Hypothesis

CONSEQUENCES OF SPATIAL SEPARATION OF PHOTOSYSTEM 1 AND 2 IN THYLAKOID MEMBRANES OF HIGHER PLANT CHLOROPLASTS

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1. Introduction

The thylakoid membranes of most higher plants and some green algae are structurally organized into a network of closely contacting, appressed membranes, the grana thylakoids, which are interconnected with single, unstacked membranes, the stroma thylakoids [1–5]. The inner surface of these thylakoid membranes encloses a space which is continuous between the grana and stroma thylakoids. As shown schematically in fig.1, thylakoids have two distinct membrane regions, termed here exposed and appressed membranes. The exposed thylakoids whose outer surfaces are in direct contact with the stroma, include stroma thylakoids and the end membranes and margins of the grana stacks. In contrast, the outer surfaces of the appressed membranes of the grana partitions have limited access to the stroma. Freeze-fracture electron microscopy reveals a difference in the size, shape and density of freeze-fracture particles located in appressed and exposed membranes [5–8]. This reflects a difference in the distribution of the main intrinsic macromolecular complexes of thylakoid membranes in the two regions. This striking structural organization of thylakoids is paralleled by a differentiation of function. Fractionation of thylakoids into grana and stroma thylakoid fractions by detergent [9] or mechanical methods [10], shows that the large subchlo-

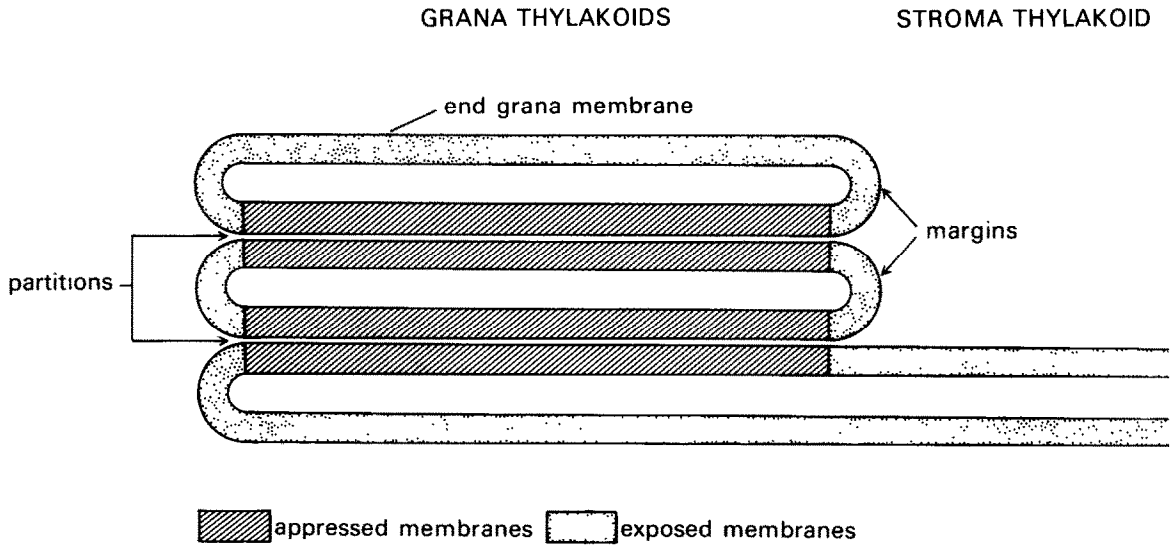

Fig.1. Schematic representation of grana and stroma thylakoids. There are two structurally different regions: the appressed membrane region (grana partitions) and the exposed membrane region (stroma thylakoids, and the grana end membranes and margins).

roplast fractions derived from grana stacks are enriched in PS 2, while the small vesicles derived from stroma thylakoids are enriched in PS 1 [1–5].

Recently, Andersson and Anderson have proposed that there is an extreme lateral heterogeneity in the distribution of PS 1 and PS 2 in higher plant thylakoids [11]. We suggest that few if any PS 1 complexes are present in the appressed membranes of the grana partitions. Assuming that most or all of the PS 1 complexes are indeed excluded from grana partitions, I consider in this paper the consequences for both light-excitation energy sharing and electron transport between spatially separated photosystems.

