test for possible relationships between associated igneous rocks; to determine the conditions of formation of phenocryst assemblages; and to constrain cumulus sequence parental liquid compositions in cases for which no appropriate chilled liquid

sample is available. It is also easy to incorporate trace elements into the fractionation scheme.

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Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems

John F. Allen*[†], John Bennett[†], Katherine E. Steinback*[‡] & Charles J. Arntzen*[‡]

* USDA-SEA-AR, Department of Botany, University of Illinois, Urbana, Illinois 61801, USA
† Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK
‡ MSU/DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824, USA

In photosynthetic membranes isolated from pea leaves, the redox state of the plastoquinone pool controls both the level of phosphorylation of the chloroplast light-harvesting pigment–protein complex (LHC) and distribution of absorbed excitation energy between the two photosystems. Phosphorylation of LHC polypeptides is proposed as the regulatory mechanism by which photosynthetic systems adapt to changing wavelengths of light.

IN chloroplasts, non-cyclic photosynthetic electron transport generates ATP and NADPH by the operation of photosystem I and photosystem II acting in series. The energy that drives this process is derived partly from light absorbed by antenna chlorophylls associated closely with each photosynthetic reaction centre and partly from the more peripheral light-harvesting pigments[1] which are organized into specific chlorophyll–protein complexes[2-4]. The most abundant of the chlorophyll-binding proteins in green plants is a 25,000 molecular weight (MW) polypeptide known as the light-harvesting chlorophyll *a/b* protein (LHCP).

In non-cyclic electron transport, the two photosystems must operate at the same rate. If absorbed radiant energy is distributed between the photosystems in such a way that one photosystem is excited more than the other, the excess excitation energy is dissipated, with a consequent loss of photosynthetic quantum efficiency. However, it is clear from studies with algal cells exposed to red and far-red light that chloroplasts can detect such an imbalance in the excitation of the photosystems and at least partially correct it by increasing the proportion of absorbed excitation energy that is distributed to the rate-limiting photosystem[5-8]. These adaptive changes are referred to as state 1–state 2 transitions. State 1 is the adaptive state which results from over-excitation of photosystem I (as, for example, by far-red light) and is characterized by an increase in the proportion of total absorbed excitation energy that is transferred to photosystem II relative to photosystem I. State 2 results from over-excitation of photosystem II by red light and is characterized by increased energy transfer to photosystem I.
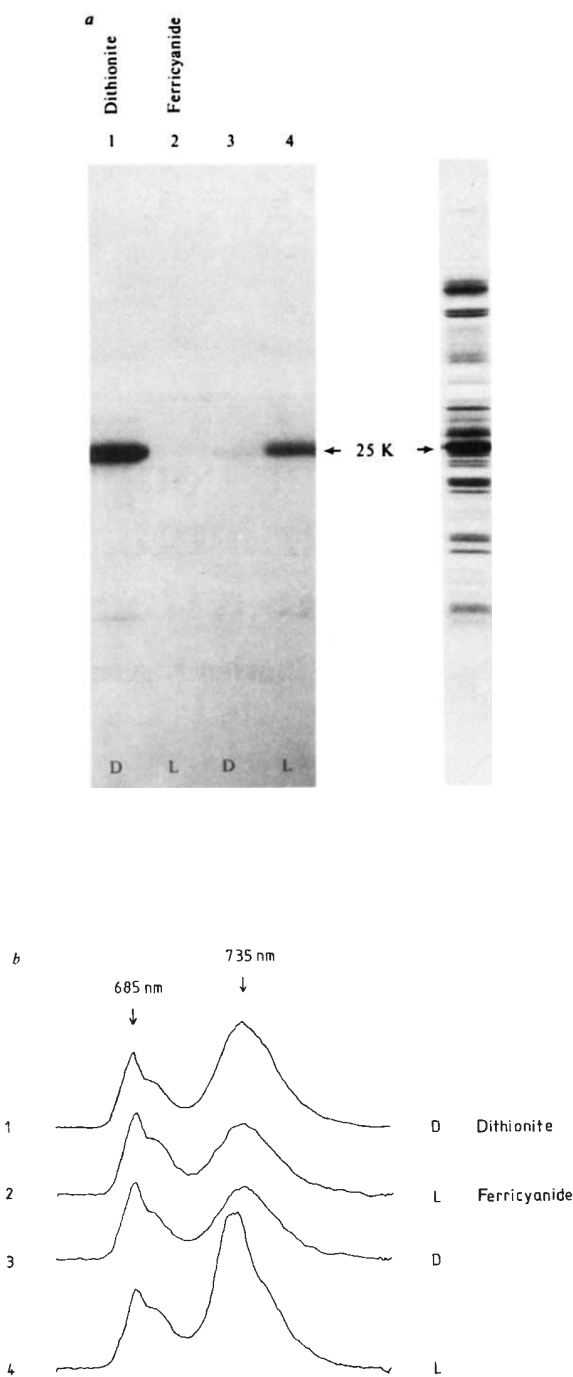
The biochemical events underlying state 1–state 2 transitions are poorly understood. However, a related phenomenon that has been extensively studied at the biochemical level is the ability of metal cations to affect the distribution of absorbed

