Review

Lateral heterogeneity of plant thylakoid protein complexes: early reminiscences

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The concept that the two photosystems of photosynthesis cooperate in series, immortalized in Hill and Bendall's Z scheme, was still a black box that defined neither the structural nor the molecular organization of the thylakoid membrane network into grana and stroma thylakoids. The differentiation of the continuous thylakoid membrane into stacked grana thylakoids interconnected by single stroma thylakoids is a morphological reflection of the non-random distribution of photosystem II/light-harvesting complex of photosystem II, photosystem I and ATP synthase, which became known as lateral heterogeneity.

Keywords: grana; lateral heterogeneity; plant thylakoids; photosystem II; stroma thylakoids

Few phenomena in natural science equal photosynthesis in sweep and grandeur.

Martin Kamen [1, p. 1]

1. INTRODUCTION

My scientific odyssey with the molecular organization of plant thylakoid membranes began when I came to Canberra in 1961 to work on photosynthesis, appropriately in the Division of Plant Industry, CSIRO. I was inspired by reading Robin Hill and Faye Bendall's famous 'hypothesis in barest outline' that is summarized thus: 'if the cytochrome in the chloroplasts is directly involved in hydrogen (or electron transfer), the system must require more than one light-driven reaction to act in opposition to the thermal gradient' (figure 1) [2]. Recognition of their hypothesis became the Z scheme that was further refined as additional components were discovered and characterized, but the structure, composition and function of the photosynthetic apparatus were still almost a black box. Neither the Z scheme nor its precursor, the photosynthetic unit, defined either the structural or spatial organization of the photosystems within thylakoids. My challenge then was twofold: (i) to determine whether there were indeed two discrete photosystems as implied by Hill and Bendall's hypothesis and (ii) to ascertain whether these photosystems were associated with the fascinating stacked and/or non-stacked thylakoid membrane domains of higher plants. Explanation of 'the complicated and visible with the simple and invisible' is particularly challenging for the thylakoid membranes of higher plant chloroplasts which form an elaborate continuous three-dimensional network comprised of regular cylindrical, tightly stacked membranes, the grana thylakoids that are interconnected with single stroma thylakoids whose outer membrane surface face the stroma.

My first approach to prove the reality of two photosystems was to follow the development during the greening of etiolated bean leaves. Naively, Keith Boardman and I hoped that photosystem I (PSI) might have been formed before PSII during chloroplast development, thereby allowing us to characterize the properties of one photosystem. It was impossible. Following the removal of contaminating mitochondria from isolated bean etioplasts and greening chloroplasts, all photochemical activities were vanishingly low. Although PSI activity appeared before oxygen evolution, we had no idea if this were so in leaves.

Our next strategy involved the non-ionic detergent, digitonin, to fragment spinach chloroplasts derived from hydroponically grown plants from which functional chloroplasts had been obtained overseas. Digitonin fragmentation of isolated thylakoids followed by differential centrifugation yielded a heavy fraction enriched in Chl *b* and PSII activity, whereas the lighter fraction was highly enriched in PSI activity, P700, cyt b_6 and cyt *f* [3,4]. With the arrival of a Cary model 14R spectrophotometer and a Plant Industry workshop copy of the Bonner type cuvette assembly, I determined the cytochrome difference spectra at 20°C and 77 K in the digitonin fractions. My isolation and characterization of a third cytochrome cyt *b* type, the *enigmatic* cyt *b559*, glimpsed in a hand spectrograph by Lundegårdh [5], was especially pleasing; it was tightly bound within the PSII-enriched fraction and clearly an integral component of PSII, whereas cyt b_6 and cyt *f* were located mainly in the PSI fraction [6]. Fortunately, John Thorne, a former naval radio engineer, built a homemade spectrofluorometer capable of correcting for the wavelength-dependence of instrumental response, so we were able to characterize the fluorescence properties

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Figure 1. Robin and Priscilla Hill in Canberra in 1973.

of the PSII-enriched and PSI fragments at 20°C and 77 K. Most of the chlorophyll fluorescence at 20°C was emitted by PSII with Chl *b* contributing to both PSII-enriched and PSI-enriched fractions [7]. Thus, this first partial separation of the photosystems proved there were indeed two photosystems.

