Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Do Plant Photoreceptors Act at the Membrane Level?

J. A. Raven

Phil. Trans. R. Soc. Lond. B 1983 **303**, 403-417
doi: 10.1098/rstb.1983.0102

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1983 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Phil. Trans. R. Soc. Lond. B **303**, 403–417 (1983)
Printed in Great Britain

Do plant photoreceptors act at the membrane level?

By J. A. Raven
*Department of Biological Sciences, University of Dundee, Dundee DD*1 4*HN, U.K.*

All of the photoreceptors involved in the absorption and transduction of light energy in photosynthesis are integral (carotenoid, chlorophyll) or peripheral (phycobilin) membrane proteins. The informational photoreceptors (phytochrome) and the flavoprotein (carotenoprotein?) cryptochrome, could be integral (carotenoprotein, flavoprotein) or peripheral or soluble (phytochrome, flavoprotein) pigment–protein complexes. The primary activity of the informational photoreceptors is unlikely to involve energization of primary active transport: the solute fluxes produced in this way would not form a quantitatively significant link in the perception–transduction–response sequence. By contrast, regulation of mediated solute fluxes at the plasmalemma could effect a substantial amplification of the absorbed photon signal, i.e. a large change in moles of solute transported could result from the absorption of 1 mol of photons. Modulation of the passive influx (or active efflux) of protons or calcium ions at the plasmalemma are likely targets for regulation by photoreceptors. Calcium flux regulation is particularly attractive in view of the ubiquity of calmodulin activity in eukaryotes, although problems could arise in maintaining the uniqueness of phytochrome messages *vis-à-vis* cryptochrome messages. Temporal analysis of the relation between photoreceptor changes and electrical effects resulting from changes in ion fluxes cannot, in general, rule out the involvement of intermediates between the redox or conformational change in the photoreceptor and the observed change in ion flux. Although slow in terms of the potential rate of change on solute fluxes resulting from direct interaction of a photoreceptor and a solute porter, the observed rates of signal transduction are well in excess of any obvious 'need' on the part of the plant in terms of rates of response to environmental changes.

1. Introduction

The explicit or implicit hypothesis underlying much recent work on photoperception by plants requires that membranes are involved at an early stage in the perception–transduction–response sequence (Marmé 1977; Raven 1981; Senger 1980). It is to these stages in photoperception that this paper is addressed, with particular emphasis on the possibility that trans-membrane fluxes of solutes are early and essential events in the photoperception process. The analysis of the close temporal (and spatial?) coupling of light absorption and solute transport requires some discussion of both the nature of the photoreceptors and the sorts of transport systems with which they could interact. Particular emphasis will be placed on the distinction between direct (within a protein, or protein–protein) and indirect interaction in coupling photon absorption to changes in solute fluxes, and to the distinction between energetic and informational coupling of light absorption to solute fluxes. In addition to the permissible 'if' and 'how' of membrane involvement, the paper finally addresses the less widely acceptable question of *why* membranes are involved in photoperception in plants.

The discussion will centre mainly on oxygen-evolving photolithotrophs, and on the role of pigments other than the main photosynthetic pigments; however, these restrictions will not be rigidly adhered to when important results or hypotheses have originated from work on other organisms.

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

TABLE 1. SOME PROPERTIES OF PIGMENT–PROTEIN COMPLEXES THAT MAY BE INVOLVED IN PHOTOPERCEPTION IN PLANTS

chromophore	absorption maxima†/nm	specific absorption coefficient $m^3 mol^{-1} m^{-1}$	lifetime of excited singlet state/s	photoredox reactions *in vivo*	light-powered ion pump *in vivo*	antenna role *in vivo*	light-induced conformational change *in vivo*	references
carotenoids	445–515	1.3×10^4 (β-carotene, 451 nm)	10^{-15}	no	no	yes	(yes)	Shropshire (1980), Song (1980)
retinol[1]	500–550	4×10^3 (500 nm)		no	yes: H^+/Cl^-	?	yes	Birge (1981), Shropshire (1980), Schobert & Lanyi (1982)
flavins	450	1.5×10^3 (450 nm)	$0.6–6.0\times10^{-9}$	yes	(no?)	no	yes	Raven (1981), Schmid (1980), Song (1980)
chlorophylls[2] (and other Mg-porphyrins)	430–450; 630–700 (chlorophylls *a*, *b*, c_1, c_2)	$1.0–1.3\times10^4$ (430–450 nm); $0.12–0.80\times10^4$ (620–700 nm)	$0.5–5.0\times10^{-9}$	yes	no	yes	yes	Meeks (1974), Nobel (1974), Giaquinta *et al.* (1975)
Fe porphyrins	415, 553, 522 (reduced Cyt c_{553} of *Petalonia fascia*)	2×10^4 (reduced Cyt c_{553} of *Petalonia fascia* at 415 nm)		yes[3] (phototaxin)	(no)	no	(yes)	Lemberg (1975), Yakushiji (1971), Poff *et al.* (1974)
phycobilin chromophore	540 (phycoerythrin) 625 (phycocyanin)	1.5×10^4 (phycoerythrin, 540 nm) 1.25×10^4 (phycocyanin, 625 nm)	$0.1–1.0\times10^{-9}$	no	no	yes	(yes)	Gantt (1981), Nobel (1974)
phytochrome chromophore	660 (P_r) 730 (P_{fr})	10^4 (P_r at 660 nm; P_{fr} at 730 nm)		no	no	?[4]	yes, in $P_r \rightarrow P_{fr}$, and $P_{fr} \rightarrow P_r$	Smith (1975), Raven (1981)

The data are mainly taken from pigments found in oxygen-evolving photolithotrophs; exceptions are denoted by superscript numbers: [1] rhodopsin found in metazoan photoreceptors; bacteriorhodopsin, halorhodopsin in the bacterial genus *Halobacterium*; [2] phototaxin occurs in the slime-mould *Dictyostelium*; [3] bacteriochlorophylls in photosynthetic bacteria; [4] the likelihood of such an antenna role for phytochrome *in vivo* is greatly decreased by the absence of a spectroscopically detectable reaction partner to which excitation energy could be transferred (W. Rüdiger, personal communication).

† In decreasing order of absorbance.