excitation energy in isolated thylakoids[9-11]. In the presence of salts of monovalent and divalent metal cations[12], the light-harvesting chlorophyll–protein complexes that contain LHCP are structurally and functionally associated with photosystem II, whereas in the absence of salts the association between the light-harvesting apparatus and the photosystems is altered and excitation energy transfer to photosystem I increases.

A major factor governing the response of thylakoids to cations *in vitro* is the LHC itself. Experiments using thylakoids have shown that trypsin can selectively remove a surface-exposed segment of the LHCP and that this removal is concomitant with the loss of the ability of cations to affect the distribution of excitation energy[13]. In addition, antibodies raised against isolated, purified LHC were found to react with surface-exposed segments of the membrane-bound complex and to prevent such cation effects[14]. These studies indicate that the regulatory mechanism controlling the distribution of absorbed excitation energy in chloroplasts involves a surface-exposed segment of the LHCP.

It has recently been established that the LHCP is reversibly phosphorylated on a threonyl residue located within this surface-exposed segment[15-18]. The LHCP belongs to a group of thylakoid polypeptides shown to be phosphorylated by a membrane-bound, light-activated protein kinase[19,20] and dephosphorylated by a membrane-bound phosphatase[17]. Bennett *et al.*[21] have demonstrated that the reversible phosphorylation of LHCP changes the light-harvesting properties of isolated thylakoids: phosphorylation increases (and dephosphorylation decreases) the proportion of absorbed excitation energy transferred to photosystem I.

This article shows that the activity of the protein kinase is regulated by the redox state of plastoquinone. We propose a molecular mechanism for state 1–state 2 transitions *in vivo* that

is consistent with these data and we argue that the presence of cations is merely a prerequisite for these transitions rather than a decisive regulatory factor in itself.

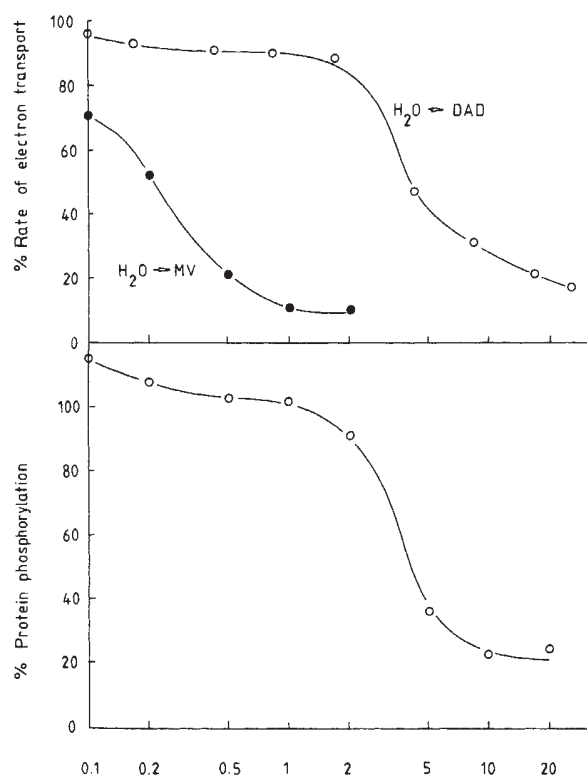## Activation of the thylakoid protein kinase by light and reducing agents