2. LATERAL HETEROGENEITY OF PLANT THYLAKOID COMPLEXES

(a) *Proteins and lipids in lively membranes*

Although there was almost immediate recognition of two sequential light reactions, understanding of the structural organization and composition of the components both across and along the thylakoids was very meagre until the 1970s. This was due to ignorance not only of the detailed composition and molecular structure of thylakoid components but, more importantly, of a credible model for membrane architecture. The generalized 'sandwich membrane model', proposed in 1925 by Gorter and Grende and widely accepted before 1970 had the phospholipids arranged in a continuous bilayer with their acyl chains occupying the interior membrane core, while the proteins were spread out on both sides of the membranes blanketing the polar phospholipid head groups (reviewed in Singer [8]). Fortunately, the concepts of protein arrangement within lipid bilayers introduced by Singer [8] led Singer & Nicolson [9] to formulate the fluid protein–lipid mosaic model where the membrane consisted of a lipid bilayer into which membrane spanning intrinsic protein complexes are embedded and to which extrinsic proteins are attached.

My greatest serendipitous opportunity coincided with my arrival in Cambridge in 1973. Owing to the coalminers' strike, electricity was severely rationed, and research in Derek Bendall's laboratory became impossible for months. Luckily, I was able to retire to a perfect ivory tower, my attic room in the Pightle, at Newnham College. With the luxury of time to think, and inspired by the brilliant fluid mosaic model of cell membrane structure [9], I dreamt that thylakoid multi-protein complexes spanned the entire thylakoid membrane and danced in the lipid bilayer in the light. My review [10], replete with speculations galore, discussed thylakoid membranes in terms of the fluid protein–lipid mosaic model [9]. It was clear that the astonishing economy of locating molecules asymmetrically across thin membranes imparts order by removing random three-dimensional movement, thereby allowing a spectacular enhancement of effective concentration of the many pigment molecules so exquisitely ordered within their proteins to regulate light harvesting and energy dissipation [10]. 'This organization allows for rapid changes in the conformation and the distribution of the macromolecular complexes which are essential for the function and structure of the intricate chloroplast membrane' [10, p. 193].

Although the molecular structure of the thylakoid protein complexes was still very limited, a new era for molecular organization arrived. Trebst [11] focused on the need for vectorial electron and proton transport across the membrane, whereas Anderson [10] assigned chloroplast components within the lipid–protein mosaic membrane model: both emphasized an asymmetry of function along thylakoid membranes. The elegant structural studies of Staehelin and co-workers [12] definitively revealed that ATP synthase with its large head group and possibly PSI were located only in unstacked stroma thylakoids. Slowly, the black box was being opened.

(b) *Green gels and aqueous polymer two-phase liquid partition*

Photosynthesis researchers have always been intrigued by the way the chlorophylls and carotenoids are arranged within photosynthetic membranes [13]. Although investigators, beginning with Emil Smith in 1941, had used detergents or solvents to disrupt thylakoids and isolate pigment-proteins, the idea still persisted that not all the chlorophyll and carotenoid molecules were non-covalently bound to proteins. In 1966, Phillip Thornber separated two chlorophyll-proteins using SDS–PAGE; by 1975, at least 60–75% of chlorophyll had been resolved as Chl-proteins by green gels [13]. Finally, my extensive struggles to improve green non-denaturing gel procedures were rewarded; over 90 per cent of both chlorophylls and carotenoids were indeed associated with six different Chl-protein complexes [14].