2. Model for the localization of chlorophyll–protein complexes

The chlorophyll (chl) and carotenoids of thylakoids are distributed between three intrinsic macromolecular complexes [4,5,12–14]. These are the PS 1 complex, which includes P700 and the antenna chl *a* molecules of PS 1, as well as the immediate electron donors and electron acceptors of PS 1, the PS 2 complex, which includes P680 and the antenna chl *a* molecules of PS 2, and the immediate electron donors and electron acceptors of PS 2, and the light-harvesting complex which includes the chl *a*/*b*-proteins.

Two recent techniques have enabled us to determine more precisely the localization of chl–protein complexes, and hence of the photosystems in appressed and exposed membranes [11].

(1) Since the amounts of chlorophyll associated with these three main intrinsic complexes can be reliably analysed by SDS–polyacrylamide gel electrophoresis, it is possible to quantify the actual amounts of chlorophyll associated with PS 2 and PS 1 [12–14].

(2) In addition to grana stack and stroma thylakoid fractions, it is now possible to isolate by aqueous polymer two-phase partition [15] inside-out vesicles which are derived mainly from grana partitions [16]. These grana partition vesicles which are enriched in PS 2 [16–18], are more enriched in appressed membranes than the traditional grana stack fractions derived from digitonin [9] or mechanical fractionation studies [10].

We analysed the relative amounts of chlorophyll associated with PS 1 complex, PS 2 complex and LHCP in unfractionated spinach thylakoids and various subchloroplast membrane fractions [11]. We find [11] that stroma thylakoids have more PS 1 complex, less PS 2 complex and less LHCP, and grana stack fractions have more PS 2 complex, more LHCP and less PS 1 complex compared to unfractionated thylakoids, confirming the results of earlier fractionation studies based on photochemical activities and other compositional data [9,10]. The even greater depletion of PS 1 complex, and enrichment of LHCP and PS 2 complex in grana partition vesicles suggests, however, that grana PS 1 is located mainly in the exposed membrane regions of grana stacks rather than in the appressed grana partitions [11] (fig.2). Further, our results suggest

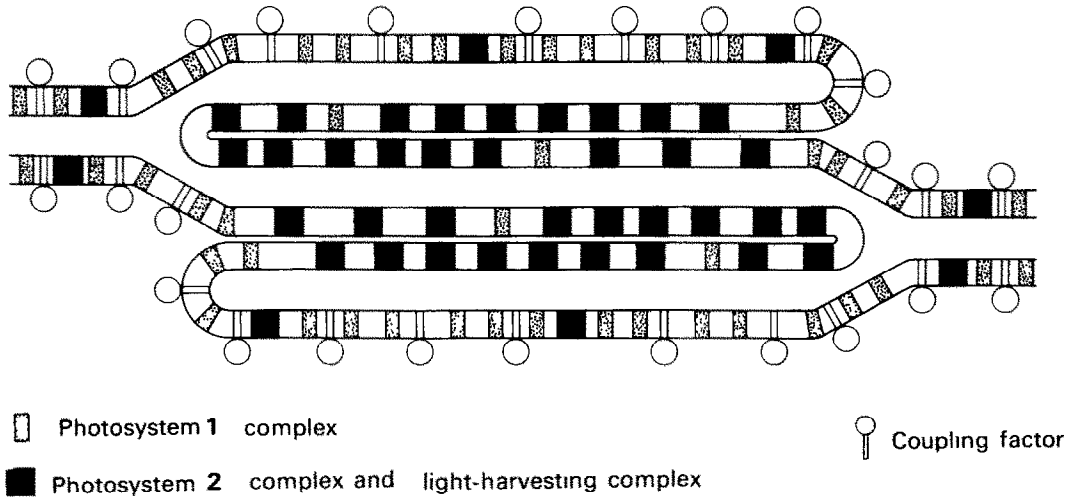

Fig.2. Proposed localization of photosystem 1 complex and photosystem 2 complex and associated light-harvesting chlorophyll *a*/*b*–protein complex in grana-containing chloroplasts [11].

