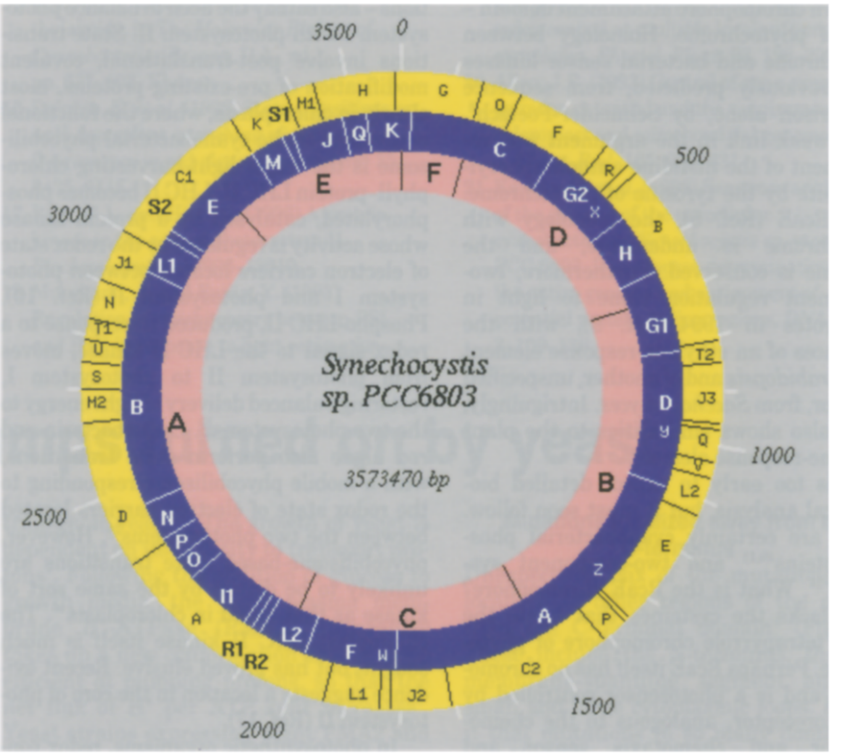
Complementary adaptations, photosynthesis and phytochrome

Cyanobacteria, formerly termed blue-green algae, are prokaryotes and hence better fit the blueprint of a bacterium than that of a plant or alga[1]. They do have the ability, shared with plants, of using water as the source of hydrogen atoms for photosynthetic electron transport, with molecular oxygen as a waste product. However, the consensus is that cell structure and genetics are a better base for taxonomy than photosynthesis and, in any case, certain species of cyanobacteria are now known to be able to throw off the aerobic, oxygenic mask by abstracting hydrogen from hydrogen sulphide instead of water, carrying out strictly anaerobic photosynthesis characteristic of other bacteria.



The circular map of *Synechocystis* sp. PCC 6803, from the CyanoBase site on the World Wide Web (see http://www.kazusa.or.jp/cyano/).

Cyanobacteria and plant research

But plant scientists cannot safely delegate the cyanobacteria[2]. First, oxygen-evolving photosynthesis is totally intact – the electrons pass through exactly the same chain as in plant chloroplasts – and photosynthesis is inherently more amenable to molecular genetic analysis in cyanobacteria than in plants. Second, the chloroplast, the essential requirement of the plant cell, is clearly the descendant of a free-living prokaryote that was closely related to the modern cyanobacteria. The question as to why the chloroplast should have retained a core genetic system to synthesize a handful of photosynthetic proteins (likewise the mitochondrion for respiratory proteins) is a major problem in cell evolution[3]. Third, cyanobacteria adapt, and it is now becoming clear that the mechanisms by which they detect and respond to environmental change are typically 'prokaryotic', while, at the same time, a foundation from which eukaryotic signal transduction pathways must have evolved.

A molecular description of complementary chromatic adaptation

Described almost a century ago, complementary chromatic adaptation is now yielding to molecular genetics, and in doing so reveals some intriguing resemblances to the light responses mediated by phytochrome in plants. If a culture of a *Calothrix* or *Fremyella* spp. is grown in red light, it appears green; when it is transferred to green light, it turns red. Teleology, endemic to biology, is now a calculated risk: the cells change colour because of changes in the relative proportions of the pigments phycoerythrin (which absorbs blue and green light, and is hence red) and phycocyanin (which absorbs red light, and is hence blue-green). Add the knowledge that these pigments function as components of the light-harvesting apparatus of cyanobacterial photosynthesis, and the functional explanation is obvious: the cells adapt in order to be able to absorb more light for photosynthesis. Thus their colour (transmitted and reflected light) becomes complementary to that of the light in which they find themselves.