Again, the stage was set for a wonderful surprise and serendipity intervened. Famously, Lawrence of Arabia lost his draft of 'Seven Pillars of Wisdom' at the Reading railway station but I found a wonderful colleague there who helped change the way we think about the molecular organization of plant thylakoids. After an otherwise

rather dull International Congress of Photosynthesis in 1977, a young Swede, Bertil Andersson bounded along the platform and declared that he was coming to Australia to work with me when he had finished his PhD thesis! Astonished, I declared that I had no money for his support and could offer only laboratory space and facilities; undaunted, Bertil replied he would secure funds. By gaining an EMBO Fellowship, Bertil eventually convinced the EMBO Chairman that his Fellowship had to be held in Canberra which was 'anyway close to Europe'. Significantly, using the aqueous two-phase polymer partition method of Albertsson [15], Bertil had partitioned a granal fraction into right-side-out and inside-out vesicles derived from non-appressed and appressed membranes, respectively [16]. Although PSII activity was greatly enriched in the appressed granal fraction, quantitation of PSII and PSI was hampered by inactivation of photochemical activities incurred by thylakoid fragmentation [17].

(c) *Partitioning photosystem II and photosystem I*

Bertil's and my joint aim was to compare the content of the main chlorophyll-protein complexes of the photosynthetic apparatus resolved by my improved 'green gel' method, using the membrane fractionation method of Albertsson [15]. Having no equipment to shake the two-phase fractions in Canberra Bertil Andersson spent many hours in the Canberra cold room (figure 2). Following Yeda-press fragmentation of thylakoids, stroma thylakoids (Y-100) were separated from granal stacks (Y-40) by differential centrifugation, and enriched inside-out vesicles (B3) were isolated by aqueous polymer two-phase partition of the granal fraction (Y-40 fraction). Stroma thylakoid fractions were highly enriched in PSI complex together with some 10–20% of PSII and light-harvesting complex of photosystem II (LHCII) complexes [18]. By contrast, the grana-appressed vesicles were substantially depleted in PSI complex and enriched in PSII and LHCII (figure 3) [18]. Allowing for the contamination of some right-side-out vesicles in the appressed inside-out vesicles, we proposed that PSI is exclusively restricted to non-appressed granal domains, comprising the grana end membranes, grana margins and stroma thylakoids [18]. Thus, a lateral heterogeneity of distribution of the photosystems exists with PSI exclusively in stroma-exposed thylakoid domains and PSII/LHCII supercomplexes mainly, but not exclusively located in appressed membranes [18]. We were delighted that our paper was speedily accepted for *Biochimica et Biophysica Acta*, then the most prestigious journal for photosynthesis research [18]. Also, molecular organization was included for the first time at the splendid Fifth International Photosynthesis Congress at Halkadiki, 1980, attracting over 60 posters. To our astonishment and frustration, however, our concept of lateral heterogeneity presented by a symposium lecture and poster was met with horror or ignored. Neither Bertil nor I were allowed to speak at two relevant Discussion series: an English chairman introduced one session by stating that there were two crazy ideas, fit only for the waste paper basket which would not be discussed: Andersson &
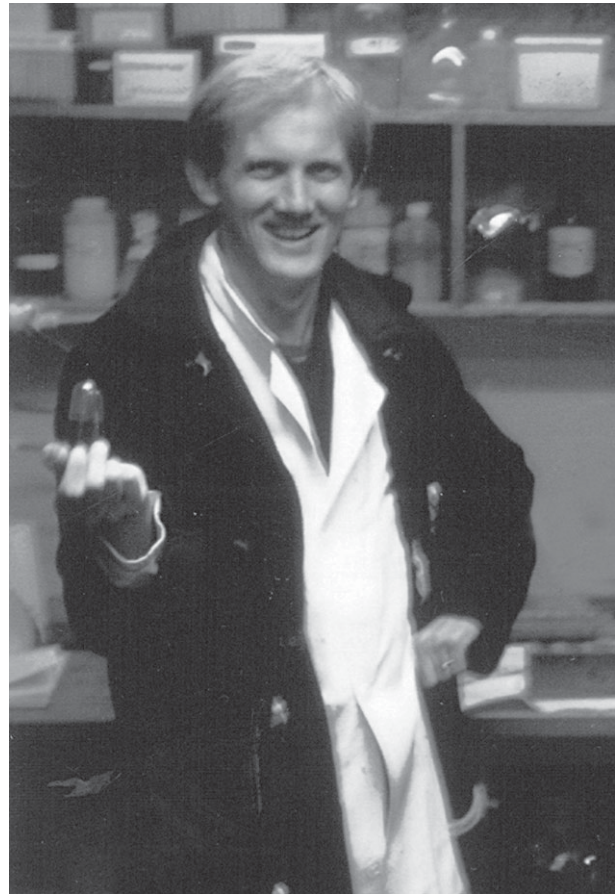

Figure 2. Bertil Andersson as usual in the Plant Industry cold room in 1979.