The protein kinase activity of isolated thylakoids was assayed by measuring the transfer of ^{32}P from $[\gamma\text{-}^{32}P]ATP$ to total membrane protein (Table 1). Light stimulated the kinase about fourfold compared with a dark-incubated control. The herbicide 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU), which blocks electron transport between the primary acceptor (Q) of photosystem II and plastoquinone, completely abolished the stimulatory effect of light. The stimulation could not be restored by addition of 2,6-dichlorophenolindophenol (DCPIP) in the reduced form (DCPIPH$_2$), which donates electrons to the intersystem chain after plastoquinone. Thus, the kinase seemed to be stimulated by reduction of certain intersystem electron carriers. This idea was supported by the effects of oxidizing and reducing agents on kinase activity (Table 1). Ferricyanide and methyl viologen accept electrons from photosystem I and consequently tend to oxidize the intersystem carriers. These oxidants inhibited light-activation of the kinase. In contrast, the low-potential reductant dithionite[22] activated the kinase in the dark, as did tetramethylhydroquinone (TMQH$_2$, a specific donor to plastoquinone in the intersystem electron transport chain[23,39]). Although dithionite was a somewhat more effective activator than light, their effects were equivalent rather than additive or synergistic. It is likely, therefore, that light and dithionite activated the kinase by the same basic mechanism, that is, by reduction of the intersystem carriers.

When activated by light, the protein kinase of the thylakoid phosphorylates predominantly the 25,000 MW LHCP[16]. Figure 1a shows that the same protein was phosphorylated when the kinase was activated in the dark by dithionite. Dithionite also stimulated phosphorylation of the 8,000 MW polypeptide reported to be the proton channel of the thylakoid ATP

Fig. 1 Effects of light, dithionite and ferricyanide on phosphorylation of LHCP (*a*) and on 77 K fluorescence emission spectrum of thylakoids (*b*). *a*, Thylakoids that had been pre-equilibrated with or without dithionite (10 mM) in darkness and with or without ferricyanide (2 mM) in the light were incubated with $[\gamma\text{-}^{32}P]ATP$ as described in Table 1 legend. Total ^{32}P incorporation into acid-insoluble material was determined and then each sample was subjected to SDS-polyacrylamide gel electrophoresis, followed by staining (lane 5) and autoradiography (lanes 1–4). The identities of the lanes (together with total protein kinase activity of the thylakoids, expressed as a percentage of the light control) are: lane 1, dark + dithionite (124%); lane 2, light + ferricyanide (33%); lane 3, dark (16%); lane 4, light (100%). The position of the 25,000 MW LHCP is indicated (25 K). *b*, Thylakoids were pre-equilibrated and incubated as described above except that the dithionite concentration was 20 mM and the ATP was not radioactive. After incubation, thylakoids were quickly diluted 10-fold with a medium containing 50 mM Tricine-NaOH (*p*H 7.6), 30% (v/v) glycerol and 10 mM MgCl$_2$ and then frozen to 77 K. Fluorescence emission spectra were obtained as described previously[21] using 440 ± 10 nm light for excitation. Spectra were normalized to give equal relative fluorescence at 685 nm using a Hewlett-Packard 9825 computer.

Fig. 2 Inhibition of electron transport and protein kinase activity by 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB). Whole chain electron transport was measured as oxygen uptake in the presence of methyl viologen (MV, 100 μM), sodium azide (5 mM) and ATP (100 μM). The control rate in the absence of DBMIB was 23 μmol (mg chlorophyll h)$^{-1}$. Photosystem II-mediated electron transport was measured as oxygen evolution in the presence of $K_3Fe(CN)_6$ (2.5 mM), diaminodurene (DAD, 1.2 mM) and ATP (100 μM). The control rate was 97 μmol (mg chlorophyll h)$^{-1}$. All electron transport measurements were carried out at saturating light intensity (1 mE m^{-2}s^{-1}) in a reaction medium (2 ml) containing 50 mM Tricine (*p*H 7.8), 100 mM sorbitol, 10 mM $MgCl_2$ and chloroplasts equivalent to 200 μg chlorophyll. Protein kinase activity was measured as described in Table 1 legend. DBMIB was present during the equilibration period before the addition of [γ-^{32}P]ATP. Values for phosphorylation were calculated by subtraction of the activity of a dark control and are expressed as a percentage of the activity in the light in the absence of DBMIB.