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 2. Intracellular location of pigment–protein complexes in plants

pigment–protein complex	soluble in N phase	soluble in P phase	peripheral on N side of membrane	peripheral on P side of membrane	integral in membrane	references
carotenoproteins	no	no	(no) (light-harvesting peridinin–chlorophyll *a* complex of thylakoid membranes of Dinophyceae?)	no	light-harvesting and reaction centre chlorophyll–carotenoid complexes of thylakoid membranes	Song (1980), Prezelin (1981), Anderson *et al.* (1982)
retinol–opsins	no	no	no	no	rhodopsin in metazoan photoreceptor membrane; bacteriorhodopsin, halorhodopsin in *Halobacterium* plasmalemma	Birge (1981)
flavoproteins	nitrate reductase (cytosol)	glycollate oxidase (microbodies)	flavodoxin (ferredoxin replacement in some algae)	no	NADH–UQ, succinate–UQ oxidoreductase of inner mitochondrial membrane; ferredoxin–$NADP^+$ oxidoreductase in thylakoids (N side); flavoprotein–cytochrome *b* complex of *Zea*, *Neurospora* plasmalemma	Ragan (1976), Trebst (1974), Caubergs *et al.* (1983), Tolbert (1981), Husain *et al.* (1976), Ninnemann & Klemme-Wolframm (1976)
chlorophylls	no	no	(no) (see entry for carotenoproteins)	no	yes: see entry for carotenoproteins	see entry for carotenoproteins
Fe-porphyrins	phototaxin? (cytosol) catalase (plastid stroma)	catalase (microbodies)	?	*c*-type cytochromes in algal thylakoids, between mitochondrial membranes	cytochrome b–c_1 and a–a_3 complexes in inner mitochondrial membrane; cytochrome *b*–*f* complex in thylakoid membrane, Cyt *b* in plasmalemma	Hauska *et al.* (1982), Wikstrom *et al.* (1981), Caubergs *et al.* (1983), Tolbert (1981), Poff *et al.* (1974), Halliwell (1981)
photosynthetic phycobilins	no	inside thylakoid of Cryptophyceae	outside thylakoid of Cyanobacteria, Rhodophyceae	no	no	Gantt (1981), MacColl & Burns (1978)
phytochrome	yes (cytosol)	no	(yes)	no	no	Smith (1975), Haupt (1982), Marmé (1977)

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

2. Properties and location of photoreceptors

Almost all of the protein-associated chromophores that have visible absorption bands have been implicated at some time or other in the perception of light by plants. Table 1 shows some of the characteristics of pigment–protein complexes that are known to be, or may be, involved in photoperception. All of the chromophores have high specific absorption coefficients, thus making them effective photon absorbers at their respective absorption maxima. The lifetime of the excited singlet state varies with the environment of the chromophore, but is uniformly short for carotenoid and retinol pigments; despite this, the carotenoids can act as photosensitizers (Song 1980). The carotenoids and phycobilins seem to have no usable photoredox activities, unlike the rest of the pigments listed in table 1. The retinol–protein complexes are distinguished by their capacity to carry out (when membrane-associated) active proton transport apparently unrelated to internal redox reactions. Conformational changes related to photon absorption are widespread among the pigment–protein complexes.

Table 2 gives some information on the location of the major classes of pigment–protein complexes in cells, distinguishing between 'soluble', 'peripheral to membrane' and 'integral in membrane', as well as differentiating between the N and the P sides of the membranes (Singer 1974; Mitchell 1979). The carotenoproteins and retinoproteins are all integral membrane proteins, as are the Mg porphyrin-proteins; the flavoproteins and Fe porphyrin-proteins have a more catholic distribution, with different representatives of the two classes occurring as integral, peripheral and soluble pigment–protein complexes. The phycobilins, and phytochrome, are peripheral or soluble; the categories are not rigid, an example being the occurrence of phytochrome as both a soluble and a membrane-peripheral entity.

We shall see in § 3 that some integral membrane proteins can function as catalysts of solute transport across the membrane in which they occur: if these proteins are associated with a chromophore, then photon absorption by the chromophore *could* give a direct informational or energetic coupling to transport within a single polypeptide. In all other cases, a less direct coupling between photon absorption and solute transport must be envisaged, involving the direct transfer of excitation energy or conformational energy from the photoreceptor protein to the porter (by protein–protein interaction), or some less direct interaction involving intermediates between the photoreceptor and the porter.

3. Porters and their relation to photoreceptors

If photoreceptors influence transmembrane transport of solutes as a 'primary' event, this must involve catalysed (mediated) transport. The timescale (10^{-6} to 10^2 s) of these primary events is too short for any substantial synthesis or degradation of membrane components. This constraint rules out the modulation of 'lipid solution' transport of solutes through the lipid portion of the membrane as a mechanism of the primary action of photoreceptors. Transport of a neutral solute (such as carbon dioxide or oxygen) by 'lipid solution' can be described by

$$J_{oc} = P(C_o - C_i),$$

where J_{oc} is the net solute flux from phase o to phase i (mol m^{-2} s^{-1}) through a membrane whose permeability coefficient for the solute is P(m s^{-1}) when the concentrations of the solute in phases o and i respectively are C_o and C_i (mol m^{-3}). Since P for a given solute is determined, at

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

a given temperature, by the lipid composition of the membrane, short-term effects of light on J_{oc} must reflect changes in C_o or C_i, or both, and cannot be construed as reflecting a *membrane* effect on the flux J_{oc}, although the changes in C_o or C_i could be products of some other membrane effect of the photoreceptor. An example is light absorption by chlorophyll in the thylakoid membrane, which, by generating NADP and ATP, leads to net carbon dioxide fixation and thus to a net influx of carbon dioxide across the plasmalemma.

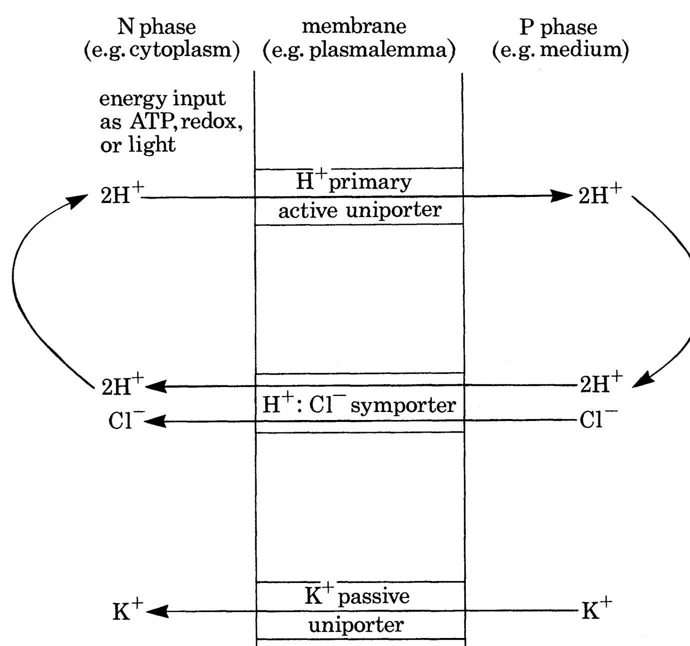

FIGURE 1. Examples of primary active transport (ATP-powered H^+ flux from N to P phase) with H^+ recirculation coupled to the secondary active transport of Cl^- from P to N phase, and electrically driven mediated passive uniport flux of K^+ from P to N phase.