Anderson's lateral heterogeneity [18] and Dan Arnon's concept [19] of the photosystems operating in parallel rather than in series, with PSII alone driving linear electron transport from water to ferredoxin and NADP, without the collaboration of PSI, whose role was limited to cyclic electron transport and cyclic phosphorylation: Dan adopted his new concept for life [19]. In 1980, Barber [20] recognized that a lateral separation of some PSI from PSII in the grana would explain his observations on the relationship between salt-induced changes in spillover from PSII to PSI; his proposed extent of lateral heterogeneity of the photosystems was not as extreme as we had demonstrated [18].

Our figure for the lateral distribution of the thylakoid protein complexes (figure 3) should have been termed a schematic model or cartoon; no mention was made of it in the text as a possible three-dimensional model of the thylakoid membrane network and that was not intended [18]. Cytochrome b_6f complex was not placed in the original model [18]. Separate proof of the uniform distribution of cyt b_6f complexes in both stacked and unstacked regions followed extensive quantitation of cyts f and b_6 [21,22]. We assigned the task of long-range transport of electrons between PSII and PSI to plastocyanin rather than plastoquinone (figure 4) [23].

(d) *Why was the concept of lateral heterogeneity such a heretical idea?*

Prior to the 1980s, it was assumed that most PSII and LHCII were located in granal stacks, yet evidence that PSI was excluded from the granal-stacked domain

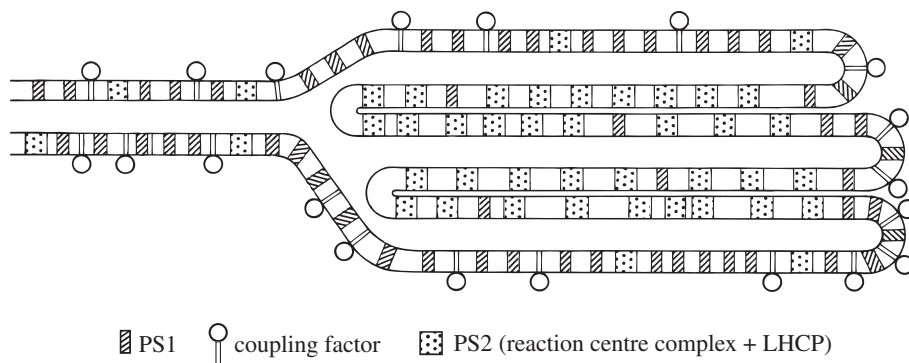▨ PS1   ♀ coupling factor   ▦ PS2 (reaction centre complex + LHCP)