synthase[20]. In contrast, ferricyanide completely inhibited phosphorylation of these thylakoid polypeptides.

Changes in the distribution of absorbed excitation energy

Bennett *et al.*[21] reported that light-induced protein phosphorylation altered the 77 K chlorophyll fluorescence emission spectrum of isolated thylakoids. The finding that emission at 735 nm (F_{735}; derived mainly from the chlorophylls of photosystem I) was increased relative to emission at 685 nm (F_{685}; derived mainly from light-harvesting chlorophylls associated with photosystem II[1]) was one of several lines of evidence[21] indicating that protein phosphorylation changed the distribution of excitation energy within the pigment bed of the membrane in such a way as to favour distribution to photosystem I at the expense of photosystem II.

If the above conclusion is correct, changes in the distribution of absorbed excitation energy should occur whenever the level of protein phosphorylation is altered regardless of how the latter is brought about. Figure 1*b* shows that the 77 K chlorophyll fluorescence emission spectrum of isolated thylakoids was changed by ferricyanide and dithionite in accordance with the effects of these reagents on protein phosphorylation. Ferricyanide inhibited the light-dependent increase in F_{735} while

dithionite increased F_{735} in darkness, thus providing evidence for the control of excitation energy distribution by the redox state of the electron transport chain.

Although the dithionite-induced fluorescence change occurred in the presence of ATP, the data in Fig. 1 do not in themselves indicate that the change required ATP or involved protein phosphorylation. To explore this point in more detail, thylakoids were incubated in various conditions in the presence and absence of ATP and dithionite. Values for the ratio F_{735}/F_{685} were calculated from the resulting 77 K fluorescence emission spectra (Table 2). An ATP-dependent increase in the ratio was observed only in conditions which also permitted protein phosphorylation. Thus, there was no significant effect of ATP on fluorescence in the dark or in the light in the presence of DCMU, unless dithionite was also present. It is also clear from Table 2 that dithionite exerted two effects on the F_{735}/F_{685} ratio, one depending on the presence of ATP and the other not. The effect which required both ATP and dithionite represented the change in the distribution of excitation energy that could be attributed to dithionite-activated protein phosphorylation. The effect of dithionite in the absence of ATP may result from a direct alteration in fluorescence yield.