Thus primary effects of light on solute transport must be sought in mediated fluxes of solutes, catalysed by intrinsic (transmembrane) protein porters. Figure 1 shows, for a case in which the proton is the working ion, primary active, secondary active, and mediated uniport processes. Light can act as energy source for primary active transport, so that a primary effect of light could be in energizing active transport (the term 'primary event' is taken to be the first detectable obligatory event in the perception–transduction–response sequence, other than the production of the excited state of the chromophore and its return to the ground state). Light could also have informational effects on the activity (i.e. the specific reaction rate of the porter under constant conditions of substrate supply) of any of the three kinds of porter. We note that, as with 'lipid solution' transport, changes in the driving forces acting on the solutes that are being transported (i.e. the chemical activity difference for the transported solute(s) between the two sides of the membrane and, for charged solutes, the electrical potential difference across the membrane) and changes in the free energy available from chemical driving reactions of primary active transport (e.g. increased free energy of ATP hydrolysis under conditions *in vivo*) cannot be construed as primary effects of light.

Table 3 indicates some of the ways in which the photoreceptors mentioned in tables 1 and 2 could influence, by energetic or regulatory means, the rate of solute transport as a primary

Downloaded from rstb.royalsocietypublishing.org

TABLE 3. POSSIBILITIES FOR PHOTORECEPTOR–PORTER INTERACTIONS IN A SINGLE MEMBRANE SYSTEM

nature and location of photoreceptor	mechanism of energy or information transfer from photoreceptor to porter	possibility of photoenergization of primary active transport	possibility of direct† photoregulation of primary or secondary active transport, or of mediated passive uniport	references
bacteriorhodopsin, halorhodopsin; integral membrane proteins	in same protein (but may function as trimer) for primary active uniporter; large lateral separation from other porters means direct (protein–protein) interaction is unlikely	yes: of protons by bacteriorhodopsin; chloride ions by halorhodopsin from P to N side of membrane	yes: bacteriorhodopsin and halorhodopsin are laterally segregated from other transporters, so the only direct photoregulation would be of the two rhodopsins themselves	Birge (1981), Schobert & Lanyi (1982)
reaction-centre chlorophyll (P_{700}, P_{680}) proteins (flavoproteins? haem-proteins?); integral membrane proteins	in same protein for primary active transport	yes: of electrons from P to N side of membrane	yes: within molecules, and (?) via conformational change effects on other porters; excitation energy migration out of reaction centres not via antenna pigments	Junge (1977), Raven (1981)
antenna chlorophyll, carotenoid pigment–protein complexes; integral membrane proteins	mostly not in same protein as photochemically active chlorophylls (P_{700} and P_{680}); excitation energy transfer to reaction centre from antenna pigment-proteins in the reaction centre complexes or light-harvesting complexes	yes: by excitation energy transfer to reaction centres	yes: in 'artefactual' protochlorophyll–Ca^{2+} porter membrane systems	Raven (1981)
antenna phycobilins; peripheral thylakoid phycobilisomes, soluble in intrathylakoid space of Cryptophyceae	not in same protein as P_{700} and P_{680}; excitation energy transfer to reaction centre	yes: by excitation energy transfer to reaction centres	yes, but not demonstrated	Gantt (1981)
any integral photoreceptor complex	result of photoinduced conformational change in photoreceptor: alters porter	unlikely that enough energy could be transferred	yes, but not demonstrated	Lemberg (1975), Giaquinta *et al.* (1975)
any peripheral photoreceptor complex (e.g. phytochrome)	result of photoinduced conformational change in photoreceptor: alters porter	unlikely that enough energy could be transferred	yes, but not demonstrated	Smith (1975)

† Direct means, in this context, an interaction between an integral protein porter and a photoreceptor protein in or on the same membrane by means of excitation energy transfer or conformational energy transfer (see Lemberg 1975).

Downloaded from rstb.royalsocietypublishing.org

action of the photoreceptor. By far the best understood of the various couplings of photon absorption to transmembrane fluxes are the energetic couplings of the bacteriorhodopsin-halorhodopsin and the chlorophyll–bacteriochlorophyll systems (see references in table 3). The analysis of these processes has been aided by the relative ease with which the relevant membranes can be extracted and purified, and the large fraction of the membrane protein that consists of the pigment–protein complexes; these factors greatly facilitate the investigation of both the photophysical and photochemical (and the transmembrane flux) aspects of the primary action of light. The much more 'dilute' nature of membrane-associated phytochrome or of flavoprotein (see table 2 of Raven (1981), and § 4*a*) means that any primary effect of solute transport is less readily investigated; other experimental problems with these two systems will be noted as the discussion proceeds.

4. Quantitative constraints on photoreceptor action at membranes

The two main groups of constraints with which I shall deal are those related to the density of photoreceptors and porters in (or on) membranes, the stoichiometry between photons absorbed and molecules of solute transported by an associated porter and thus to the relation between incident photons and transmembrane solute flux, and the problems of temporal analysis of the relation between photon absorption and solute transport, which is crucial to determining if the effect on solute transport is a primary action of the photoreceptor. It is important to note that such constraints as the low density of photoreceptors on membranes, or the long time taken to complete a photochemical cycle, which are found with phytochrome for example, and not intrinsic to photoreceptors, but are specialities of some of the informational, as opposed to the energetic, photoreceptors (Birge 1981).

(*a*) *Density and specific reaction rates of porters and photoreceptors*

Raven (1981) has attempted to relate the density of photoreceptors in (or on) membranes, by means of the specific absorption coefficients of the photoreceptors, to the rate of photon absorption per unit membrane area at a given incident photon flux density. Table 2 of Raven (1981) shows that the density of pigment molecules in the thylakoid membrane (chlorophylls plus carotenoids plus, where they are present, the phycobilins on the membrane) is some 2–3 μmol m^{-2}, while that of flavoprotein at the plasmalemma is some 2.5 nmol m^{-2}, and that of phytochrome at the plasmalemma is only some 0.33 nmol m^{-2} even if all of the cell phytochrome is associated with the plasmalemma. To illustrate the magnitude of fluxes which could be achieved at various photon flux densities if these photoreceptors were to energize active transport, I shall take the hypothetical case of a stoichiometry of 1 photon absorbed for each 1 proton transported for primary active proton transport. The assumed stoichiometry is mechanistically rather than thermodynamically constrained, in that there is much more energy per mole of photons (some 180 kJ mol^{-1} in the red region of the spectrum, and 260 kJ mol^{-1} in the blue region) than is required to pump one mole of protons from an N to a P phase (typical minimum energy requirements of 20–30 kJ mol^{-1}) (see Raven & Smith 1980).