that LHCP is associated mainly with the PS 2 complex. As shown in our model for the distribution of chl–protein complexes between appressed and exposed membrane regions, PS 2 complexes and their associated LHCP are located mainly in grana partitions, but a small amount of PS 2 complex and LHCP (10–20% of the chlorophyll of PS 2 in spinach thylakoids) is also in the exposed membranes. The most significant feature of this model, however, is the marked depletion of PS 1 complex in the grana partitions. While it was generally agreed that most of PS 2 complex and LHCP were located in grana stacks which had more PS 2 than PS 1 [1–5], direct evidence [11] that most, perhaps all, of PS 1 complex is excluded from grana partitions is a new and initially startling result.

A functional heterogeneity in the distribution of other chloroplast components between appressed and exposed thylakoids has also been demonstrated. It is established that both chloroplast ATPase (CF_1) [19] and ferredoxin-$NADP^+$ reductase [20,21] are excluded from grana partitions and located only in exposed thylakoid regions. In contrast, other studies [21,22] suggest that PS 2 is restricted mainly to grana partitions in agreement with our recent results [11].

The proposed localization of chlorophyll–protein complexes and hence of PS 2 and PS 1 in different membrane regions must have important consequences for our current understanding of photosynthesis. Assuming that PS 1 is indeed excluded from grana partitions, I shall consider:

(1) The consequences for electron transport between spatially separated PS 2 and PS 1;
(2) Correlations of structure with function;
(3) The mechanism of exclusion of PS 1 from grana partitions;
(4) The consequences for the sharing of light energy (spillover) between PS 2 and PS 1.

3. Consequences for electron transport between spatially separated photosystems

Clearly, if most PS 1 complexes are located in non-appressed membrane regions separated from the PS 2 complexes located in grana partitions (fig.2), there must be a mobile electron-transport carrier to transport electrons from granal PS 2 to PS 1. Is there any advantage for electron transport in having PS 1 complex located only in exposed membranes? Is the possibility of an intermediate electron transport carrier diffusing laterally in the membrane plane in accordance with the known properties of biological membranes? What is the most likely candidate for such a mobile electron carrier?

Berzborn [20] first suggested in 1969 that ferredoxin-$NADP^+$ reductase was located at the outer surface of non-appressed thylakoids, and this has been shown recently to be correct [21]. If some PS 1 complexes were located in grana partitions as hitherto assumed, electrons from these PS 1 complexes would have to be transported out to ferredoxin-$NADP^+$ reductase. There are no candidates for either a water-soluble electron carrier (which could not function efficiently anyway as it would be able to diffuse throughout the stroma space) or for a mobile extrinsic electron carrier at the outer thylakoid surface (which might have difficulty in diffusing laterally in the restricted area between the appressed grana paritions). The transport of electrons from PS 1 complex to the flavoprotein and ultimately to NADP is not a problem if PS 1 complexes are indeed located in the same membrane region as the flavoprotein enzyme. Since both $NADP^+$ and ATP are being synthesized on exposed thylakoid membranes, they are immediately released to the stroma where they are required for the reduction of CO_2. Thus, there is an advantage in having PS 1 complexes located only in exposed membrane regions.

There is good evidence that the lipid matrix of thylakoids is fluid at physiological temperatures. The lipids of the thylakoid bilayer matrix [monogalactosyldiglyceride (MGDG) (50%), digalactosyldiglyceride (DGDG) (25%), phospholipids (17%) and sulpholipid (8%)] have a remarkably high degree of unsaturated acyl groups, particularly MGDG and DGDG, since 97% of MGDG and 92% of DGDG acyl groups have three unsaturated bonds [3]. This very high unsaturated acyl group content, together with the absence of cholesterol, is consistent with the low exothermic phase transition of the ordered gel state to the fluid liquid-crystalline state which occurs in thylakoids below 0°C [23] as is found also with inner mitochondrial membranes [24]. The concept of a fluid lipid matrix for thylakoids is supported also by freeze-fracture studies [5–8]. The characteristic distribution of intramembranous particles associated with appressed and exposed membranes (see section 2) is lost and the particles are uniformly distributed along the entire membrane [6,7] when thylakoids are artifically destacked by suspension in low-salt

