



Minireview

Chloroplast structure: from chlorophyll granules to supra-molecular architecture of thylakoid membranes

L. Andrew Staehelin

Department of Molecular, Cellular and Developmental Biology, UCB 347, University of Colorado, Boulder, CO 80309-0347, USA (e-mail: staeheli@spot.colorado.edu; fax: +1-303-492-7744)

Received 15 August 2002; accepted in revised form 6 December 2002

Key words: ATP synthase, Daniel Branton, chloroplast, cytochrome b_6f , etioplast, grana, Brian Gunning, lateral heterogeneity, Werner Menke, Hugo von Mohl, molecular organization, Photosystem I, Photosystem II, prolamellar body, proplastid, David Simpson, state 1, state 2, thylakoid, thylakoid stacking, Diter von Wettstein

Abstract

This review provides a brief historical account of how microscopical studies of chloroplasts have contributed to our current knowledge of the structural and functional organization of thylakoid membranes. It starts by tracing the origins of the terms plastid, grana, stroma and chloroplasts to light microscopic studies of 19th century German botanists, and then describes how different types of electron microscopical techniques have added to this field. The most notable contributions of thin section electron microscopy include the elucidation of the 3-D organization of thylakoid membranes, the discovery of prolamellar bodies in etioplasts, and the structural changes in thylakoid architecture that accompany the light-dependent transformation of etioplasts into chloroplasts. Attention is then focused on the roles that freeze-fracture and freeze-etch electron microscopy and immuno electron microscopy have played in defining the extent to which the functional complexes of thylakoids are non-randomly distributed between appressed, grana and non-appressed stroma thylakoids. Studies reporting on how this lateral differentiation can be altered experimentally, and how the spatial organization of functional complexes is affected by alterations in the light environment of plants are also included in this discussion. Finally, the review points to the possible uses of electron microscope tomography techniques in future structural studies of thylakoid membranes.

Introduction

During the past 50 years, progress in structural photosynthesis research has been synonymous with the introduction of new research techniques that have increased the spatial resolution of thylakoid structures by three orders of magnitude, i.e., from 0.2 μm to 2 Å. In the late 1940s, photosynthesis researchers were still limited by the same 0.2 μm resolution of light microscopes encountered by the German botanist Hugo von Mohl (Figure 1), who, in 1837, provided the first definitive description of ‘Chlorophyllkörnern’ (chlorophyll granules) in green plant cells. Between 1950 and 1960 the introduction of the electron microscope (EM) and of thin specimen preparation methods

improved the 2-D resolution to < 100 Å, which led to the discovery of thylakoids and to the first characterization of their 3-D architecture. This was followed in the 1970s by a period in which freeze-fracture (-etch) electron microscopy (~50 Å resolution) became the most popular method for investigating the spatial organization of protein complexes in grana and stroma thylakoid membrane regions. The 1980s and 1990s, finally, brought crystallographic research techniques to the forefront, which have pushed in some cases the resolution of isolated protein complexes to the atomic level (~2 Å; Deisenhofer et al. 1995; Zouni et al. 2001; Jordan et al. 2001).

Do crystal structures represent both the ‘crowning achievement’ as well as ‘the end of the road’ for struc-



Figure 1. Hugo von Mohl (1805–1872), German botanist, who provided the first detailed description of ‘Chlorophyllkörnern’ (chlorophyll granules) in green leaves in 1837.

tural photosynthesis research? From my perspective the answer is ‘no.’ While crystal structure studies of the protein complexes of photosynthetic membranes are continuing to make headlines, photosynthesis researchers are becoming increasingly aware of the fact that the crystal structures alone cannot explain many known functional properties of thylakoid membranes. What is needed now is more precise information on how the different complexes are organized in thylakoid membranes and how this organization is altered in response to short-term (seconds to hours) and long-term (days to weeks) changes in the environment. Fortunately, two forms of EM tomography have emerged recently that have the potential of providing answers to these and other questions. In particular, dual axis EM tomography of cryofixed, freeze-substituted and resin-embedded samples provides a means for obtaining information about the *in situ* organization of chloroplast membranes with a 3-D resolution of about 70 Å, and electron tomographic studies of isolated and frozen thylakoids should provide information on thylakoid architecture with a resolution approaching 10 Å (McIntosh 2001). The latter resolution should provide researchers with the means of fitting crystal structure information into real thylakoids. To date, however, no EM tomographic studies of thylakoid membranes have been published.