Figure 3. The original schematic of the lateral heterogeneity in the distribution of PSII, PSI and ATP synthase between appressed and non-appressed thylakoid of plant chloroplasts [18].
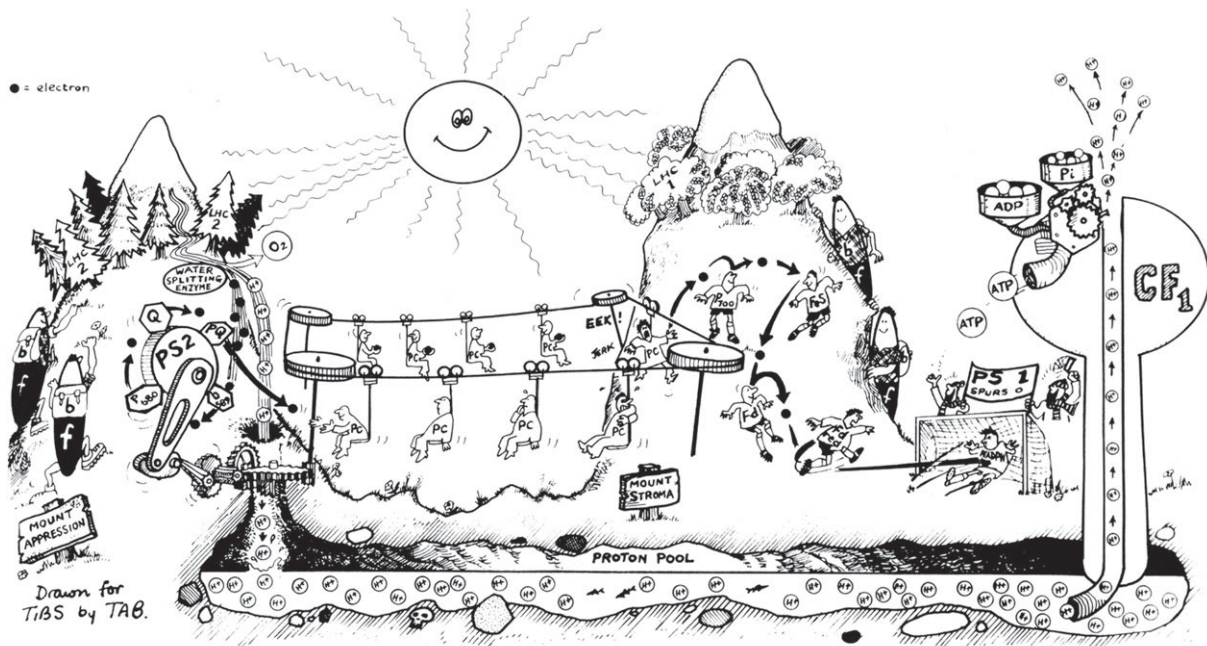

Figure 4. Cartoon drawn by TAB of *Trends Biochem. Sci.* (inspired by Bertil Andersson after visiting Queenstown, New Zealand, where J.M.A. grew up) depicting the lateral separation of PSII/LHCII on Mt Appression from PSI of Mount Stroma, while mountaineering cyt b_6f complexes receive electrons from PSII and laterally transport them to PSI then return back empty-handed for more [23].

was initially a heretical idea. Two extreme models for the regulation of light excitation energy distribution between the photosystems were in vogue in the 1970s: continuous array versus separate packages. Continuous array models were strongly favoured with a common light-harvesting antenna (LHCII) being shared between PSII and PSI (earlier studies [24,25] contain references for earlier models), whereas Boardman *et al.* [26] proposed a separate package model with limited interaction between the two photosystems to distribute some excitation energy between them. Part of the difficulty of accepting lateral heterogeneity of the photosystems was the notion that PSI did not have its own Chl *b*. However, noting that the LHCP/ PSII ratios were similar in appressed and non-appressed subchloroplast fragments, Andersson & Anderson [18] suggested that it was more reasonable to assume a close structural linkage of LHCII with PSII rather than it being shared between the photosystems. Mullet *et al.* [27] first showed that 'native'

PSI had its own antenna Chl-proteins, although they suggested that the low Chl *b* content present was due to contamination with LHCII. Later Chl *b* was shown to be an integral component of PSI, when a Chl *a/b*-protein was first isolated from *Chlamydomonas* [28] and then several Chl *a/b*-proteins were isolated from spinach [29].

Challenged by early scepticism about the lateral heterogeneity of thylakoid protein complexes for the functional and structural consequences of excluding PSI and ATP synthase from stacked granal domains that store the large PSII/LHCII supercomplexes, I focused early attention on the significance of grana stacking [23,30–34]. Fortunately, many others joined the challenge and the 1980s heralded a decade of excitement concerning 'why grana?' which is still continuing.

This paper is dedicated to my wonderful colleague and friend, Bertil Andersson, whose boundless curiosity and passion has brightened my 'why grana?' journey. I greatly appreciate the splendid meeting at the Royal Society at

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