buffers [25]. Restacking can be induced by the addition of cations and the particles then resegregate [5–8,26]. This reversible lateral redistribution of freeze-fracture particles shows that the main intrinsic complexes of thylakoids, which include chl–protein complexes [3], are able to diffuse laterally in the membrane plane. Further, fractionation of destacked thylakoids gives rise to subchloroplast membrane fractions which have the same amounts of chl *a* and chl *b*, demonstrating that the chl–protein complexes are distributed uniformly along destacked thylakoids [18,27,28], which is clearly not so in the differentiated thylakoid membrane system.

Actual rates of lateral diffusion for any of the components of thylakoids have not been determined. Nevertheless, very fast rates of lateral diffusion should be feasible in the plane of the extremely fluid lipid matrix of thylakoids. In some biological membranes whose bilayer matrices are not as fluid as that of thylakoids, the lateral diffusion coefficients of some lipids range from 10^{-8}–10^{-9} $cm^2 . s^{-1}$ and of those of some proteins range from $1–5 \times 10^{-9}$ $cm^2 . s^{-1}$ [29–31]. Lipids and proteins with diffusion coefficients in this range could undergo a linear displacement of some 40 $nm . ms^{-1}$. This is well within the rate-limiting step of photosynthesis which is 20 ms [32]. If PS 2 and PS 1 were linked by the components involved in this rate-limiting step considerable distances could be traversed.

Taken together, the following points strongly suggest that plastoquinone is the most likely candidate for a mobile electron carrier to link PS 2 and PS 1.

(i) The rate-limiting step for photosynthetic electron transport is the oxidation of reduced plastoquinone by plastocyanin, which has a half-time of 20 ms [32]. This step is very slow compared to the other time constants of the electron transport chain.

(ii) The rate-limiting time constant is 20-times greater than the combined time constants for electron transfer either from water through to plastoquinone, or from plastocyanin to $NADP^+$ [33].

(iii) There is a large pool of plastoquinone molecules (10–14 equiv.) present for each molecule of P680 and P700 [33].

(iv) It is known also that at least 10 PS 2 reaction centres are interconnected by the plastoquinone pool [34].

(v) We had suggested [35] that pools of intermediate electron carriers such as plastoquinone, cytochrome (cyt) *f* and plastocyanin link discrete reaction centre complexes, because no universal stoichiometry exists between plastoquinone, cyt *f* and P700 in plants grown at different light intensities which have very different saturated rates of electron transport. Since 3–4-fold increases in the rates of electron transport were accompanied by 1.5–3-fold increases in the amounts of these intermediate electron carriers, we assumed a collision mechanism was involved for the interaction of successive electron transport carriers [35]. These ideas are consistent with the new hypothesis of spatially separated reaction centre complexes [11].

(vi) Plastoquinone molecules should have very rapid rates of lateral diffusion, of at least 40 $nm . ms^{-1}$ in the fluid lipid matrix of the thylakoid bilayer. Indeed in the inner hydrophobic space between the acyl chains of each bilayer half, reduced plastoquinone (PQ^{2-}) which readily binds protons to form the hydrophobic PQH_2 [36], might have even faster rates of diffusion than the bilayer lipids. Hence reduced plastoquinone could rapidly migrate along from appressed regions to non-appressed regions, well within the rate-limiting time of 20 ms.

(vii) The ratio of chemically determined plastoquinone to chlorophyll is similar in grana and stroma subchloroplast membrane fractions [37].

(viii) The other intrinsic macromolecular complex involved in electron transport is the intermediate electron-transport carrier complex referred to as the cyt $f–b_6$ complex [5,38]. This complex includes also the Rieske iron–sulphur protein and bound plastocyanin. I suggest that the cyt $f–b_6$ complex is likely to be located in the same region as PS 1 complex, that is in exposed membranes. Since the amounts of P700 and cyt *f* are comparable in subchloroplast fractions derived from grana stacks and stroma thylakoids of chloroplasts [9,10], it seems that cyt *f* is located in the same membrane region as P700. If the cyt $f–b_6$ complex is located mainly in non-appressed membranes, then plastoquinone would be the only possible electron carrier from granal PS 2 to the exposed region.

