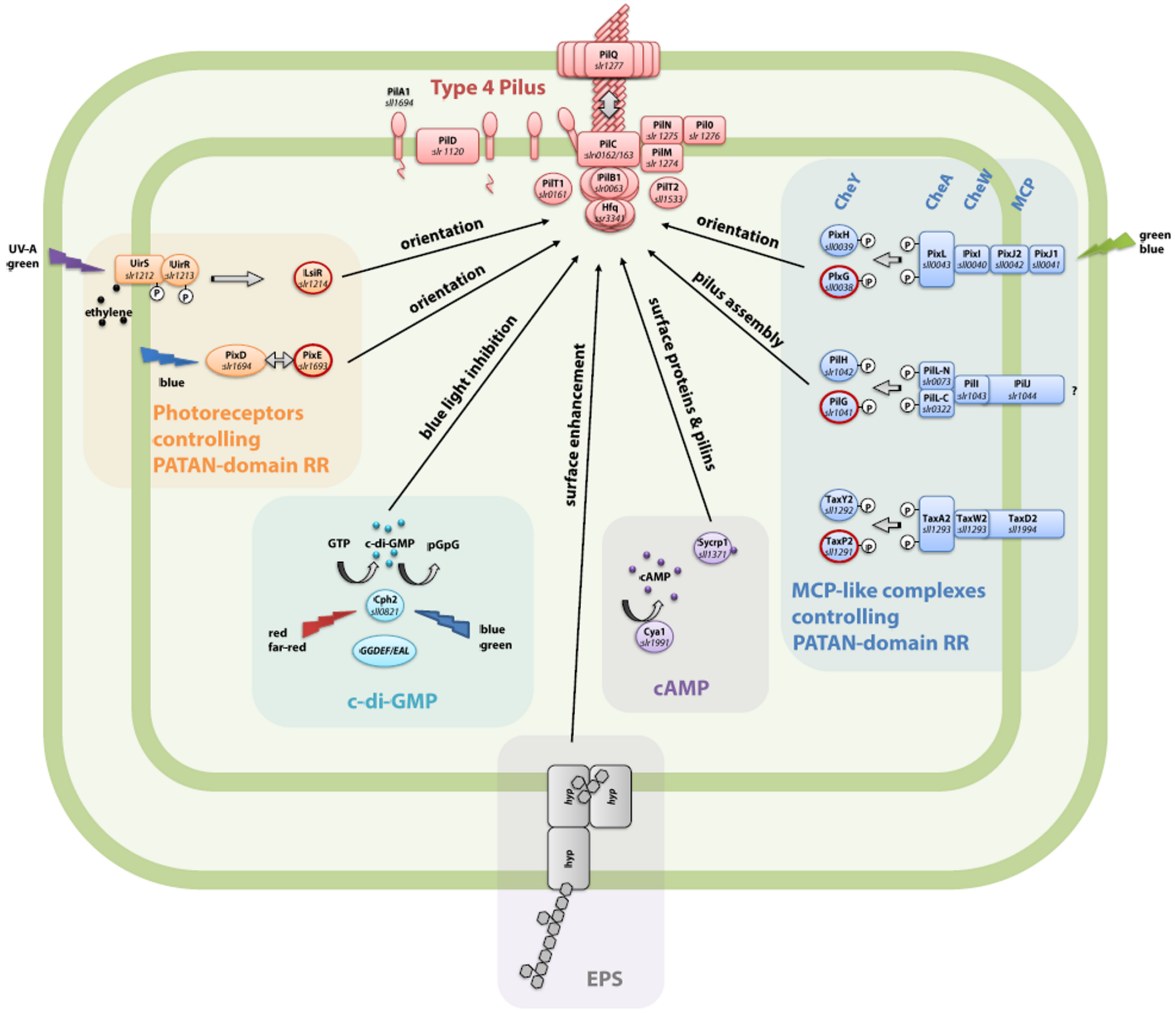

Figure 2. Components involved in regulation of phototaxis in *Synechocystis* 6803, showing putative taxis proteins and a schematic cyanobacterial type IV pilus. Three photoreceptors (PixJ1, UirS and PixD) are known to regulate phototaxis. Inactivation of each of them or of some of the downstream signal transduction components leads to a change in direction of movement in comparison to the wild type. It is not firmly established how the light signals (flash symbols) are transduced to the pilus base, but we hypothesize that this response is mediated by differentially localized CheY-like response regulators. These include PATAN-domain proteins shown with bold outlines. The second messenger molecules cAMP and c-di-GMP regulate motility of the cells, but not directional movement. Dynamic localization of PilB1 at the front side of the cell was demonstrated in [22]. The binding of the putative RNA chaperone Hfq to PilB1 was shown in [49].

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