Raven (1981) computed that, with an incident photon flux density of 500 μmol $m^{-2}\,s^{-1}$ at the wavelength of maximum absorption by the pigment, the flavoprotein and phytochrome densities mentioned above would give proton fluxes of 4.0–4.5 nmol $m^{-2}\,s^{-1}$, the similarity of the two fluxes in the face of the different density per unit membrane area being explained by

Downloaded from rstb.royalsocietypublishing.org

the compensating differences in specific absorption coefficient (see table 1; also Raven 1981). These values are some four orders of magnitude lower than the proton fluxes at the same incident photon flux density in thylakoid membranes. In all cases, however, I have assumed that photon absorption rate determined the proton flux; in reality this is unlikely to be true at an incident photon flux density of 500 μmol m^{-2} s^{-1}, and the proton flux across the thylakoid membrane is likely to be restricted to some 5 μmol m^{-2} s^{-1} by limitations in the reoxidation rate of reduced plastoquinone (Raven 1980; Raven & Smith 1980). For phytochrome the restrictions on the ratio of photons absorbed to protons moved are likely to be less severe, in that the absence of a 'phytochrome unit' analogous to 'photosynthetic units' means that each photon absorbed by phytochrome is used independently rather than being transferred to reaction centres present at some 1 per 400–500 chlorophylls for each of the two photoreactions; however, if energization by phytochrome involves the full P_r–P_{fr}–P_r cycle, I would envisage a specific reaction rate of some 0.83 protons moved per phytochrome molecule per second (see §4*b*) rather than the 12.7 s^{-1}, corresponding to a flux of 4.1 nmol m^{-2} s^{-1}. Accordingly, the flux might be reduced to 0.27 nmol protons m^{-2} s^{-1}.

Substantial photobehavioural or photomorphogenetic responses occur at a photon flux density of 1 μmol m^{-2} s^{-1} at the wavelength of maximum absorption of the photoreceptor; here the proton fluxes are reduced to 8.2–8.6 pmol m^{-2} s^{-1} at the plasmalemma for phytochrome and flavoprotein, while the thylakoid proton flux would be some 60 nmol m^{-2} s^{-1}. The context in which all of these fluxes should be viewed is that of the 'background' proton recirculation (cf. figure 1) at the eukaryote plasmalemma of 1 μmol m^{-2} s^{-1}, and an irreducible minimum of uncatalysed 'leak' plus mediated 'slippage' downhill proton fluxes at biological membranes of some 10–20 nmol m^{-2} s^{-1} (Raven 1980; Raven & Smith 1980; Raven & Beardall 1981, 1982; Richardson *et al.* 1983). It is very unlikely that the cell could distinguish between the light-powered proton fluxes of some 10 pmol m^{-2} s^{-1} at a photon flux density of 1 μmol m^{-2} s^{-1} from the 'background' proton flux of up to 1 μmol m^{-2} s^{-1}: it is very likely that such small photoreceptor fluxes of protons would be lost in the noise associated with the background fluxes. However, it is still possible that photoperception of the informational (as opposed to the energetic) type could result from photons absorbed by a photoreceptor having active transport as their primary action. The ciliate *Stentor coeruleus* uses the pigment–protein complex stentorin (with a hypericin-like *meso*-naphthodianthroquinone chromophore) as photoreceptor for its photophobic response (Wood 1970, 1973, 1976; Walker *et al.* 1979, 1981; Song *et al.* 1980). It is possible that the primary action of this chromoprotein is the energizing of active proton transport into cortical vesicles whose bounding membranes contain the stentorin (Walker *et al.* 1981). This possibility is supported by the large amount of stentorin present in the ciliate: the references cited above suggest that *Stentor coeruleus* contains some 0.2 mol m^{-3} of stentorin (specific absorption coefficient 5×10^3 m^3 mol^{-1} m^{-1}), which permits 10% of the maximum photophobic response to be exhibited at an incident photon flux density of 0.5 μmol m^{-2} s^{-1} of 'red' light. Thus a photobehaviour photoreceptor acting by energizing primary active transport must be present in cells at substantially higher concentrations (0.2 mol m^{-3}) than the phytochrome (0.1 mmol m^{-3}) or plasmalemma-associated flavoprotein (1 mmol m^{-3}) pigments (cf. Raven 1981). The stentorin concentration in *Stentor coeruleus* is, in fact, at the lower end of the range of concentrations found for photosynthetic pigments in oxygen-evolving organisms (table 1 of Raven *et al.* 1979). It is accordingly likely that photoperception by energizing primary active transport requires as a necessary (but not a sufficient) condition that the cells be almost as densely pigmented as

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

phototrophic cells are. Pigments whose primary functions are the harvesting and transduction of light energy for growth of phototrophs also have an important informational role in regulating behaviour and metabolism (Raven 1981; Stoeckenius & Bogomolni 1982).

Turning to the more plausible mechanism (for photoreceptors present at relatively low concentrations in the cell) of the regulation of active or passive mediated fluxes, the maximum specific reaction rates range from 10^2–10^3 s^{-1} (primary active transport of protons or chloride ions), to 10^4 s^{-1} (secondary active transport) to 10^7–10^8 s^{-1} (mediated passive uniport) (Raven 1980; Raven & Smith 1980). The specific reaction rates for mediated passive uniport of 10^7 s^{-1} (calcium (Reuter 1983)) and 10^8 s^{-1} (sodium (Hille 1970)) are close to the limits imposed by the rate of collision of ions with the uniporter from the experimental ion concentrations used (Lauger 1973). Regulation of such porters could achieve very substantial amplification of the photon-induced change in the photoreceptor: the ultimate would be a single molecule of, for example, phytochrome associated with a passive uniporter that had a specific reaction rate of 0 s^{-1} when phytochrome was in the P_r form but had a specific reaction rate of 10^8 s^{-1} when phytochrone was in the P_{fr} form (assuming that the ion concentration were in excess of 100 mol m^{-3} to prevent collision limitation!). Even a modest uniporter specific reaction rate of 10^3 s^{-1} for P_{fr}-activated uniporters present at the same density as phytochrome (0.33 nmol m^{-2}) would give an ion flux of 330 nmol m^{-2} s^{-1}, which (if the ion were the proton) would be greatly in excess of the minimum leakage plus slippage flux of 10–20 nmol m^{-2} s^{-1} (see above), and not much less than the maximum proton recirculation flux of 1 μmol m^{-2} s^{-1}. With a specific photon absorption rate by phytochrome of 0.025 s^{-1} with an incident photon flux density of 1 μmol m^{-2} s^{-1}, only 40 s would be needed to convert all the P_r to P_{fr} and thus activate all the uniporters (cf. Raven 1981). The magnitude of such mediated uniport (downhill) influxes is such that they would have large electrical effects (depolarized potential difference; increased conductance), which could be part of the transduction process. More specific effects result from the effects of the net ion fluxes on the intracellular free calcium and proton concentrations (cf. the much lower 'normal' bidirectional calcium fluxes at the plant cell plasmalemma (Macklon & Sim 1981)).