The light microscopic era of chloroplast research produced names we still use

Several terms used in photosynthesis research today can be traced to 19th century light microscopic studies of von Mohl’s chlorophyll granules. Thus, the term ‘plastid’ was introduced by A.F.W. Schimper in 1883 as a substitute for chlorophyll granule, and the term ‘grana’ was coined the same year by A. Meyer to describe the dense, dot-like structures embedded in the semi-transparent material called ‘stroma’ of these plastids (Schimper 1885). While the terms grana and stroma are still being used today for the structures seen by Meyer, the term ‘chloroplast’ supplanted plastid as a name for the green organelles of leaves by the turn of the century. The term plastid, however, has survived as the name for the family of organelles of which chloroplasts are the best known member. By 1900, essentially all of the structural elements of chloroplasts resolvable with the light microscope had been reported.

Another notable achievement of the light microscopic era was the demonstration by C. Zirkle (1927) that chloroplasts were derived from colorless precursor organelles called ‘primordia’ (proplastids). This feat was achieved by showing that ‘primordia’ could be distinguished from the similarly sized mitochondria by the presence of minute starch granules and by demonstrating that mitochondria but not plastid ‘primordia’ were able to change the color of the dye Janus Green B.

Thin section electron microscopy revealed the ultrastructural features of chloroplasts

The chloroplasts of higher plants are lens-shaped organelles with a diameter of $\sim 5 \mu\text{m}$ and a width of $\sim 2.5 \mu\text{m}$ (Figure 2). Each chloroplast is delineated by two envelope membranes, which encompass an aqueous matrix, the stroma, and the internal photosynthetic membranes, the ‘thylakoids,’ a name introduced by Wilhelm Menke (1962, 1990). The envelope membranes control the transport of metabolites, lipids and proteins into and out of chloroplasts. They also contain multi-subunit bridging complexes that transport cytoplasmically synthesized proteins into the chloroplasts. Components of the stroma include the enzymes involved in carbon fixation, circular DNA anchored to the thylakoids, ribosomes, starch granules and plastoglobuli.

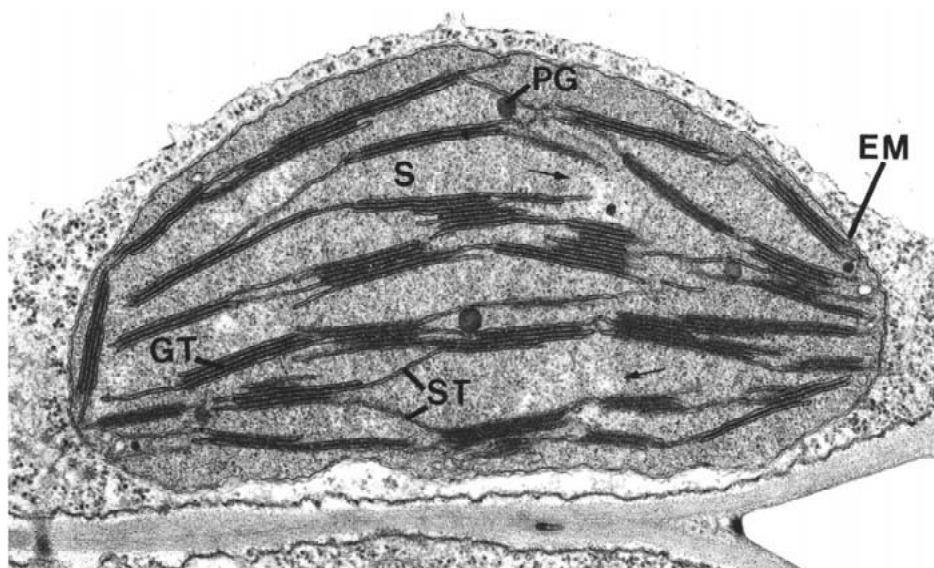


Figure 2. Thin section electron micrograph of a young tobacco chloroplast. Two envelope membranes (EM) surround the chloroplast stroma (S), within which stacked grana thylakoids (GT) and unstacked stroma thylakoids (ST) can be recognized. Plastoglobuli (PG) and DNA-containing regions (arrows) are also seen. Reproduced from Staehelin (1986).

Within each chloroplast, the thylakoids form a continuous 3-D membrane network that surrounds a single, anastomosing chamber, the thylakoid lumen. In thin section electron micrographs, the most striking morphological feature is the differentiation of the thylakoid membranes into stacked and non-stacked membrane domains (Figure 3). The cylindrical stacks of appressed membranes correspond to the grana structures described by A. Meyer. The non-stacked thylakoids are known as stroma thylakoids, because they are in direct contact with the stroma. According to this definition, the top and bottom membranes of the grana stacks are also stroma thylakoids. Mature chloroplasts may contain 40 to 60 grana stacks with diameters of 0.3 to 0.6 μm . The number of thylakoids per stack in mature thylakoids varies from <10 in high light chloroplasts to as many as 100 thylakoids in the extreme shade plant *Alocasia macrorrhiza* (Goodchild et al. 1972). The stacked thylakoid regions typically account for 50 to 60% of the membrane surface area in plants grown under high light, and for about 70% in those grown under low light conditions. From a historical perspective, it is interesting to note that the very first electron micrographs of chloroplasts published by G.A. Kausche and Helmut Ruska in 1940 already hinted that grana possessed a lamellar substructure.

