

A Novel Phototaxis Receptor Hidden in the Cyanobacterial Genome

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Many microorganisms, plants and animals control their movements in response to light. The simplest motility response to light, bacterial phototaxis, was described more than a century ago (Engelmann, 1883). Although bacterial taxis is now the best-understood simple sensory system, no phototaxis receptors in bacteria have been described yet. More complex organisms are better studied in this respect: phototaxis in unicellular algae (Foster, 1984; Ebnet *et al.*, 1999) and fungi (Saranak and Foster, 1997), as well as in Archaea (Spudich, 1998) was shown to be dependent on rhodopsin-like receptors.

Complete genome sequencing of the photosynthetic cyanobacterium *Synechocystis* sp. PCC6803 (Kaneko *et al.*, 1996) should have resulted in the identification of a phototaxis receptor. However, the bioinformatics methods used for the genome annotation in 1996 were not sensitive enough. An increased power of new algorithms for genomics, especially the introduction of the PSI-BLAST program (Altschul *et al.*, 1997), provided new opportunities for discoveries in completely sequenced genomes. Therefore, I have revisited the *Synechocystis* genome in order to identify the first bacterial phototaxis receptor.

BLAST searches against the *Synechocystis* genome database (available at www.kazusa.or.jp/cyano/cyano.html) by using a highly conserved signaling domain of bacterial chemotaxis receptors (Le Moual and Koshland, 1996) as queries have identified three genes coding for taxis receptors: sll0041, sll1294 and slr1044. Further *in silico* domain analysis including PSI-BLAST searches (Altschul *et al.*, 1997) and multiple alignments revealed that the gene sll0041, which has been annotated as coding for a methyl-accepted chemotaxis protein I or a serine chemotaxis receptor (Kaneko *et al.*, 1996), coded in fact for a phototaxis receptor. In its C-terminal region, the sll0041 protein contains a conserved signaling module typical of bacterial taxis receptors (Figure 1). This sequence region is homologous to the signaling module from the serine chemoreceptor and other methyl-accepting chemotaxis proteins from *Escherichia coli* that caused the erroneous gene annotation in 1996. I have identified two GAF domains and two HAMP domains in the N-terminal region of the sll0041 protein (Figure 1). The GAF domains are sensor modules in various phototransducing proteins in both eukaryotes and prokaryotes (Aravind and Ponting, 1997). The best known examples of GAF-domain-containing photoreceptors are plant phytochromes (Reed, 1999) and ethylene response sensors (Chang and Shockey, 1999)

and mammalian rod photoreceptor phosphodiesterases (Soderling and Beavo, 2000). HAMP domain is found in various signal transduction proteins. Aravind and Ponting have recently suggested that the HAMP domain is not just a linker between a transmembrane region and a signaling domain, as it was previously thought, but it is an important signaling domain itself (Aravind and Ponting, 1999). The presence of the second HAMP domain between the GAF2 domain and the signaling module in the cyanobacterial photoreceptor (Figure 1) is in a good agreement with their idea. All sensory and signaling domains of the sll0041 protein are predicted to be located in the cytoplasm (Figure 1).