For protons, the net passive influx of 330 nmol m^{-2} s^{-1}, with a 500 nm thick layer of cytosol and a proton buffer capacity of 10 mol proton m^{-3} $(pH)^{-1}$ would give a pH change in the cytosol at 0.066 pH s^{-1}, by no means negligible in terms of the 'permitted' change in cytosol pH of perhaps 1 pH (Smith & Raven 1979). An analogous calculation for calcium would, with a calcium buffer capacity of 10 mmol calcium m^{-3} $(pCa)^{-1}$ m^{-1}, yield the same rate of change as for protons, i.e. 0.066 pCa s^{-1} (cf. Raven 1977). While there are homeostatic mechanisms for pH and pCa (both normally about 7.0) in plant cytosol in addition to 'passive buffering', and (as a more temporally pressing matter) some ionic countermovements must occur in the face of a postulated calcium influx of 330 nmol m^{-2} s^{-1}, which, if unbalanced, would give a cell depolarization rate of 3.3 V s^{-1}, modulation of passive uniport by photoreceptors could yield significant electrical and cytosol activity effects that could act as part of a signal transduction sequence. There is evidence that both proton movements and calcium movements across the plasmalemma are important early (tens of seconds or more) products of activation of phytochrome and cryptochrome in plant cells (Marmé 1977; Raven 1981; Senger 1980), so that modulation of active or passive fluxes of these ions could be a primary action site for these photoreceptors.

31-2

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

(b) *Temporal analysis of photoreceptor action in relation to membrane processes*

The time course of photoreceptor action on solute fluxes is of clear importance in establishing whether the changed solute fluxes are indeed the primary effect of photoreceptor activity. Ideally, the time course of phototransformation of the pigment would be compared with the time course of the change in solute flux, with the latter (for charged solutes) being measured by microelectrode techniques for transplasmalemma electrical potential difference or conductance, or both, to overcome problems of extracellular diffusion lags in the chemical or radiochemical estimation of fluxes. To illustrate how complex such temporal analyses can be, even under very favourable circumstances, I shall first consider a photoelectrical phenomenon of no known photobehavioural or photomorphogenetic significance, i.e. the effect of high photon flux densities of green light on the electrical potential difference at the plasmalemma of the marine coenocytic algae *Acetabularia mediterranea* and *A. crenata* (Schilde 1968; Gradmann 1978). The elegant analysis by Gradmann (1978) showed that green light inhibits the ATP-driven primary active chloride influx of these cells with a lag of less than 40 μs. Even such a short lag is consistent with the diffusion of a low molecular mass chemical messenger from the photoreceptor to the chloride pump over a distance of up to 200 nm, provided that other reactions in the transduction sequence are essentially instantaneous. We note that the slowest reaction in the *Acetabularia* chloride pump reaction sequence has a rate constant in excess of $10^3\ s^{-1}$ when the pump is working at its maximum rate (Gradmann 1978). Although it is likely that the green light effect on the *Acetabularia* chloride pump does involve light absorption by a pump component, or an adjacent membrane component, the possibility of a 200 nm diffusion path being compatible with a lag of 70 μs between initiation of illumination and the first measurable electrical response is salutary in the context of the precision of location 'plasmalemma' photoreceptors by polarized light and microbeam experiments (Haupt 1982), and of the 500 nm thickness of the cytosol of many vacuolate higher plant cells (Macklon 1975).

The shortest lag is an electrical response that can be related to a photobehavioural event is the 1 ms lag between blue light irradiation and a calcium-dependent transcellular electrical potential difference in the chlorophycean flagellate *Haematococcus pluvialis* (Litvin *et al.* 1978). This electrical effect appears to be an essential part of the organism's phototactic response mechanism (cf. Raven 1981). The 1 ms lag is consistent with a diffusion distance inside the cell of 2.8 μm, i.e. a significant fraction of the cell radius and 14 times the diameter of the flagella axes. We note that the total time from 'light on' to the peak electrical potential difference, i.e. about 5 ms at saturating light, is very short compared with the 5 s half-time for the flavoprotein-sensitized reduction of cytochrome *b* in eukaryote plasmalemma, which *may* be a part of the cryptochrome reaction (Brain *et al.* 1977). Although such a slow cytochrome reduction might be a part of the reaction sequence for the cryptochrome effect on electrical potential difference in *Phaseolus vulgaris* hypocotyls (1–5 s lag time (Hartmann & Schmid 1980)) or *Onoclea sensibilis* protonemata (10 s or more (Racusen & Cooke 1982)), the involvement of the much slower (half time of 35 s) reoxidation of the cytochrome by molecular oxygen (Brain *et al.* 1977) in the reaction sequence is dubious, particularly in view of reports of the absence of an oxygen requirement for the induction (Gressel *et al.* 1973) or even the whole response (Kowallick & Gaffron 1967) of certain cryptochrome effects.

The fastest reported phytochrome effect on electrical potential difference (4.5 s after initiation of the red light treatment in *Avena sativa* coleoptiles (Newman 1981)) is slower than some effects

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

of chloroplast-absorbed light on the plasmalemma electrical properties of *Nitella translucens* (1–2 s (Vredenberg 1969)). Thus signals that have to pass from the thylakoid membrane, through the 'tight' inner plastid envelope membrane and the 'leaky' outer plastid envelope membrane, to the plasmalemma have no longer lag time in inducing electrical effects at the plasmalemma than does phytochrome, which may be associated with the N (cytosol) side of the plasmalemma. However, the delay in electrical response to light absorption by P_r does not necessarily imply a substantial diffusion path for a chemical messenger from photoreceptor to porter, because the time taken for the P_r–P_{fr} conversion is by no means negligible in the context of lags of seconds. Briggs & Fork (1969) showed that the half-time for the P_r–P_{fr} conversion was some 0.6 s (as was that for the P_{fr}–P_r photoconversion). This temporal constraint does not apply to reactions in which there is a direct transfer of excitation energy from P_r to for example a primary active transport porter (cf. the assumption made earlier that energization might require the full P_r–P_{fr}–P_r photocycle with, implicitly, a conformational mechanism of energy transfer to a pump); however, the excitation energy transfer mechanism precludes the demonstration of phytochrome's involvement by the criterion of red–far-red reversibility. It is significant that in a number of cases the effect of red light on electrical phenomena, and not merely their induction, can be reversed by far-red light, suggesting that the ion-transport effects are reversibly regulated by the phytochrome system.

We may conclude that the temporal data available do not rule out a direct interaction of cryptochrome or phytochrome with mediated transport at the plasmalemma, but they do not exclude the involvement of an intermediate between photoreceptor and porter that can diffuse over 1 μm or more. We note that the vertebrate retinal rod, a much-studied photoreceptor system, seems to have guanine nucleotide interconversions as the first part of the perception–transduction–response sequence following light absorption by rhodopsin; calcium and calmodulin are involved later, followed by a decrease in the sodium permeability of the plasmalemma (Miller 1981; Birge 1981). Here it seems (Birge 1981) that speed of response is sacrificed to precision, i.e. to decreasing the likelihood of spurious signals, an interesting contrast to the much more rapid response of the energy-transforming bacteriorhodopsin system (see §5).