Activation of protein kinase by reduced plastoquinone

The above data raised the possibility that plastoquinone was the electron transport component whose reduction led to kinase activation. This possibility was tested by the use of 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB), which inhibits electron transport at two sites—it inhibits oxidation of plastohydroquinone at a high-affinity site[24] and reduction of plastoquinone at a secondary, low-affinity site[25]. Figure 2 illustrates both inhibitory activities. At a concentration of 1 μM,
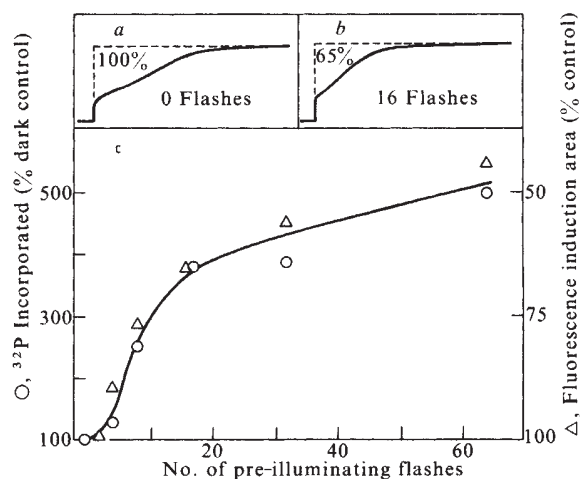
Fig. 3 Thylakoid protein phosphorylation and redox state of plastoquinone as functions of the number of pre-illuminating single-turnover flashes. Thylakoids were diluted with a medium containing 50 mM Tricine (*p*H 7.8), 100 mM sorbitol, 10 mM $MgCl_2$ and 10 mM NaF to a chlorophyll concentration of 50 μg ml^{-1} for protein kinase assays or 5 μg ml^{-1} for room temperature chlorophyll fluorescence induction transients. The dilution medium had been bubbled with nitrogen before the addition of thylakoids. The samples were pre-illuminated with a flashlamp (General Radio 'Stroboslav', type 1539A) delivering one intense, 8-μs flash per s. A fibre-optics bundle carried light from the flash-lamp directly into either cuvettes (for the induction transients) or polyethylene microcentrifuge tubes (for kinase assays). After the indicated number of flashes, fluorescence induction was measured in the cuvettes as previously described[3]. Direct tracings of oscillographic recordings; broken lines delimit the area above the induction curve, a measure of the proportion of the plastoquinone pool that is in the oxidized state. *c*, [γ-^{32}P]ATP was added to the microcentrifuge tubes for a 2-min, dark, anaerobic incubation which was terminated by addition of trichloroacetic acid to a concentration of 10%. Protein phosphorylation was then measured as described in Table 1 legend. Fluorescence measurements and additions of [γ-^{32}P]ATP were made within 5 s of the final pre-illuminating flash.
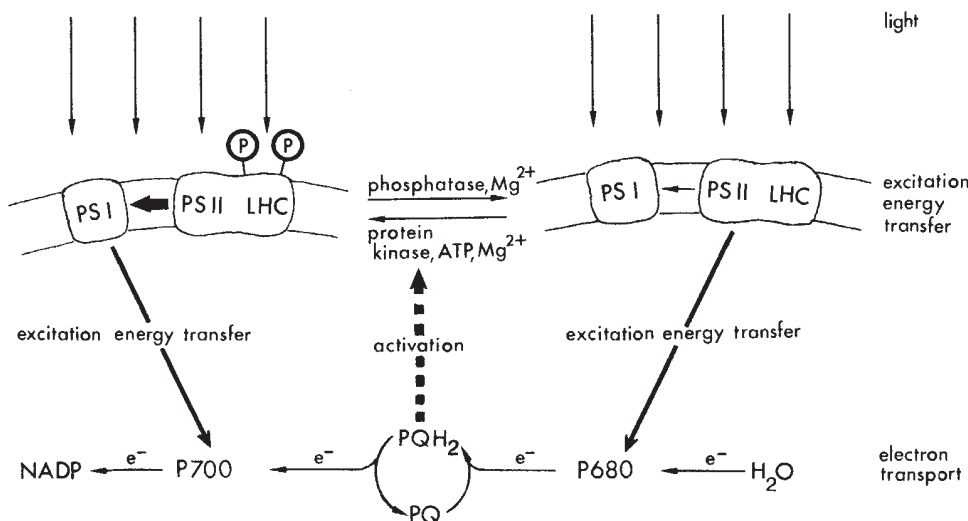
Fig. 4 A model for control of distribution of excitation energy in photosynthesis, in which reversible phosphorylation of LHC couples the redox state of plastoquinone (PQ) to the distribution of excitation energy between the photosystems. The mechanism we propose ensures maximal quantum efficiency of non-cyclic electron transport from water to NADP, mediated by P680 and P700, the reaction centres of photosystem II (PSII) and photosystem I (PSI), respectively. Reduction of plastoquinone by photosystem II leads to kinase activation and LHC phosphorylation, and hence to increased excitation of photosystem I. Conversely, oxidation of plastohydroquinone (PQH_2) by photosystem I inactivates the kinase; the phosphatase then dephosphorylates LHC and hence excitation of photosystem II is increased relative to that of photosystem I. Since the redox state of plastoquinone will be determined in part by the distribution of excitation energy between the two photosystems, reversible LHC phosphorylation completes a feedback loop by means of which any imbalance in this distribution will tend to be self-correcting.