In the present model it is envisaged that plastoquinone not only serves as a mobile carrier in the sense that both electrons and protons are transported from the outer to the inner thylakoid surface [9], but also

that electrons and protons are carried laterally from the grana partitions to the exposed membrane regions.

Another possibility that should be considered is the transport of electrons by a water-soluble electron carrier diffusing in the continuous inner space of grana and stroma thylakoids. Sane et al. [10] considered that the extra PS 1 found in stroma thylakoids (then thought to have no PS 2) could be linked with PS 2 in the grana by a water-soluble electron carrier (diffusion coefficient at 10^{-5} $cm^2 . s^{-1}$). Plastcyanin which mediates electron transfer from cyt *f* to P700 is located at the inner thylakoid surface [33]. The reduction of P700 by plastoquinone has a half-time of 0.2 ms which is 100-times faster than the rate-limiting step [40]. Plastocyanin acts as an electron shuttle between the cyt f–b_6 complex and PS 1 complex in the same way that cyt *c* links complexes III and IV in mitochondria. However, it is unlikely that plastocyanin is the mobile electron carrier from PS 2 to PS 1 if the cyt f–b_6 complex is located in non-appressed membranes (see (viii) above).

The proven and suggested localization of the 5 main intrinsic macromolecular complexes of thylakoids is summarized in table 1. Only PS 2 complex and associated LHCP are located in both appressed and exposed membranes, although only minor amounts of both complexes are present in the exposed membrane region. Recently, evidence has accumulated for the existence of two different, independent PS 2 complexes, termed PS II_α and PS II_β [40–43]. PS II_α complexes have more antenna chl *a* molecules compared to PS II_β complexes; several PS II_α complexes are connected, but PS II_β complexes are independent of each other. An increase in the amount of PS II_β complex is correlated both with an increase in chl *a*/chl *b* ratios, and a decreased proportion of appressed membranes [43]. Melis and Thielen [43] suggest that the PS II_β complexes are located in exposed membranes, while the PS II_α complexes are found in appressed membranes. The reported difference in antenna size and electron transport properties of PS II_α and PS II_β [40–42] may be partly explained by our model, since the PS II_β complexes would be in closer proximity to PS 1 complexes than the PS II_α complexes.

In summary, the present model can accommodate electron transport from the PS 2–LHCP complexes in grana partitions to the cyt f–b_6 complex and PS 1 complex located in the exposed thylakoid membranes, if reduced plastoquinone can move laterally in the plane of the membrane. The variability of the amounts of intermediate electron carriers compared to reaction centre complexes in different chloroplasts [35] suggested early on that electron transport did not proceed through single structural electron-transport chains. Melis and Brown [37] have shown that the stoichiometric relationship between the two photosystems is not necessarily unity as generally assumed. They found that the ratio of PS 2 reaction centres to PS 1 reaction centres varied from 0.43–3.3 in different thylakoids [37]. The localization of most of PS 2, and most or all of PS 1 in spatially separated membrane regions allows different thylakoids to possess varying photosynthetic capacities.

4. Correlations of structure and function

Does the idea of PS 1 complex being restricted to exposed membranes agree with circumstantial evidence from structural studies? The structural differentiation of higher plant and green algal thylakoids in exposed and appressed membrane regions is clearly revealed

Table 1
Localization of intrinsic macromolecular complexes of chl *b*-containing thylakoids

Exposed membranes	Ref.	Appressed membranes	Ref.
PS 1 complex	[11]	PS 2 complex	[11]
		Light-harvesting complex	[11]
Chloroplast ATPase	[20]		
Cytochrome f–b_6 complex (?)			
PS 2 complex[a] and LHCP	[11]		

[a] Minor amounts only (10–20% of the total amount in spinach thylakoids)

by freeze-fracture and freeze-etch electron microscopy [5–8]. High densities of intramembranous particles of varying sizes which represent the main intrinsic macromolecular complexes [3,5] are located on four distinct fracture faces. Large EF particles are concentrated in the appressed membranes of grana partitions, while many smaller PF particles of the outer fracture face are present in both appressed (PFs) and exposed membranes (PFu).