A recent breakthrough by Grossman and co-workers provides the outline of a molecular description of what is going on in complementary chromatic adaptation[4,5]. It was already known that the phycoerythrobilin and phycocyanobilin chromophores are attached to proteins to form the pigment–proteins phycoerythrin and phycocyanin. Transcription of the genes encoding the apoproteins is induced by light with a spectral composition matching that of the absorption spectrum of the holoproteins, the light-harvesting biliprotein gene products themselves. Mutants of *F. diplosiphon* were obtained that were unable to adapt, and among these were some mutants that stayed red. Some of these mutations were found to be complemented – chromatic adaptation was restored – upon transformation of the red-only cells with the *rcaC* gene ('regulator of chromatic adaptation C')[4].

The predicted 73 kDa RcaC polypeptide is a typical bacterial response regulator, which means that it has a conserved pattern of motifs seen elsewhere in proteins involved in a wide variety of bacterial responses to specific environmental change[6]. These motifs include a predicted α–β structure with an aspartate on a surface-exposed loop between the first α-helix and the first β-strand. The paradigm for such response regulators is the CheY protein, which dictates clockwise or anticlockwise rotation of the *Escherichia coli* flagellum, and hence switches on 'tumbling', the behavioural response at the centre of bacterial chemotaxis. A high-resolution structure for unmodified CheY has been obtained by X-ray crystallography[7]. The CheY protein is modified by phosphorylation – all response regulators are phosphorylated on the aspartate of the surface-exposed loop. The phosphate group is accepted from a phosphohistidine side chain of the sensor[6]. The sensor becomes phosphorylated (on histidine) in response to the relevant environmental signal. The sensor is thus a response regulator kinase, mediating transfer of phosphate from ATP to the response regulator only if the environmental conditions are right (and not otherwise). This is not teleology, but chemistry plus natural selection. Without two-component (sensor and response regulator) signal transduction, bacteria are completely unable to sense their environment.

The photosensor

What is the photosensor for complementary chromatic adaptation? In general terms, this is a foregone conclusion – a histidine sensor kinase. But the latest part of the breakthrough, the discovery of complementation of other mutations with rcaE (Ref. 5), brings us back to plants, and introduces something

few would have expected. The predicted chromatic adaptation sensor RcaE indeed contains the four C-terminal motifs expected for a histidine sensor kinase, including the histidine itself. However, the N-terminal domain contains a region with similarity to a known chromophore attachment domain – that of phytochrome. Homology between phytochrome and bacterial sensor kinases was previously predicted, from sequence comparison alone, by Schneider-Poetsch[8], but a weak link in the argument was replacement of the histidine autophosphorylation site by the tyrosine of phytochrome. With RcaE (Ref. 5), the homology with phytochrome is undeniable, and the histidine is conserved. Furthermore, two-component regulation came to light in eukaryotes in 1994 (Ref. 9), with the sequences of an ethylene-response element from *Arabidopsis* and of another, unspecified receptor, from *Saccharomyces*. Intriguingly, RcaE also shows similarities to the plant ethylene-response element[9].

It is too early to expect detailed biochemical analysis, but it must soon follow. There are certainly cyanobacterial phosphoproteins[10,11] and two-component systems[12–14]. What is the RcaE chromophore? RcaE lacks the cysteines that ligate the linear tetrapyrrole chromophore of phytochrome. Perhaps RcaE itself has no chromophore, and is a photosensor controlled by a photoreceptor, analogous to the chemoreceptor and chemotaxis sensor and response regulators of *E. coli*. The phycocyanin and phycoerythrin chromophores themselves are also linear tetrapyrroles. It is tempting to think that the evolutionary prototype of the photoreceptor phytochrome was itself a photosynthetic light-harvesting protein, capable of regulating its own synthesis. While red light repression of phycoerythrin synthesis may well be explained by the phytochrome-like properties of RcaE, the green light activation of phycoerythrin synthesis occurs over a spectral range outside of the response of phytochrome. It remains to be seen whether the biliprotein spectral window is modified in the photoreceptor, or whether another photosensory system, for which the rhodopsins are especially suited, is responsible for effects of green light.