DBMIB inhibited whole chain electron transport (from water to methyl viologen) by 90%, whereas photosystem II-mediated reduction of diaminodurene (DAD) was strongly inhibited only at concentrations greater than about 10 μM (DAD accepts electrons at or before plastoquinone[26]). When light-dependent kinase activity was measured as a function of DBMIB concentration (Fig. 2), strong inhibition was obtained only at concentrations equal to or greater than 10 μM. Thus, the electron transport component that links the activity of the protein kinase to the redox state of the intersystem carriers is situated between the primary and secondary sites of action of DBMIB; the most obvious candidate is plastoquinone itself.

To characterize further the possible relationship between the redox state of plastoquinone and kinase activation, we subjected a series of thylakoid samples to short, intense flashes of light. The redox state of the plastoquinone pool was assessed by recording chlorophyll fluorescence induction transients (Fig. 3). The area above the curve of the transient is proportional to the fraction of the plastoquinone pool that is in the oxidized state[27]. As shown in Fig. 3a and b, pre-illuminating flashes decreased the area above the curve of the transient, indicating that the plastoquinone pool had accumulated reducing equivalents. In parallel measurements, [γ-^{32}P]ATP was injected in the dark into preflashed samples which were then incubated for 4 min to permit kinase activity assay. As shown in Fig 3c, there was a striking similarity between the extent of kinase activation and that of reduction of the plastoquinone pool, as measured by the decrease in the area above the curve of the transient. Thus, the activity of the kinase is linked to the redox state of plastoquinone.

The previous observation[19] that NADPH and ferredoxin could together activate the protein kinase in darkness may now

be explained as an indirect effect, resulting from the ability of reduced ferredoxin to reduce plastoquinone[28,29]. We do not believe that activation of the thylakoid-bound protein kinase is mediated by either the thioredoxin system[30] or the 'LEM' system[31], both of which have been implicated in the control of stromal enzyme activity and require electron transport via components on the reducing side of photosystem I.

Regulation of photosynthetic efficiency by reversible protein phosphorylation

The involvement of plastoquinone in the activation of the thylakoid protein kinase is probably of fundamental importance in the regulation of photosynthesis, for it provides a physiological mechanism for an adaptation which will correct any imbalance in the distribution of excitation energy between the two photosystems (Fig. 4).

The operation of this mechanism may be illustrated by reference to state 1–state 2 transitions. In green algae and higher plants, that is, in photosynthetic organisms containing LHCP, exposure to red light (of wavelength <680 nm) will excite photosystem II to a greater extent than photosystem I, and thereby lead to reduction of plastoquinone. The protein kinase will then be activated, LHCP will become phosphorylated and an increased proportion of absorbed excitation energy will be transferred from the light-harvesting complex to photosystem I at the expense of photosystem II. Thus, the biochemical event underlying the transition to state 2 is phosphorylation of the LHCP. Conversely, under far-red light (of wavelength >680 nm), photosystem I will be excited to a greater extent than photosystem II, plastoquinone will be oxidized and the kinase will be inactivated. Any phosphorylated LHCP will be dephosphorylated by the thylakoid phosphatase and excitation energy transfer to photosystem I will be decreased. Thus, the biochemical event underlying the transition to state 1 is dephosphorylation of the LHCP.

It has often been suggested that state 1–state 2 transitions reflect changes in the cation content of the stroma surrounding the thylakoids[9–11,32], a suggestion based on the effect of cations on the distribution of excitation energy in isolated thylakoids[9–11]. One problem with this idea is that state 2 transitions would have to be correlated with a massive loss of cations from the stroma (to either the thylakoid or the cytoplasm), but there is no reason to believe that the cation concentration of the stroma changes greatly when higher plants or algae are transferred from far-red light to red light or vice versa. However, it should not be assumed that cations are not involved in the

Table 2 Effects of light, dithionite, DCMU and ATP on the ratio of 77 K fluorescence emissions at 735 nm and 685 nm

Incubation conditions*	F_{735}/F_{685}		
	+ATP	−ATP	
	(a)	(b)	(a)/(b)†
Dark	1.00	0.95	1.05
Light	1.90	1.20	1.58
Dark + dithionite	1.45	1.10	1.32
Light + dithionite	1.85	1.30	1.42
Light + DCMU	0.85	0.90	0.94
Light + DCMU + dithionite	1.90	1.30	1.46

* As described in Fig. 1b; DCMU concentration, 10 μM.

† Values greater than 1 indicate an ATP-dependent change in excitation energy distribution in favour of photosystem I.

regulation of excitation energy distribution *in vivo*. Two roles need to be emphasized. First, cations such as Mg^{2+} ions are required to establish the pattern of excitation energy distribution seen in dephosphorylated membranes, including the close structural and functional association between the LHCP complex and photosystem II[10,12,33]. The second role for Mg^{2+} ions derives from the fact that these cations are cofactors of both the kinase and the phosphatase (Fig. 4)[17,19,20]. Thus, the presence of cations should be thought of as a prerequisite for state 1–state 2 trasitions rather than as a regulatory factor *per se*.

The mechanism depicted in Fig. 4 provides an explanation for the original results of Bonaventura and Myers[5] and of Murata[6] and also for more recent contributions to the study of state 1–state 2 transitions. Several authors have drawn attention to the involvement of the high energy state of the membrane in these transitions[9,34,35]. This would certainly be required for the generation of ATP consumed in protein phosphorylation (transition to state 2). Duysens[36] suggested that the redox state of a component of the intersystem electron transport chain controls these transitions. Plastoquinone would clearly fulfil this role. Horton and Black[37,38] have recently established state 1–state 2 transitions *in vitro*. The transition to state 2 required ATP and was controlled by a membrane-bound factor with the redox potential of plastoquinone. The idea that protein phosphorulation might provide the connection between plastoquinone and ATP is fully in agreement with our present results.

It is important to recognize that state 1–state 2 transitions are also observed in organisms that lack LHCP. For example, Murata[6] and Ried and Reinhardt[8] have shown that these transitions occur in red algae, which contain phycobiliproteins instead of LHCP complexes. Ried and Reinhardt[8] conclude from their studies that the transitions are controlled by the redox state of a component of the intersystem electron transport chain. It will be interesting to discover whether state 1–state 2 transitions in the red algae are brought about by a mechanism analogous to that shown in Fig. 4; that is, a mechanism in which reversible phosphorylation of the phycobiliproteins is controlled by the redox state of plastoquinone.

Note added in proof: Another piece of evidence for the mechanism in Fig. 4 has now been obtained. Potentiometric redox titrations of plastoquinone, of LHCP phosphorylation and of ATP-dependent chlorophyll fluorescence quenching give identical results[40].

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IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts

Patricia J. Gearhart

Department of Embryology, Carnegie Institution of Washington, 115 W. University Parkway, Baltimore, Maryland 21210, USA

Nelson D. Johnson, Richard Douglas & Leroy Hood

Division of Biology, California Institute of Technology, Pasadena, California 91125, USA

An amino acid sequence analysis of the N-terminal immunoglobulin heavy and light chain variable regions (V_H and V_L) from 16 hybridoma proteins which bind phosphorylcholine as well as the complete sequence analysis of 9 of these V_H regions is presented. There seem to be more V_H regions participating in the phosphorylcholine response than can be encoded directly by germ-line V_H gene segments. Moreover, the V regions from IgG antibodies are considerably more variable than those from their IgM counterparts. These observations raise the possibility that a somatic mechanism for V region diversification produces greater diversity in IgG than in IgM antibodies.

ONE goal in molecular immunology is to understand the mechanisms responsible for antibody gene diversification. Insight into antibody diversity has been obtained by comparing sequence information from both immunoglobulin genes and proteins[1-8], and our present level of understanding may be summarized as follows. (1) Antibody molecules are composed of heavy (H) and light (L) chains, each of which contain variable (V) and constant (C) regions. (2) Comparative analyses of many variable regions have demonstrated that amino acid sequence variability is unevenly distributed throughout the V region. The antigen-binding site is composed of extremely variable sections termed hypervariable regions (three for each chain), whereas