Lest it be thought that this discussion of the temporal analysis of the interaction of photoreceptors with transmembrane fluxes has ended on a pessimistic note, i.e. that the time course of the effects of phytochrome or cryptochrome is such that the question of 'direct' or 'indirect' interaction of photoreceptor and porters cannot be resolved, it is important to point out that there are other ways of approaching this problem. The most direct (but most technically demanding) would be the 'classical' extraction and reconstitution approach; here isolated and purified membranes (and, if non-integral, photoreceptors) would be tested for the occurrence of photoreceptor–porter coupling. Such experiments could show what components were needed to obtain this coupling; even more convincing would be reconstitution experiments with purified photoreceptor and porter in liposomes (Racker 1976). However, such experiments cannot be conducted until we know a great deal more about cryptochrome and plasmalemma porters in plants.

5. Why membranes?

Finally, we may ask why membranes should be involved in photoperception in plants: is it 'evolutionary inertia' or can some selective advantage be construed in membrane-associated photoperception in extant plants? Tackling the 'evolutionary inertia' question first, a plausible

Downloaded from rstb.royalsocietypublishing.org

evolutionary speculation has been presented by Carlile (1980; cf. Seliger & McElroy 1965; Presti & Delbrück 1978). Early organisms would have been chemoorganotrophs, growing on abiologically photosynthesized organic compounds. As burgeoning life used these organic compounds, the increasingly thin primeval soup might mean that motile organisms with chemotaxis would be at a selective advantage: the chemoreceptors were probably in the plasmalemma of these organisms (cf. Lengeler 1982). Eventually the evolution of (biological) photosynthesis overcame this primeval energy crisis, with membrane-associated mechanisms for light-energy transduction (Raven & Smith 1981). Carlile (1980) points out that the association of the membrane-associated photosynthetic photoreceptor with the pre-existing chemotactic system, with its chemosensory apparatus in the plasmalemma, could have led to a phototactic system, with a selective advantage in terms of optimizing the position of the organism in the aquatic photon flux density gradient. This optimization is construed by Carlile (1980) in photosynthetic terms, although it is also possible that an involvement of the photoreactivation system (Presti & Delbrück 1978) as a photoreceptor for phototaxis related to the avoidance of high photon flux densities of u.v. could have occurred. At all events, the intervening 2×10^9 or so years would seem to have given ample time for evolutionary change of the location of photoreceptors, particularly if the mechanism did not involve net storage of light energy as chemical energy (note that even bioluminescence, which deals in the production of blue quanta of some 260 kJ mol^{-1} energy content, is not membrane-associated (Hastings 1975)).

Having bought forward evidence that phytochrome and cryptochrome do not operate via net membrane-associated energy storage (§4*a*), it is worth considering what selective pressures might be involved in keeping a photoreceptor system associated with membranes. Birge (1981) and Miller (1981) have recently discussed the attributes of photosensory systems: it would appear that the maintenance of a high signal:noise ratio and the necessary amplification of the signal in the transduction mechanism militates against extremely rapid responses in photosensory systems. We note that the very rapid effect of green light on the *Acetabularia* chloride pump does not involve amplification, or a large signal:noise ratio (Schilde 1968; Gradmann 1978). I have already shown that large amplification factors are needed to get good signal:noise ratios for photosensory proton fluxes: the same is true for photosensory calcium fluxes when there are large net calcium fluxes associated with intracellular $CaCO_3$ precipitation (Raven 1981). Overall, unless 'non-nutrient' solute fluxes are being regulated, there would not seem to be any advantage in using transmembrane fluxes as part of a photosensory mechanism in terms of maximizing the signal:noise ratio.

Granted the intrinsic slowness of some photoreceptor events (e.g. phytochrome phototransformations), are there situations in which the rapid response (milliseconds rather than seconds) of some photoreceptors can be of advantage to the organism? A good case (see above) is the rapid (5 ms or less) photoelectric response, related to phototaxis, in *Haematococcus pluvialis* (Litvin *et al.* 1978). An important potential 'use' of phototaxis of motile microorganisms, and of plastids in non-motile plants as well as of leaf photonasty in terrestrial plants, is the avoidance of photoinhibition of photosynthesis (Samuelsson & Richardson 1982; Björkman & Powles 1981; Powles & Björkman 1981; cf. Nultsch *et al.* 1981). In *Oxalis oregana* a very good case has been made out for cryptochrome-mediated photonasty in reducing light interception by leaves, and hence in reducing photoinhibition. When a sunfleck (photon flux density 1600 μmol m^{-2} s^{-1}) replaces the normal diffuse forest-floor visible radiation (4 μmol m^{-2} s^{-1}), leaf folding can be completed in 6 min, in which time very little photoinhibition has occurred (Björkman &

Downloaded from rstb.royalsocietypublishing.org

BIOLOGICAL SCIENCES THE ROYAL B SOCIETY PHILOSOPHICAL TRANSACTIONS OF

Powles 1981; Powles & Björkman 1981). However, the difference between a lag of milliseconds and one of seconds before the arrival of a sunfleck is translated into detectable leaf movement would not seem to be of great moment in this situation. This is true *a fortiori* of the phototaxis of motile phototrophs. Samuelsson & Richardson (1982) showed that the accumulation of *Amphidinium carterae* in a particular region of a photon flux density gradient could be interpreted in terms of maximizing photosynthesis while preventing photoinhibition, which can set in at 80 μmol m^{-2} s^{-1} in this shade-adapted dinoflagellate. However, even with a swimming velocity of 0.5 mm s^{-1}, and a large (for open water (Spence 1981)) vertical attenuation coefficient of visible radiation of 0.5 m^{-1}, it would take over half an hour for a dinophyte in stratified water to swim from a definitely photoinhibitory photon flux density of 80 μmol m^{-2} s^{-1} to a non-photoinhibitory 40 μmol m^{-2} s^{-1}, again making the difference between millisecond and second lags seem irrelevant. For photomorphogenetic rather than photobehavioural responses an even stronger case can be made out for the irrelevance of reducing lag times to below a second.

In conclusion, it is not easy to see any overwhelming advantage in having photoreceptors in plants associated with membranes: their messages are not generally destined for rapid transmission by action potentials, because plants with rapid responses to environmental changes (e.g. carnivores like *Dionaea*) wisely rely on touch rather than shading (Bentrup 1979), thus avoiding the embarrassment of closing their traps on shadows. The hypothesis that photoreceptor action is membrane-associated has generated many useful ideas and experimental results; however, one cannot help feeling that it is a cruel irony that makes the first measureable effects of photoreceptor activity on membrane activity faster than the plant (to our imperfect perception) 'needs', yet not fast enough to avoid ambiguity as to the interaction (direct or indirect) between photoreceptor and porter.

Dr K. Richardson has contributed vigorous discussion of the material in this article. Professor W. Rüdiger has provided important counsel on the possibility of phytochrome's acting as an antenna (sensitizer) *in vivo*.

References

Anderson, J. M., Barrett, J. & Thorne, S. W. 1982 Chlorophyll–protein complexes of photosynthetic eukaryotes and prokaryotes: properties and functional organisation. In *Photosynthesis*, vol. 3 (*Structure and molecular organisation of the photosynthetic apparatus*) (ed. G. Akoyonoglou), pp. 301–315. Glenside, Pennsylvania: Balaban International Scientific Services.