Originally, it was suggested from fractionation studies [10,45] and a developmental study [8] that the EF particles represent PS 2 (a core PS 2 complex and LHCP) and the PF particles represent PS 1. However, if PS 1 complexes are excluded from the appressed membranes of grana partitions (fig.2) they could not be associated with the PFs particles of grana partitions. Recently, the earlier idea that the PFs particles of grana partitions represent PS 1 complex has been challenged.

(i) Simpson [46] showed that the EFs particles of a chl *b*-less mutant barley which lacks one of the LHCP polypeptides [47] were only 12% smaller than those of wild type barley. Since there was a 50% decrease in the number of PFs particles in mutant barley, he concludes that some LHCP is located in PFs particles which are closely associated with EFs particles.

(ii) Some PS 2 mutants have greatly reduced numbers of EFs particles and the associated tetrameric structures seen at the inner thylakoid surface, yet they have marked grana stacking and LHCP [48,49]. Formation of grana with few EFs and normal PFs particles is difficult to explain if LHCP is located only in EFs particles, and is consistent with Simpson's view that some PFs particles contain LHCP [46].

(iii) Miller [50] has shown that a PS 1 mutant of maize lacking two specific PS 1 polypeptides has normal stacked fracture faces but the particle size on the PFu fracture face is substantially reduced. He suggests that PS 1 is confined to non-appressed membranes.

(iv) Simpson [51] has shown also that the PFu particles are decreased in size in a PS 1 mutant of barley.

Thus, the recent structural studies of Simpson [46,51] and Miller [49,50] support our model which excludes PS 1 complex from grana partitions. In the grana partitions, the close association of EFs and PFs particles (seen best in ordered arrays [8,46,49]) suggests that 1 EFs and 4 PFs particles are derived from a single structural membrane-spanning unit comprising PS 2 complex and LHCP. On freeze-fracture, the PFs particles with greater mass at the outer surface cleave to the PF fracture face, and the larger EFs particles with greater mass towards the inner thylakoid surface cleave with the inner fracture face [3]. (The tetrameric structures seen on the outer thylakoid surface would then be due to PFs particles rather than EFs particles as originally suggested [8].) The PFu particles of exposed membranes could represent either PS 1 complex, the intrinsic membrane sector (CF_0) of chloroplast ATPase or the cyt f–b_6 complex. Thus, the evidence derived from freeze-fracture studies for the structural organization of particles of varying sizes between appressed and exposed membranes is at least consistent with the proposed spatial separation of PS 1 from grana PS 2 (fig.2).

5. Mechanism for the exclusion of PS 1 complex from grana partitions

The lateral heterogeneity in the distribution of components along the plane of membranes seems to be as important a feature of thylakoids as the more widely recognized asymmetric distribution of components across the membrane [39]. During membrane–membrane interactions, protein complexes that are highly charged at the outer membrane surface would be expected to migrate away; in contrast, the less-charged protein complexes would be drawn into the appressed region [52,53], thereby resulting in a lateral redistribution of membrane proteins and lipids [52,53]. Barber and Chow [54] postulate that stacking of thylakoids by electrostatic screening involves such a redistribution of intrinsic membrane complexes by lateral diffusion. They suggest that highly charged complexes will migrate outwards, so as to allow net attraction between adjacent membranes at less polar regions and membrane repulsion at more electrically charged surface regions. They suggest that PS 1 particles may be highly charged and migrate to unstacked membrane regions, and LHCP may be less charged and thus remain in appressed membrane regions. This is consistent with our model.

It is clear that a surface-exposed portion of some polypeptides are involved in thylakoid stacking. It has been shown that some of the polypeptides of LHCP are involved in thylakoid stacking in vitro [55–59].

A small segment (2000 M_r) of both the 25 000 and 23 000 M_r polypeptides of LHCP exposed at the outer thylakoid surface is readily released by mild proteolytic treatment of destacked thylakoids [55,56] or reconstituted LHCP–proteoliposomes [57–59]. Since removal of this segment prevents cation-induced restacking of destacked thylakoids, or aggregation of LHCP–proteoliposomes, this suggests that the surface-exposed regions of LHCP are involved in stacking in vivo. Thus, it is not surprising that most of LHCP is located in the grana partitions.

6. Consequences for the sharing of light energy (spillover) between PS 2 and PS 1

The concept of 'spillover', that is the transference of light energy from PS 2 to PS 1, has been widely accepted as a regulatory mechanism which ensures optimal light energy distribution and hence electron transport between photosystems (cf. [60–62]). The spillover hypothesis [63–65] requires that the antenna pigment molecules of each reaction centre may be in contact with each other. It has been assumed that spillover occurs in grana partitions.

The studies of Arntzen et al. (cf. [5]) suggest that LHCP is required for the regulation of light energy distribution between the photosystems. Due to similarities between state 1–state 2 light interconversions in plants and algae, whereby light absorbed by PS 2 involves a change which increases the efficiency of PS 1 and vice versa [65], and the Mg^{2+} control of distribution of excitation energy in isolated chloroplasts, it was proposed that light-induced cation fluxes across thylakoids alter local cation concentrations thereby altering the association of LHCP with PS 2 and PS 1 complexes [5,60–62]. However, would cation efflux necessarily be a selective regulatory process when both photosystems participate in it? Further, there is a difference in the time involved in the cation-induced changes in fluorescence yields of isolated chloroplasts (10–20 s) compared to state 1–state 2 transitions (5–10 min) [61,62,64].

Recently, Bennett et al. [66] suggest that reversible phosphorylation by a membrane kinase of surface-exposed segments of the main polypeptides of LHCP alters the properties of its interaction with both photosystems, such that the distribution of excitation energy increasingly favours PS 1. Interestingly, the regulation of light energy may be mediated by the redox state of the plastoquinone pool, since reduced plastoquinone serves to activate the kinase which catalyses the phosphorylation of LHCP [67]. It should be noted that if this phosphorylation reaction is important for the physiological regulation of light energy distribution between PS 1 and PS 2, according to our model, it would apply only to the portion of LHCP which is located in the exposed thylakoid membranes, where contact between PS 2 and PS 1 is feasible.

If indeed there is little or no PS 1 complex in grana partitions as we have proposed [11], there could be little or no spillover in appressed membrane regions. Thus, for spillover in chl *b*-containing chloroplasts attention must be focussed on the exposed thylakoids, since it is only or mainly here that PS 2 and PS 1 complexes may be in contact. As the exposed membranes of spinach thylakoids have 10–20% of the total amounts of PS 2 complex and LHCP [11], this may be sufficient to account for the 'spillover values' calculated by Butler [68]. Since different plants have varying ratios of appressed to exposed membranes, the degree of chlorophyll available for 'spillover' can be roughly regulated. Small changes in the lateral organization of chl–protein complexes could result in changes in the fine structure of thylakoids. For example, if some PS 2 complexes and LHCP near the periphery of grana partitions migrate out to the exposed membrane region, the degree of stacking would be slightly reduced, and there would be greater opportunity for spillover. Indeed, Bennoun and Jupin [69] found a 20% decrease in thylakoid stacking in *Chlamydomonas* after a state 1–state 2 transition, which is from minimum to maximum spillover. The change from grana-containing chloroplasts to destacked thylakoids in *Egeria densa* observed by Punnet [70] was paralleled by a decrease in photosynthetic enhancement, which could be interpreted now as due to the chl–protein complexes becoming randomly dispersed along the membranes. Recently, Barber [71] has suggested that the relationship between salt-induced thylakoid stacking and associated fluorescence changes in isolated thylakoids is controlled by electrostatic screening. When electrostatic screening is low, the thylakoids are unstacked, the chl–protein complexes are randomized, fluorescence is low and spillover is high. With increased electrostatic screening, PS 2–LHCP complexes are predominantly in the partition regions of grana together with some PS 1 complexes, and some PS 1 complexes are now located in stroma thylakoids, resulting in a decrease in energy transfer from PS 2 to

PS 1, and an increase in fluorescence [71]. Our model (fig.2) is different from that of Barber [71] in one important respect. We envisage that spillover in grana-containing chloroplasts is taking place mainly in the exposed membrane regions (fig.2), whereas Barber has spillover occurring in the appressed membranes of grana partitions. In either event, it seems that in most grana-containing thylakoids the amount of chlorophyll available to participate in spillover is not large.