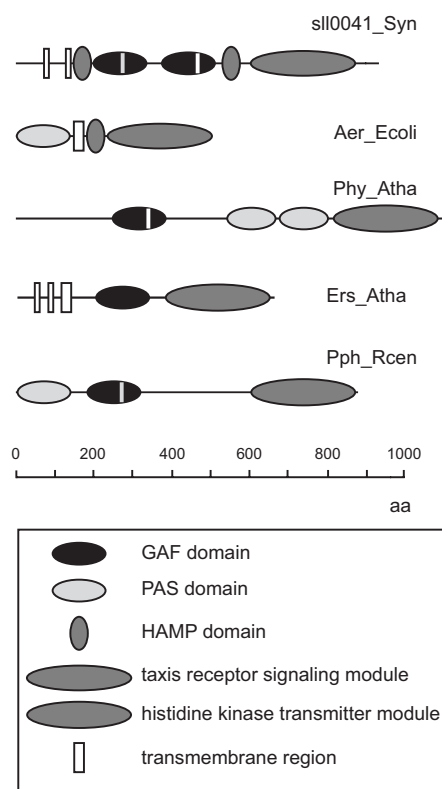


Figure 1. Domain structure of the cyanobacterial phototaxis receptor sll0041 in comparison with related proteins. Aer_Ecoli, aerotaxis receptor from *Escherichia coli* (g1703222); Phy_Atha, phytochrome E from *Arabidopsis thaliana* (g1172498); Ers_Atha, ethylene response sensor from *Arabidopsis thaliana* (g1046225); Pph_Rcen, photoreceptor histidine kinase from *Rhodospira rubra* (g4545094). Grey vertical line in GAF domains indicates a conserved histidine residue (His311), a known chromophore attachment site in bacteriophytochromes. White vertical line in GAF domains indicates a conserved cysteine residue (Cys483), a known chromophore attachment site in plant phytochromes. PAS domain is a sensor module typical of oxygen, light, and redox potential responsive proteins from prokaryotes and eukaryotes (Taylor and Zhulin, 1999). HAMP domain is a signaling module typical of taxis receptors, histidine kinases and adenylate cyclases from prokaryotes and low eukaryotes (Aravind and Ponting, 1999). Transmembrane regions have been predicted by using the dense alignment surface (DAS) algorithm (Cserzo *et al.*, 1997).

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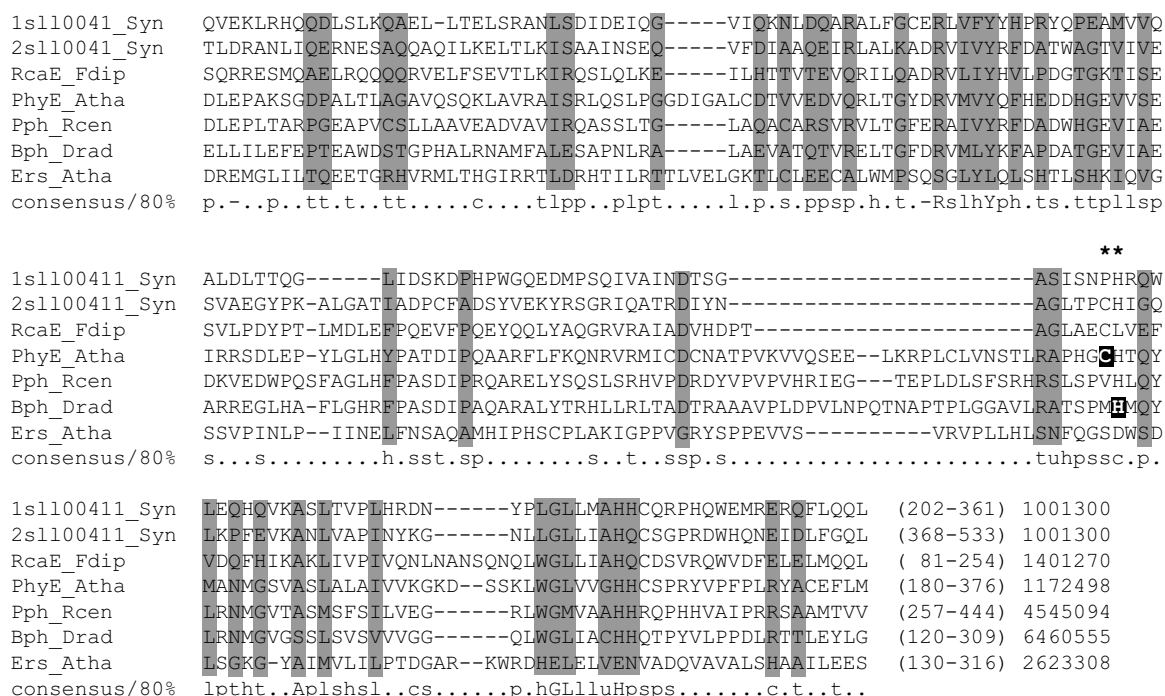


Figure 2. Multiple alignment of selected GAF domains. Alignment was constructed by using the CLUSTAL X program (Thompson *et al.*, 1997) and manual adjustments. The first and the last residue of each domain and gene identification numbers for each protein in the GenBank database are shown after each sequence. Abbreviations are as follows: 1s110041_Syn and 2s110041_Syn, GAF1 and GAF2 domains of s110041 from *Synechocystis* sp., respectively; RcaE_Fdip, chromatic adaptation sensor from *Fremyella diplosiphon*; PhyE_Atha, phytochrome E from *Arabidopsis thaliana*; Pph_Rcen, photosensor histidine kinase, *Rhodocista centenaria*; Bph_Drad, bacteriophytochrome from *Deinococcus radiodurans*; Ers_Atha, ethylene response sensor from *A. thaliana*. Stars indicate positions of known and putative chromophore attachment sites. Conserved cysteine and histidine residues that are known sites for a chromophore attachment in plant phytochromes and bacteriophytochromes are shown in reverse shading. Consensus line underneath the alignment indicates conservation of amino acid residues in all but one sequence: -, negatively charged residues (D, E); c, charged residues (D, E, K, R, H); l, aliphatic residues (L, I, V, M); h, bulky hydrophobic residues (L, I, V, M, F, W, Y, H); u, tiny residues (A, G, S); s, small residues (A, C, D, G, N, P, S, T, V); p, polar residues (C, D, E, H, K, N, Q, R, S, T); t, turn-like residues (A, C, D, E, G, H, K, N, Q, R, S, T). Residues conserved in all sequences are shown in grey shading.

The GAF1 domain of s110041 has a conserved histidine (His311), the known tetrapyrrole chromophore binding site in eubacterial photoreceptors (Davis *et al.*, 1999), whereas the GAF2 domain has a conserved cysteine residue (Cys483), the known tetrapyrrole chromophore binding site in plant phytochromes (Reed, 1999) (Figure 2). Thus, both GAF domains of s110041 may contain the chromophore. Tetrapyrrole chromophores in plant and bacterial photoreceptors mediate responses to red/far red light. Recent finding that *Synechocystis* has a positive phototactic response to red/far red light (Choi *et al.*, 1999) suggests the presence of a tetrapyrrole-containing sensor and supports our *in silico* evidence that the s110041 protein is indeed a phototaxis receptor. A GAF-domain-containing sensor module has been recently reported in light-responsive histidine kinases from *Rhodocista centenaria* (Jiang *et al.*, 1999), *Deinococcus radiodurans* and *Pseudomonas aeruginosa* (Davis *et al.*, 1999) indicating that GAF photosensors are present in distantly related bacteria. The GAF-domain-containing phototaxis receptor from cyanobacteria is a member of the emerging subclass of taxis receptors that sense stimuli intracellularly. This involves the PAS-domain-containing Aer protein from *E. coli* (Bibikov *et al.*, 1997; Rebbapragada *et al.*, 1997) and myoglobin-like oxygen sensors from *Halobacterium salinarum* and *Bacillus subtilis* (Hou *et al.*, 2000).

References

- Altschul, S.F., Madden, T.N., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25: 3380-3402.
- Aravind, L., and Ponting, C. P. 1997. The GAF domain: an evolutionary link between diverse phototransducing proteins. *Trends Biochem. Sci.* 22: 458-459.
- Aravind, L., and Ponting, C. P. 1999. The cytoplasmic helical linker domain of receptor histidine kinase and methyl-accepting proteins is common to many prokaryotic signalling proteins. *FEMS Microbiol. Lett.* 176: 111-116.
- Bibikov, S.I., Biran, R., Rudd, K.E., and Parkinson, J.S. 1997. A signal transducer for aerotaxis in *Escherichia coli*. *J. Bacteriol.* 179: 4075-4079.
- Chang, C., and Shockey, J.A. 1999. The ethylene-response pathway: signal perception to gene regulation. *Curr. Opin. Plant Biol.* 2: 352-358.
- Choi, J.-S., Chung, Y.-H., Moon, Y.-J., Kim, C., Watanabe, M., Song, P.-S., Joe, C.-O., Bogorad, L., and Park, Y.M. 1999. Photomovement of the gliding cyanobacterium *Synechocystis* sp. PCC 6803. *Photochem. Photobiol.* 70: 95-102.
- Cserzo, M., Wallin, E., Simon, I., von Heijne, G., and Elofsson, A. 1997. Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method. *Protein Eng.* 10: 673-676.
- Davis, S.J., Vener, A.V., and Vierstra, R.D. 1999. Bacteriophytochromes: phytochrome-like photoreceptors from nonphotosynthetic eubacteria. *Science* 286: 2517-2520.
- Ebnet, E., Fischer, M., Deininger, W., and Hegemann, P. 1999. Volvoxrhodopsin, a light-regulated sensory photoreceptor of the spherical green alga *Volvox carteri*. *Plant Cell* 11: 1473-1484.
- Engelmann, T.W. 1883. Bacterium photometricum: ein beitrag zur vergleichenden physiologie des licht und fabensinnes. *Pflugers Arch. Gesamte Physiol. Menschen Tiere* 42: 183-186.
- Foster, K.W., Saranak, J., Patel, N., Zarilli, G., Okabe, M., Kline, T., and Nakanishi, K. 1984. A rhodopsin is the functional photoreceptor for

- phototaxis in the unicellular eukaryote *Chlamydomonas*. *Nature* 311: 756-759.
- Hou, S., Larsen, R.W., Boudko, D., Riley, C.W., Karatan, E., Zimmer, M., Ordal, G.W., and Alam, M. 2000. Myoglobin-like aerotaxis transducers in Archaea and Bacteria. *Nature* 403: 540-544.
- Jiang, Z.Y., Swem, L.R., Rushing, B.G., Devanathan, S., Tollin, G., and Bauer, C.E. 1999. Bacterial photoreceptor with similarity to photoactive yellow protein and plant phytochromes. *Science* 285: 406-409.
- Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., Hirose, M., Sugiura, M., Sasamoto, S., Kimura, T., Hosouchi, T., Matsuno, A., Muraki, A., Nakazaki, N., Naruo, K., Okumura, S., Shimpo, S., Takeuchi, C., Wada, T., Watanabe, A., Yamada, M., Yasuda, M., and Tabata, S. 1996. Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Res.* 3: 109-136.
- Le Moual, H., and Koshlan, D.E., Jr. 1996. Molecular evolution of the C-terminal cytoplasmic domain of a superfamily of bacterial receptors involved in taxis. *J. Mol. Biol.* 261: 568-585.
- Rebbapragada, A., Johnson, M.S., Harding, G.P., Zuccarelli, A.J., Fletcher, H.M., Zhulin, I.B., and Taylor, B.L. 1997. The Aer protein and the serine chemoreceptor Tsr independently sense intracellular energy levels and transduce oxygen, redox, and energy signals for *Escherichia coli* behavior. *Proc. Natl. Acad. Sci. USA* 94: 10541-10546.
- Reed, J.W. 1999. Phytochromes are Pr-kinases. *Curr. Opin. Plant Biol.* 2: 393-397.
- Saranak, J., and Foster, K.W. 1997. Rhodopsin guides fungal phototaxis. *Nature* 387: 465-466.
- Soderling, S.H., and Beavo, J.A. 2000. Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Curr. Opin. Cell Biol.* 12: 174-179.
- Spudich, J.L. 1997. Variations on a molecular switch: transport and sensory signalling by archaeal rhodopsins. *Mol. Microbiol.* 28: 1051-1058.
- Taylor, B.L., and Zhulin, I.B. 1999. PAS domains: internal sensors of oxygen, redox potential, and light. *Microbiol. Mol. Biol. Rev.* 63: 479-506.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997.
- The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25: 4876-4882.