Bentrup, H. W. 1979 Reception and transduction of electrical and mechanical stimuli. In *Encyclopedia of plant physiology* (*new series*) (ed. W. Haupt & M. Feinberg), vol. 7 (*Physiology of movements*), pp. 42–70. Berlin: Springer-Verlag.

Birge, R. R. 1981 Photophysics of light transduction in rhodopsin and bacteriorhodopsin. *A. Rev. Biophys. Bioengng* **10**, 315–354.

Björkman, O. & Powles, S. B. 1981 Leaf movements in the shade species *Oxalis oregana*. I. Response to light level and light quality. *Yb. Carnegie Instn Wash.* **80**, 59–62.

Brain, R. D., Freeberg, J. A., Weiss, C. V. & Briggs, W. R. 1977 Blue light-induced absorbance changes in membrane fractions from corn and *Neurospora*. *Pl. Physiol.* **59**, 948–952.

Briggs, W. R. & Fork, D. C. 1969 Long-lived intermediates in phytochrome transformations. I. *In vitro* studies. *Pl. Physiol.* **44**, 1081–1089.

Carlile, M. J. 1980 The biological significance and evolution of photosensory systems. In *The blue light syndrome* (ed. H. Senger), pp. 3–4. Berlin: Springer-Verlag.

Caubergs, R., Widell, S., Larsson, C. & De Greef, J. A. 1983 Comparison of two methods for the preparation of a membrane fraction of cauliflower inflorescences containing a blue light reducible *b*-type cytochrome. *Physiologia Pl.* **57**, 291–295.

Gantt, E. 1981 Phycobilisomes. *A. Rev. Pl. Physiol.* **32**, 327–347.

Downloaded from rstb.royalsocietypublishing.org

Giaquinta, R. T., Ort, D. R. & Dilley, R. A. 1975 The possible relationship between a membrane conformational change and a photosystem two dependent H^+ ion accumulation and ATP synthesis. *Biochemistry, Wash.* **14**, 4392–4396.

Gradmann, D. 1978 Green light (550 nm) inhibits electrogenic Cl^- pump in the *Acetabularia* membrane by permeability increase to the carrier ion. *J. Membrane Biol.* **44**, 1–24.

Gressel, J., Bar-Lev, S. & Galun, E. 1975 Blue light induced response in the absence of free oxygen. *Pl. Cell Physiol.* **16**, 367–370.

Halliwell, B. 1981 *Chloroplast metabolism. The structure and function of chloroplasts in green leaf cells.* Oxford: Clarendon Press.

Hartmann, E. & Schmid, K. 1980 Effects of UV and blue light on the biopotential changes in etiolated hypocotyl hooks of dwarf beans. In *The blue light syndrome* (ed. H. Senger), pp. 221–237. Berlin: Springer-Verlag.

Hastings, J. W. 1975 Bioluminescence: from chemical bonds to photons. In *CIBA Foundation Symposium* no. 31, pp. 125–146.

Haupt, W. 1982 Light-mediated movement of chloroplasts. *A. Rev. Pl. Physiol.* **33**, 205–233.

Hauska, G., Gabellini, N., Hurt, E., Krinmer, M. & Lockau, W. 1982 Cytochrome *b/c* complexes with polyprenyl quinol: cytochrome *c* oxidoreductase activity from *Anabaena variabilis* and *Rhodopseudomonas sphaeroides* GA: comparison of preparations from chloroplasts and mitochondria. *Biochem. Soc. Trans.* **10**, 340–341.

Hille, B. 1970 Ionic channels in nerve membranes. *Prog. Biophys. molec. Biol.* **31**, 3–32.

Husain, A., Hutson, K. G., Andrew, P. W. & Rogers, L. J. 1976 Flavodoxin from a red alga. *Biochem. Soc. Trans.* **4**, 488.

Junge, W. 1977 Membrane potentials in photosynthesis. *A. Rev. Pl. Physiol.* **28**, 503–536.

Kowallik, W. & Gaffron, H. 1967 Enhancement of respiration and fermentation in algae by blue light. *Nature, Lond.* **215**, 1038–1040.

Lauger, P. 1973 Ion transport through pores: a rate-theory analysis. *Biochim. biophys. Acta* **311**, 423–441.

Lemberg, M. R. 1975 Conformational changes in hemoproteins of the respiratory chain. *Ann. N.Y. Acad. Sci.* **244**, 72–79.

Lengerler, J. 1982 The biochemistry of chemoperception, signal-transduction and adaptation in bacterial chemotaxis. In *Plasmalemma and tonoplast, their functions in the plant cell* (ed. D. Marmé, E. Marré & R. Hertel), pp. 337–344. Amsterdam: North-Holland.

Litvin, F. F., Sineshchekov, O. A. & Sineshchekov, V. A. 1978 Photoreceptor electric potential in the phototaxis of the alga *Haematococcus pluvialis. Nature, Lond.* **271**, 476–478.

MacColl, R. & Burns, D. S. 1978 Energy transfer studies on cryptophycean biliproteins. *Photochem. Photobiol.* **27**, 343–349.

Macklon, A. E. S. 1975 Cortical cell fluxes and transport to the stele in excised root segments of *Allium cepa* L. I. Potassium, sodium and chloride. *Planta* **122**, 109–130.

Macklon, A. E. S. & Sim, A. 1981 Cortical cell fluxes and transport to the stele in excised root segments of *Allium cepa* L. IV. Calcium as affected by its external concentration. *Planta* **152**, 381–387.

Marmé, D. 1977 Phytochrome: membranes as possible sites of primary action. *A. Rev. Pl. Physiol.* **28**, 173–198.

Meeks, J. C. 1974 Chlorophylls. In *Algal physiology and biochemistry* (ed. W. D. P. Stewart), pp. 161–175. Oxford: Blackwells Scientific.

Miller, W. H. 1981 Calcium and cyclic GMP. *Curr. Top. Membrane Tr.* **15**, 441–445.

Mitchell, P. 1979 Direct chemiosmotic ligand conduction in protonmotive complexes. In *Membrane bioenergetics* (ed. C. P. Lee, G. Schatz & L. Ernster), pp. 361–372. Reading, Massachusetts: Addison-Wesley.

Newman, I. A. 1981 Rapid electrical response of oats to phytochrome show membrane processes unrelated to pelletability. *Pl. Physiol.* **68**, 1494–1499.

Ninnemann, H. & Klemm-Wolfgramm, E. 1980 Blue light-controlled conidiation and absorbance change in *Neurospora* are mediated by nitrate reductase. In *The blue light syndrome* (ed. H. Senger), pp. 238–243. Berlin: Springer-Verlag.

Nobel, P. S. 1974 *An introduction to biophysical plant physiology.* San Francisco: W. H. Freeman & Co.

Nultsch, W., Pfau, J. & Ruffer, U. 1981 Do correlations exist between chromatophore arrangement and photosynthetic activity in seaweeds? *Mar. Biol.* **62**, 111–117.