As most of the models postulated for the organization of chl–protein complexes are based on the spillover hypothesis, the antenna chlorophyll molecules of PS 1 and PS 2 are assumed to be in contact (cf. [72]). These continuous array models include the computer array model of Seely [73], the fluorescence tri- or bipartite models of Butler [74], the compositional model of Thornber et al. [75] and structural models [5,26]. Initially, Boardman et al. [2] proposed an essentially separate package model which had LHCP associated with both PS 1 and PS 2 complexes. However, since the ratios of LHCP to PS 2 complex are comparable in both appressed and exposed membrane regions of subchloroplast fragments, it is reasonable to assume a close structural linkage between LHCP and PS 2 complex. This is indicated also by structural studies (see section 2). Thus, in the proposed model most of the chlorophyll of the photosystems of grana-containing chloroplasts is in separate packages with PS 2–LHCP complexes located mainly in grana partitions and PS 1 complex located in exposed thylakoids; only a limited amount of PS 1 and PS 2 have the possibility of close contact in exposed membranes.

Finally, it is important to note that our recent results [11] show that at most only 10% of the total chlorophyll of grana partitions belongs to PS 1 complex. Since the isolated grana partition vesicles are contaminated by some exposed membranes [11,16–18], this value is likely to be even lower. Our calculations which of necessity involved certain assumptions suggest strongly that there may be no PS 1 complex in grana partitions. Whether this is so remains to be proven, but it is clear that most of the chlorophyll of grana partitions belongs to the PS 2–LHCP complexes. Thus, the assumption that most of photosynthetic electron transport occurs with PS 2 and PS 1 located in grana partitions has to be abandoned. It seems likely that the localization of PS 2–LHCP complexes in grana partitions is a means of separating PS 2 from PS 1, rather than a way of increasing interactions between the pigment molecules of PS 2 and PS 1. Although it has been thought that most of the chlorophyll is concentrated into appressed membranes of grana partitions, this may not be so. Our results [11] suggest that the amount of chlorophyll is rather similar in both appressed and non-appressed thylakoids. Thus 35–40% of the total chlorophyll of spinach thylakoids may be present in the exposed membranes, which is about comparable to the percentage (by length) of exposed membranes [76,77]. The function of grana in chl *b*-containing chloroplasts will be discussed in another paper.

In conclusion, our proposal that most or all of the PS 1 complexes are excluded from grana partitions gives a new and I hope useful approach for viewing photosynthesis. The consequences examined here for electron transport between spatially separated PS 2 and PS 1 can be experimentally tested. For example, would the incorporation of galactolipid liposomes with thylakoids and subchloroplast membrane fractions influence their rates of electron transport and structure, as Schneider et al. [78] have shown so convincingly with inner mitochondrial membranes. If the elegent quantitative determination by optical spectroscopy of the amounts of PS 1 and PS 2 reaction centres and the photoreducible plastoquinone pool introduced by Melis and Brown [37] were applied to many different thylakoids with varying degrees of thylakoid stacking, it should be possible to see if plastoquinone is functioning as a mobile electron carrier. Are the kinetics of plastoquinone reduction biphasic? Perhaps the ideas of massive spillover in grana-containing chloroplasts should be re-examined. Neither is the ratio of PS 1 reaction centres to PS 2 reaction centres necessarily unity [37] as hitherto assumed, nor are the chlorophyll molecules about evenly divided between PS 2 and PS 1 as was often assumed, since LHCP is mainly associated with PS 2 [11]. The proposed spatial separation of most of the PS 2–LHCP complexes from most of PS 1 complexes that occurs in grana-containing chloroplasts may be vital for optimal photosynthetic efficiency at low light conditions. Above all, the dynamic nature of the fluid lipid matrix of chloroplast thylakoids should be realized.

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