A prokaryotic phytochrome

Phytochrome photoreceptors are almost certainly ubiquitous in green plants, regulating numerous aspects of development throughout their life cycle. Phytochromes were thought to exist only in plants, but the recently described sequence of the chromosome from the cyanobacterium *Synechocystis* revealed a gene that seemed to encode a phytochrome-like protein[1]. By expressing this gene in *Escherichia coli* and feeding appropriate chromophores we show that it encodes a phytochrome, which may offer an excellent starting material for crystallization and X-ray diffraction analysis.

The carboxy-terminal amphiphilic structure of phytochrome resembles that of bacterial sensory histidine-kinases, a group of enzymes used by prokaryotes to monitor and react to various aspects of their environment[2–4]. Conceptual translation of the *Synechocystis* sp. PCC 6803 open reading frame slr0473 (the putative *Synechocystis phy* gene) yields a product that shows similarity to plant phytochromes throughout its length and to bacterial sensory kinases towards the C terminus[1,5]. In particular, the chromophore-binding domain, highly conserved in all phytochromes, is clearly represented in the product (residues Val 246–Asp 280). This, however, does not prove that the gene product is a genuine phytochrome. Phycocyanin levels prevent spectral photoreversibility measurements[6,7] of phytochrome in cyanobacteria, so we investigated the putative *phy* gene product by expression-cloning in *E. coli* using the vector pQE12.

Expression of the *Synechocystis* PHY apoprotein was very efficient. Products of relative molecular mass 85,000 accumulated to about 50% total soluble protein (Fig. 1a, lane 1). This contrasts with results from similar approaches with plant phytochrome genes in *E. coli* which are usually very weakly expressed or give rise to largely insoluble products[8–10]. The clone was engineered to express a C-terminal polyhistidine tag for nickel-affinity purification. The product bound quantitatively to Ni-NTA (lane 2) and was eluted as a homogeneous apoprotein (lane 3), which could be concentrated to a 5–10 mg ml^{-1} solution.

Plant phytochrome apoproteins auto catalytically attach linear tetrapyrrole chromophores such as phycocyanobilin (PCB)[11], abundant in the cytoplasm of cyanobacteria. Indeed, the *Synechocystis* PHY apoprotein attached purified PCB, producing visibly photochromic holoprotein (phy*, Fig. 1b). In contrast, plant phytochromes expressed in *E. coli* show poor autoassembly, folding incorrectly[8,9,12]. *Synechocystis* phy* was analysed spectrophotometrically after exposure to saturating monochromatic 657 nm (red) and 731 nm (far-red) irradiation (Fig. 1c). The spectra are reminiscent of plant phytochrome–PCB adducts[11] with absorb-ance maxima at 658 and 702 nm after red and far-red irradiation, respectively, and an isosbestic point at 677 nm.

This is the first report of a spectrally functional prokaryotic phytochrome. In most lower organisms light is detected by retinal or coumaric acid-based photoreceptors. However, a *Fremyella* gene, *rcaE*, involved in chromatic adaptation and encoding a putative histidine-kinase sensor protein was recently described[13]. Although the conceptual gene product shows local similarities to PHYE in *Arabidopsis*, no significant homology to the phytochrome chromophore-binding domain is apparent[13] and photoreceptor activity has yet to be demonstrated. The *Synechocystis phy* gene product is the most phytochrome-like of the *Synechocystis* genome and among all known prokaryotic sequences.

A simple prokaryote has advantages for basic studies of phytochrome biology. For example, co-expression in the heterologous *E. coli* host might be useful in studying subsequent components of the signal transduction pathway. Furthermore, milligram amounts of homogeneous, spectrally active *Synechocystis* phytochrome holoprotein can be produced easily in our system. As concentrations of 10 mg ml^{-1} can be achieved readily — at least ten times higher than in other phytochrome overexpression systems known to us — there remains no barrier in principle to obtaining crystals for X-ray diffraction analysis of phytochrome molecular structure.

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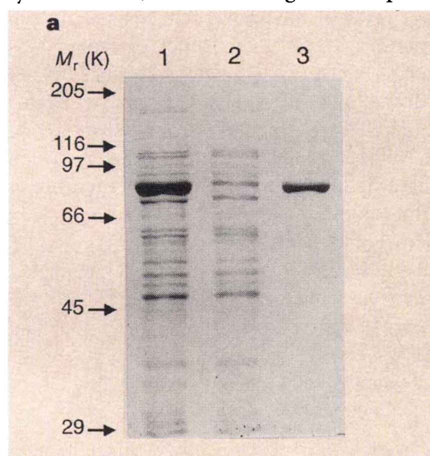

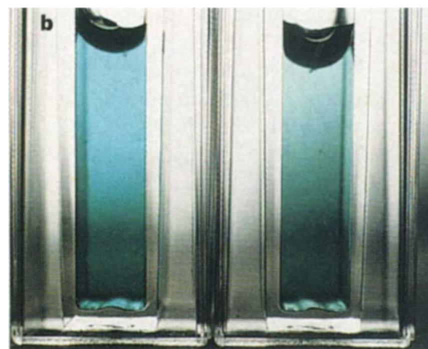

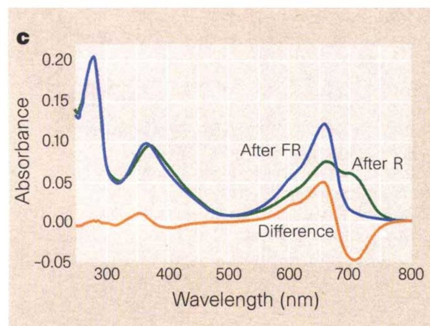

Figure 1 a, **Expression and affinity purification of recombinant PHY apoprotein in *E. coli*. SDS–PAGE 10% gel stained with Coomassie. Lane 1, total soluble protein in lysate; 2, after adsorption to Ni-NTA matrix; 3, 250 mM imidazole eluate.** b, **Photochromicity of phy* holoprotein. Stoichiometric amounts of PCB were added to PHY (3 mg ml^{-1}). After autoassembly (20 min in darkness) the sample was divided and each portion irradiated with 731 nm (far-red, left) or 657 nm (red, right) light. Note the blue or green transition associated with phytochrome photoconversion.** c, **Absorbance characteristics of phy* after irradiation with saturating red (R) or far-red (FR) light, and the calculated difference spectrum.**

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