Poff, K. L., Loomis, W. F. & Butler, W. L. 1974 Isolation and purification of the photoreceptor pigment associated with phototaxis in *Dictyostelium discoideum. J. biol. Chem.* **249**, 2164–2167.

Powles, S. B. & Björkman, O. 1981 Leaf movement in the shade species *Oxalis oregana.* II. Role in protection against injury by intense light. *Yb. Carnegie Instn Wash.* **80**, 63–66.

Presti, D. & Delbrück, M. 1978 Photoreceptors for biosynthesis, energy storage and vision. *Pl. Cell Envir.* **1**, 81–100.

Prezelin, B. B. 1981 Light reactions in photosynthesis. In *Physiological bases of phytoplankton ecology* (ed. T. Platt) (*Can. Bull. Fish. Aquat. Sci.* **210**), 1–43.

Racker, E. 1976 *A new look at mechanisms in bioenergetics.* New York: Academic Press.

Racusen, R. H. & Cooke, T. J. 1981 Electrical changes in the apical cells of the fern gametophyte during irradiation with photomorphogenetically active light. *Pl. Physiol.* **70**, 331–334.

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ragan, C. I. 1976 NADH-ubiquinone oxidoreductase. *Biochim. biophys. Acta* **456**, 249–290.
Raven, J. A. 1977 H^+ and Ca^{2+} in phloem and symplast: relation of relative immobility to the cytoplasmic nature of the transport paths. *New Phytol.* **79**, 465–480.
Raven, J. A. 1980 Nutrient transport in microalgae. *Adv. Microb. Physiol.* **21**, 47–226.
Raven, J. A. 1981 Light quality and solute transport. In *Plants and the daylight spectrum* (ed. H. Smith), pp. 375–390. London: Academic Press.
Raven, J. A. & Beardall, J. 1981 The intrinsic permeability of biological membranes to H^+: significance for low rates of energy transformation. *FEMS Microbiol. Lett.* **10**, 1–5.
Raven, J. A. & Beardall, J. 1982 The lower limit of photon fluence rate for phototrophic growth: the significance of 'slippage' reactions. *Pl. Cell Envir.* **5**, 117–124.
Raven, J. A. & Smith, F. A. 1980 The chemiosmotic approach. In *Plant membrane transport: current conceptual issues* (ed. R. M. Spanswick, W. J. Lucas & J. Dainty), pp. 161–178. Amsterdam: Elsevier/North-Holland.
Raven, J. A. & Smith, F. A. 1981 H^+ transport in the evolution of photosynthesis. *BioSystems* **14**, 95–111.
Raven, J. A., Smith, F. A. & Glidewell, S. M. 1979 Photosynthetic capacities and biological strategies of giant-celled and small-celled macro-algae. *New Phytol.* **83**, 299–309.
Reuter, H. 1983 Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature, Lond.* **301**, 569–574.
Richardson, K., Beardall, J. & Raven, J. A. 1983 Adaptation of unicellular algae to irradiance: an analysis of strategies. *New Phytol.* **93**, 157–191.
Samuelsson, G. & Richardson, K. 1982 Photoinhibition at low quantum flux densities in a marine coastal dinoflagellate (*Amphidinium carterae*). *Mar. Biol.* **70**, 21–26.
Schilde, C. 1968 Schnelle photoelektrische Effecte der Alge *Acetabularia*. *Z. Naturf.* **23b**, 1369–1376.
Schmid, G. 1980 Conformational changes caused by blue light. In *The blue light syndrome* (ed. H. Senger), pp. 198–204. Berlin: Springer-Verlag.
Schobert, B. & Lanyi, J. K. 1982 Halorhodopsin is a light-driven chloride pump. *J. biol. Chem.* **257**, 10303–10313.
Seliger, H. H. & McElroy, W. D. 1965 *Light: physical and biological action.* New York: Academic Press.
Senger, H. 1980 (ed.) *The blue light syndrome.* Berlin: Springer-Verlag.
Shropshire, W. R. Jr 1980 Carotenoids as primary photoreceptors in blue-light responses. In *The blue light syndrome* (ed. H. Senger), pp. 172–186. Berlin: Springer-Verlag.
Singer, S. J. 1974 The molecular organisation of membranes. *A. Rev. Biochem.* **43**, 805–834.
Smith, F. A. & Raven, J. A. 1979 Intracellular pH and its regulation. *A. Rev. Pl. Physiol.* **30**, 289–311.
Smith, H. 1975 *Phytochrome and photomorphogenesis.* London: McGraw-Hill.
Song, P. S. 1980 Spectroscopic and photochemical characterisation of flavoproteins and carotenoproteins as blue light photoreceptors. In *The blue light syndrome* (ed. H. Senger), pp. 157–171. Berlin: Springer-Verlag.
Song, P. S., Hader, D.-P. & Poff, K. L. 1980 Step-up photophobic response in the ciliate, *Stentor coeruleus*. *Arch. Microbiol.* **126**, 181–186.
Spence, D. H. N. 1981 Light quality and plant response underwater. In *Plants and the daylight spectrum* (ed. H. Smith), pp. 245–275. London: Academic Press.
Stoeckenius, W. & Bogomolni, R. A. 1982 Bacteriorhodopsin and related pigments of Halobacteria. *A. Rev. Biochem.* **51**, 587–616.
Tolbert, N. E. 1981 Metabolic pathways in peroxisomes and glyoxysomes. *A. Rev. Biochem.* **50**, 133–157.
Trebst, A. 1974 Energy conservation in photosynthetic electron transport of chloroplasts. *A. Rev. Pl. Physiol.* **25**, 423–458.
Vredenberg, W. J. 1969 Light-induced changes in membrane potential of algal cells associated with photosynthetic electron transport. *Biochem. biophys. Res. Commun.* **37**, 785–792.
Walker, E. B., Lee, T. Y. & Song, P.-S. 1979 Spectroscopic characterisation of the *Stentor* photoreceptor. *Biochim. biophys. Acta* **587**, 129–144.
Walker, E. B., Yoon, M. & Song, P.-S. 1981 The pH dependence of photosensory responses in *Stentor coeruleus* and model system. *Biochim. biophys. Acta* **634**, 289–308.
Wikstrom, M., Krab, K. & Saraste, M. 1981 Proton-translocating cytochrome complexes. *A. Rev. Biochem.* **50**, 623–655.
Wood, D. C. 1970 Electrophysiological studies of the protozoan, *Stentor coeruleus*. *J. Neurobiol.* **1**, 367–377.
Wood, D. C. 1973 Stimulus specific habituation in a protozoan. *Physiol. Behav.* **11**, 394–354.
Wood, D. C. 1976 Action spectrum and electrophysiological responses correlated with the photophobic response of *Stentor coeruleus*. *Photochem. Photobiol.* **24**, 261–266.
Yakushiji, E. 1971 Cytochromes: algal. *Methods Enzymol.* **23**, 364–368.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES