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Short Communication

Bipolar Localization of Putative Photoreceptor Protein for Phototaxis in Thermophilic Cyanobacterium *Synechococcus elongatus*

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We identified an open reading frame from a database of the entire genome of *Synechococcus elongatus*, the product of which was very similar to *pixJ1*, which was proposed as photoreceptor gene for phototaxis in *Synechocystis* sp. PCC6803 [Yoshihara et al. (2000) *Plant Cell Physiol.* 41: 1299]. The mRNA of *S. elongatus pixJ* (*SepixJ*) was expressed in vivo as a part of the product of an operon. *Se*PixJ was detected exclusively in the membrane fraction after cell fractionation. Immunogold labeling of *Se*PixJ in ultra-thin sections indicated that it existed only in both ends of the rod-shaped cell; probably bound with the cytoplasmic membrane.

Keywords: Bipolar localization — Methyl-accepting chemotaxis protein — Photoreceptor — Phototaxis — Phytochrome — Thermophilic cyanobacterium.

Abbreviations: MCP, methyl-accepting chemotaxis protein; ORF, open reading frame; PCC, Pasteur culture collection; RT-PCR, reverse transcription-PCR.

Many cyanobacteria display phototaxis, which guides them toward or away from light sources (Häder 1987). Recently in the unicellular cyanobacterium *Synechocystis* sp. PCC6803 [the entire genome of which has been sequenced (Kaneko et al. 1996)], gene disruption experiments have revealed that phototaxis is mediated by type IV pili (Bhaya et al. 2000, Yoshihara et al. 2001). Type IV pilus structures have been shown to play an important role in twitching and gliding motility in a number of Gram-negative non-photosynthetic bacteria (Wall and Kaiser 1999).

The rod-shaped unicellular thermophilic cyanobacterium *Synechococcus elongatus* (also called *Thermosynechococcus elongatus*) also displays phototaxis. *S. elongatus* has been used to produce suitable materials for crystallographic studies about protein complexes such as photosystem, because of the structural stability of the complexes found in this organism. We recently measured detailed action spectra of the phototaxis of *S. elongatus* on agar plates, and suggested the involvement of a phytochrome-like photoreceptor in the phenomenon (Kondou et al. 2001). Yoshihara et al. (2000) investigated the effects of disruption of the gene cluster on the phototaxis in *Synechocystis* sp. PCC6803, which was similar to the *pilG* gene cluster found in *Pseudomonas aeruginosa*. They found that the disruption of *pixJ1*, a member of the gene cluster in *Synechocystis* sp. PCC6803, dramatically affected the wavelength dependency of phototaxis (Yoshihara et al. 2002). Since the deduced product of *pixJ1* had a GAF domain, which has been shown to be a chromophore binding domain in phytochrome (Aravind and Ponting 1997), the *pixJ1* gene product was thought to be a photoreceptor involved in phototaxis.

Sequence analysis of the entire *S. elongatus* genome (Nakamura et al. 2002) has revealed that there are at least five distinct genes encoding putative proteins possessing the GAF domain. When subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) and ClustalW analysis (http://www.ch.embnet.org/software/ClustalW.html), one GAF-protein was shown to be an ortholog of PixJ1. The *pixJ1* gene was encoded in the proposed *pixGHIJL* gene cluster (Yoshihara et al. 2000). The *S. elongatus pixJ1* homologue (*SepixJ*) also appears to be in a gene cluster. The proposed gene cluster containing *SepixJ* resembles the *pixJ1* gene cluster (Fig. 1A). To confirm the expression of *SepixJ* at the mRNA level, RT-PCR analysis was performed as followings. First, mRNA extracted from the cells at early log phase was reverse-transcribed with the oligo-nucleotide (5′-GCTCACTTTCGCGGAAGGGC-3′) of the complementary sequence to the 3′-terminal region of *SepixL* (Fig. 1A). The expected *SepixJ* region was then amplified by PCR (using primers; 5′-AACAAAGCAACCGCCGGTGG-3′ and 5′-AGACTGGGCAATGACCGTTCCTT-3′). The result, shown in Fig. 2, indicates that *SepixJ* was transcribed and that the transcriptional product was extended to 3′-terminal region of *SepixL*. The *pixH* and *pixL* genes in the *pixJ1* gene cluster are homologous to genes of CheY and CheA, respectively (Yoshihara et al. 2000). CheY and CheA are

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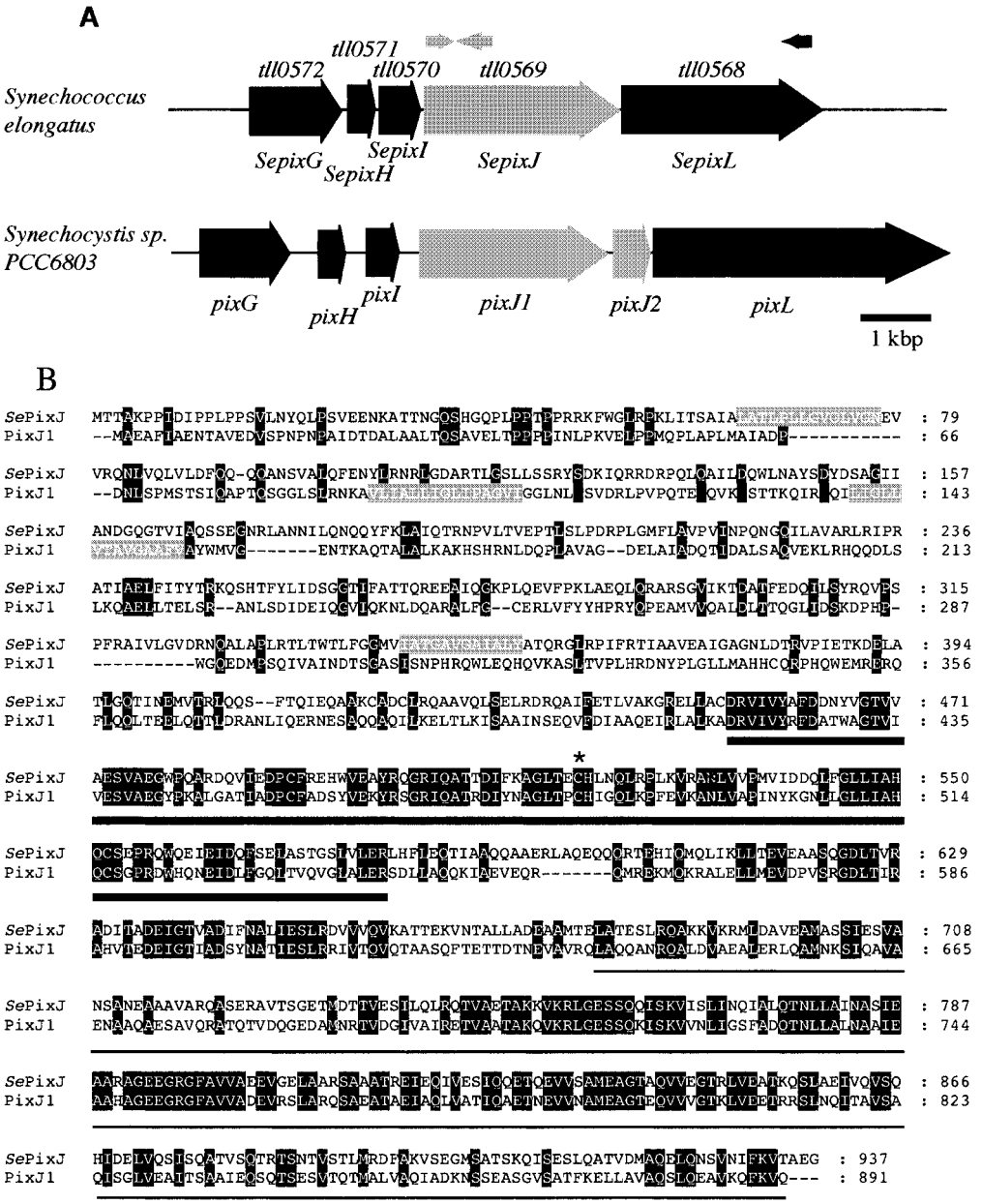

Fig. 1 Primary sequences of the *SepixJ* from *S. elongatus* aligned with *pixJ1* from *Synechocystis* sp. PCC6803. (A) The arrangements of *SepixJ* cluster and *pixJ1* cluster as annotated by the ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The name of each gene is described under the ORF (thick arrow). The accession number of each gene in Cyanobase (http://www.kazusa.or.jp/cyano/cyano.html) is described on the ORF. The direction of arrow indicates predicted transcriptional orientation. Black and gray arrows depicted on *SepixJ* gene cluster indicate primer for synthesis of cDNA and primers for amplification of PCR, respectively (used in RT-PCR shown in Fig. 2). (B) Amino acid sequence alignment of *Se*PixJ and PixJ1. The shadowed box indicates the transmembrane region predicted by DAS analysis (http://www.sbc.su.se/~miklos/DAS/maindas.html). The GAF and MCP signal domains are represented with a thick and thin underline, respectively. The cysteine predicted to be a chromophore binding residue is marked with an asterisk.

known as a response regulator and histidine kinase, respectively, of the two-component regulatory system in bacteria (Eisenbach 1996). Amino acid sequences of *Se*PixH and *Se*PixL are similar to those of CheY and CheA, respectively. Also, important residues of the proteins deduced from the gene cluster containing *SepixJ*, which are proposed to function in the phosphotransfer relay system, are conserved (data not shown). The amino acid sequence homology between *Se*PixJ and PixJ1 was 32% identical and 50% similar. Both have transmembrane regions, as well as GAF and MCP (methyl-accepting chemotaxis protein) signal domains (Fig. 1B). The C-terminal regions of the proteins, which contain GAF and MCP signal domains,

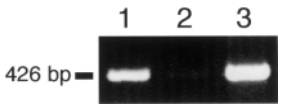

Fig. 2 RT-PCR profile of *SepixJ* in *S. elongatus*. The Following were used as templates for the PCR. (1) Reverse-transcribed RNA from *S. elongatus*, (2) RNA before reverse-transcription and (3) genome DNA isolated from *S. elongatus*. Primers used are schematically shown in Fig. 1 (see text in detail). For liquid culturing, this strain was grown at 50°C in DTN medium under continuous white light (Mühlenhoff and Chauvat 1996). RNA was extracted according to method of Mohamed and Jansson (1989).

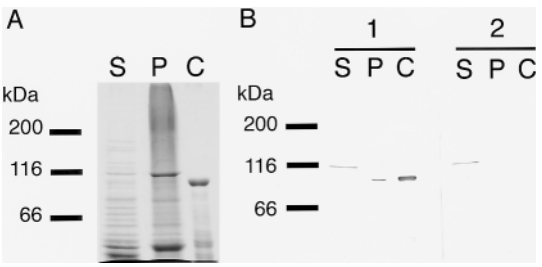

Fig. 3 SDS-PAGE profiles of extracts of *S. elongatus* cells after ultracentrifugation: (A) Coomassie Brilliant Blue staining; (B) immunoblotting. Cells harvested at late log phase were disrupted by a sonicator (Bioruptor model UCD-200, Cosmo Bio Co., Ltd., Tokyo, Japan) and ultracentrifuged at 100,000×*g* for 60 min. Lanes labeled with S, P and C indicate supernatant (soluble fraction), pellet (membrane fraction) after the ultracentrifugation and purified recombinant *Se*PixJ (positive control), respectively. (1) Anti-*Se*PixJ antibody was used as primary antibody; (2) no immune control. Since recombinant *Se*PixJ was fusion protein with Histidine-tag, mobility of specific staining band observed in C was a little lower than that observed in P. The band at 116 kDa in S is not specific to *Se*PixJ because this band is observed in both 1 and 2. SDS-PAGE and immunochemical staining was performed according to the method described previously (Nakazawa et al. 1990). Polyclonal antibodies used in immunoblotting were raised against purified recombinant *Se*PixJ. To acquire recombinant *Se*PixJ, pET expression system (Novagen Co., Ltd., WI, U.S.A.) was used. The full-length sequence encoding *SepixJ* was amplified from genomic DNA isolated from *S. elongatus* by PCR. Over-expressed protein was purified by Ni-affinity chromatography (HiTrap Chelating, Amersham Biosciences AB, Uppsala, Sweden) and further purified in SDS-PAGE. About 2 mg of the purified protein was entrusted to Sawady Technology Co., Ltd. (Tokyo, Japan) to immunize a rabbit. Before use in the experiments the rabbit anti serum was purified by antigen-affinity chromatography using the antigen conjugated to HiTrap NHS-activated HP (Amersham Biosciences AB, Uppsala, Sweden).

show a considerable degree of homology (identity = 49%, similarity = 68%). This might indicate that the C-terminal regions of the two proteins are important for a common biochemical function in cells of both strains, and that the N-terminal domains function primarily to anchor the proteins in the cell membrane.

In general, photoreceptors for phototaxis are localized in particular structures in the cell. For example, a photoactivated

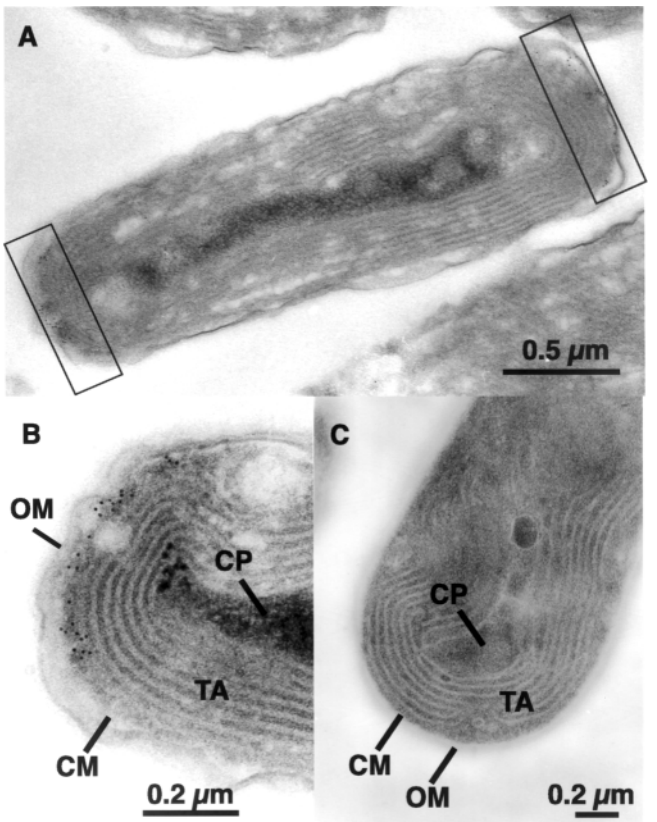

Fig. 4 Electron micrographs of immunostained cell of *S. elongatus*. (A, B) Anti-*Se*PixJ antibody was used as primary antibody and (C) primary antibody was omitted. Two squares in (A) indicate both edges of cell, in which labels are observed. (B) shows magnified edge of cell. OM, CM, TA and CP represent outer membrane, cytoplasmic membrane, thylakoid area and centroplasm, respectively.The immuno-electronmicroscopy was performed according to previously described method (Mogami et al. 2002) using *S. elongatus* cells harvested at late log phase.

adenylyl cyclase, which was the proposed photoreceptor for phototaxis of *Euglena gracilis* (Iseki et al. 2002), is located in the paraflagellar body. Also, in the cyanobacterium *Anabaena variabilis*, the photoreceptors for phototaxis have been thought to be fixed within a membrane. In the latter case, photosynthetic pigments in the thylakoid membrane were physiologically proposed as the photoreceptors for phototaxis in *A. variabilis* (Nultsch and Schuchart 1985). The photoreceptors for phototaxis in *Chlamydomonas reinhardtii*, which were the *Chlamydomonas* sensory rhodopsins A and B, were found in the membrane overlying the eyespot (Deininger et al. 1995, Sineshchekov et al. 2002). To determine whether or not *Se*PixJ is associated with a specific cellular structure, the following experiments have been carried out. Western blotting of *Se*PixJ using soluble and membrane fractions from *S. elongatus* cells was performed, and specific staining of a 102 kDa band was observed only in the membrane fraction (Fig. 3). This value is identical to the molecular mass expected from the deduced

amino acid sequence. These results indicate that *Se*PixJ is a 102 kDa membrane-bound protein.

To investigate the subcellular localization of *Se*PixJ in *S. elongatus* cell, we next performed immuno-electron microscopy. Interestingly, immunogold labels on ultra-thin sections were mainly observed near the edges of rod-shaped cell, where no particular structure except the cytoplasmic membrane could be observed; almost no labeling was observed in thylakoids and the centroplasm (Fig. 4A, B). Thus, *Se*PixJ appears to be located in the bipolar regions of the cytoplasmic membrane. Though similar bipolar localization has been shown to be a universal property of chemoreceptor proteins containing an MCP signal domain (such as Trg and Tsr in bacteria and archaea) (Alley et al. 1992, Gestwicki et al. 2000), this is the first report showing the bipolar localization of a MCP signal domain protein in cyanobacterium.

Chemoreceptor location may be involved in the sensitivity and response range of *E. coli* to ligands (Bray et al. 1998, Duke and Bray 1999). The bipolar localization of *Se*PixJ might indicate that the photoreceptor for phototaxis is strategically located to recognize light direction efficiently. Since molecular mechanisms of phototactic movement of *Synechocystis* sp. PCC6803 and *S. elongatus* appear to be similar, the subcellular localization of PixJ1 in spherical cells of *Synechocystis* sp. PCC6803 is interesting. Proteins containing both GAF and MCP signal domains have been found only in cyanobacteria. Common function among the orthologs of PixJ1 is expected. To elucidate the exact function of *Se*PixJ, gene disruption experiment of *Se*PixJ in *S. elongatus* is now in progress.

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