Other responses to spectral composition

Complementary chromatic adaptation is not the only way in which cyanobacteria sense their light environment – indeed, many species do not exhibit the phenomenon at all. A more widespread, long-term response to altered spectral composition is adjustment of the stoichiometry of photosystem I and photosystem II of the photosynthetic electron transport chain[15]. Again, the response is complementary to the change in the light regime: photosystem I absorbs more at higher wavelengths than photosystem II, and becomes relatively more abundant under blue-green illumination, as if to make good by sheer quantity its limited light-harvesting capacity. As a function of light intensity, too, the ratio of photosystem I to photosystem II changes – from near to unity in saturating light to a sixfold increase in favour of photosystem I in low light.

Short-term adaptations – state transitions – also satisfy the need to balance photosystem I with photosystem II. State transitions involve post-translational, covalent modification of pre-existing proteins, most clearly in chloroplasts, where the functional equivalent of the cyanobacterial phycobilisome is the major light-harvesting chlorophyll–protein LHC II. LHC II becomes phosphorylated, catalysed by a protein kinase whose activity is regulated by the redox state of electron carriers located between photosystem I and photosystem II (Ref. 10). Phospho-LHC II, produced in response to a redox signal to the LHC II kinase, moves from photosystem II to photosystem I, restoring balanced delivery of light energy to the two photosystems[10]. Cyanobacteria and red algae also perform state transitions, with a mobile phycobilisome responding to the redox state of electron carriers located between the two photosystems[10]. However, phycobilisome-based state transitions are unlikely to be driven by the same sort of kinase as that found in chloroplasts[16]. The chloroplast LHC II kinase itself is much sought, but has proved elusive. Recent evidence suggests a location in the core of photosystem II (Ref. 17).

In photosynthetic organisms, redox control means light control. The underlying mechanism of adjustment of photosystem stoichiometry probably involves redox control, rather than direct photocontrol, of gene expression[10,15,18,19]. The universal eukaryotic location of the genes for the core components of the two photosystems in chloroplasts rather than in the nucleus immediately suggests a reason for the maintenance of the extranuclear genomes of chloroplasts (and, by analogy, mitochondria): electron transport holds a tight rein on the expression of genes for its own components[3,20].

Cyanobacterial phytochrome

For research on cyanobacteria, and indeed biology as a whole, an important development was the completion in 1996 of genome sequencing of *Synechocystis* PCC 6803 (Ref. 21). It seems to have taken 24 people under two years to obtain the complete sequence of approximately 3.5 Mb. Thanks to the openness and generosity of the participants, the question 'is there a cyanobacterial gene with homology to x?' can be addressed immediately using the CyanoBase site on the World Wide Web (http://www.kazusa.or.jp/cyano/).

Synechocystis 6803 is not a complementary chromatic adapter. However, using CyanoBase, *Synechocystis* PCC 6803 appears to contain several histidine sensor kinases with homology to phytochrome. Perhaps colour of light does other things through two-component signal transduction, such as triggering development (this is known in other species[14]). Or maybe the photosystem stoichiometry and state transition redox sensor(s) are there at the origin of photomorphogenesis. Furthermore, *Synechocystis* PCC 6803 contains a gene, *slr0473*, the predicted product of which has 36% identity to that of the *phyC* gene product of *Arabidopsis*: cyanobacteria have no seed dormancy, no photoperiodism and no phototropism, but they certainly contain phytochrome. Unexpected certainty is unusual in science, but a complete genome sequence can give precisely that. And what would Stanier[1] have thought of CyanoBase?

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Plant pumps turned on by yeast

The plasma membrane H^+-ATPase catalyzes proton pumping out of the cell. The plasma membrane pump – which has a much simpler structure than the pumps found in the vacuolar membrane, chloroplasts, or mitochondria – plays an important role in generating the electrochemical proton gradient across the membrane. Among the important physiological processes driven by this gradient are the transport of ions into root epidermal cells, loading of organic compounds into the phloem and opening of stomata. In addition to the role of the enzyme in cytosolic pH regulation, the pH gradient that develops may also affect pH-sensitive enzymes in the cell wall and plasma membrane channel proteins. The membrane potential may also have a regulatory effect on voltage-gated channels[1–3]. The plasma membrane H^+-ATPase seems to be regulated by several hormones, light, phytotoxins and environmental stress, but the molecular mechanisms underlying this regulation are poorly understood. Recent analysis[4] has revealed that mutations in specific domains of the plasma membrane H^+-ATPase can give rise to an enzyme that is even more effective at creating an electrochemical proton gradient.

Improved coupling between H^+ pumping and ATP hydrolysis

Several plasma membrane H^+-ATPase genes have been identified in all plants tested. Because the proteins encoded by these genes may have different properties, functional studies are best performed after expressing single genes in species devoid of an endogenous H^+-ATPase. Previously, two plant ATPases – AHA2 (*Arabidopsis*) and PMA2 (*Nicotiana plumbaginifolia*) – were shown to be capable of functionally replacing the yeast (*Saccharomyces cerevisiae*) H^+-ATPase in transgenic yeast[5,6]. The growth of yeast is dependent on its capacity to transport protons[1]. Following the addition of glucose to growth media, the yeast plasma membrane H^+-ATPase (PMA1) apparently takes up a new conformation that is more efficient at pumping protons, so that the net flux of H^+ per ATP split increases[7]. Yeast strains expressing plant PMA2 and AHA2 H^+-ATPases are unstable, and spontaneous mutations readily occur. This feature has now been used as a useful means of screening for *pma2* and *aha2* mutants capable of sustaining yeast growth under nonoptimal conditions[4,8]. This results in plant enzymes with new kinetic properties, the most significant being a several-fold increase in the H^+-pumping : ATPase coupling ratio[4,8].

The plasma membrane H^+-ATPase appears to be autoinhibited by a mechanism involving the C-terminal hydrophilic domain[9]. Removal or displacement of this domain by proteolysis, lysophosphatidylcholine treatment or genetic engineering increases enzyme activity. Several of the single point mutations analyzed by Morsomme *et al.*[4], and one mutation characterized by Baunsgaard *et al.*[8], are located in the C-termini of PMA2 and AHA2, respectively. These results indicate that single point mutations can induce the same response as the removal of over 60 residues from the C-terminus[9]. Plants tissues, such as coleoptile segments, react when treated with the fungal toxin fusicoccin by extruding protons at a high rate; the H^+-ATPase isolated from such treated plants has altered kinetic properties, including a markedly increased capacity for transporting protons[10]. This suggests that plant H^+-ATPase can be activated in the intact plant by a mechanism involving a similar change in conformation as that induced by the single point mutations.

Mutations localized away from the C-terminus

Unexpectedly, six of the mutations were localized to other regions of the protein than the C-terminal domain[4] (Fig. 1a). These include the N-terminus, the first membrane-spanning segment and the small and large cytoplasmic loops. How is it that mutations in so many domains of the H^+-ATPase result in a protein with improved transport capacity? If the C-terminus of plant plasma membrane H^+-ATPase is directly involved in autoinhibition of the enzyme, it might be through an interaction with an important functional region(s) of the ATPase[4,8] (Fig. 1b). Alternatively, the C-terminus may lock the ATPase in its low-activity state, but not necessarily by binding to sites important for catalysis[8]. The study by Morsomme *et al.*[4] identified a specific domain within the C-terminus that has a concentration of point mutations (between Trp858 and Leu893). This suggests that this domain is important for regulation and might interact, either directly or indirectly, with the four other known regions. In yeast, analysis of second-site revertants implicated in glucose regulation of PMA1 likewise suggests an interaction between the C-terminus and both the small and the large cytoplasmic loops[11]. Thus, in both plant and yeast plasma membrane H^+-ATPases, several regions that are clearly separate in the linear sequence may act to form an intramolecular receptor for a C-terminal autoinhibitory domain.

The putative conversion of plant PMA2 and AHA2 between two distinct states with different H^+-pumping properties could be in association with another protein that activates the ATPase (Ref. 4) (Fig. 1b). Protein kinase-mediated phosphorylation of the plant H^+-ATPase, which is mimicked by single point mutations,