The 3-D organization of the thylakoids of higher plant chloroplasts has been investigated by means

of reconstructions of serially sectioned chloroplasts (Heslop-Harrison 1963; Wehrmeyer 1964a, b; Paolillo 1970). As depicted in Figure 4, the individual grana stacks within a chloroplast are interconnected by about eight parallel, evenly spaced stroma thylakoids, which intersect the grana membranes and form right-handed helices around the stacks. At each intersection, a narrow, neck-like membrane region connects the grana and stroma membrane domains.

How this complex 3-D architecture of thylakoids is produced is still subject of debate. According to Brangeon and Mustardy (1979), the process starts with thylakoids that are highly perforated and contain no stacked regions. Grana formation is initiated by membrane expansion at specific sites in the margins of the perforations. As these tongue-like outgrowths expand, they begin to overlap the initiating thylakoid and become attached to it, thereby giving rise to the first stacked grana membrane. The next growth site most likely arises in the perforation margin adjacent to the first site, leading to a second grana thylakoid overgrowing the first one. During this overgrowth the initiating stroma thylakoid has been postulated to be pulled up and around the margin of the forming grana stack, and as more grana thylakoids are added to the stack the spiral-like arrangement of the stroma thylakoid becomes established. Yet to be determined is whether the multiple parallel stroma thylakoids con-

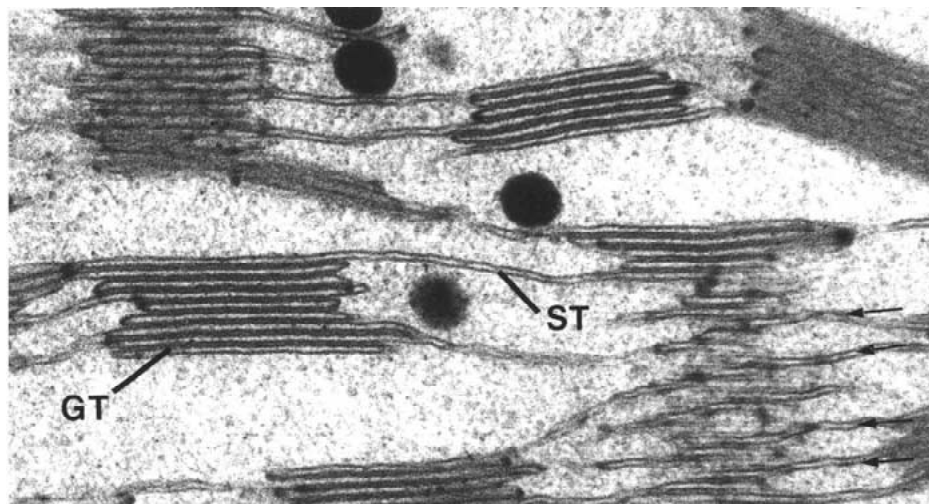


Figure 3. Higher magnification view of thin sectioned grana (GT) and stoma (ST) thylakoids of a spinach chloroplast. Reproduced from Staehelin (1986).

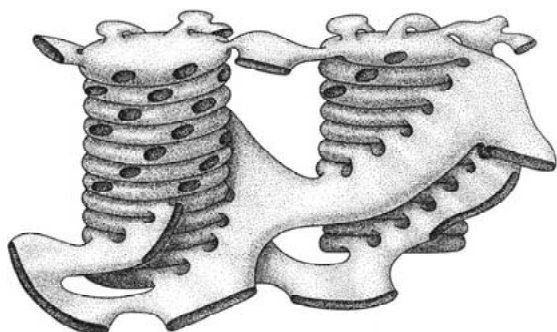


Figure 4. Diagram illustrating the spatial relationship between stacked grana and interconnecting stroma thylakoids. This model is based on the analysis of serial thin sections through chloroplasts and micrographs of freeze-fractured chloroplasts. Reproduced from Staehelin and van der Staay (1996).

nected to a single grana stack are formed by splitting of the first stroma thylakoid, or by the secondary attachment of separate stroma thylakoids to the already formed stack (reviewed by Mustardy 1996).

Light-induced conversion of etioplasts into chloroplasts

When angiosperm plants are grown in the dark, the light-dependent synthesis of chlorophyll is inhibited, which, in turn, blocks the assembly of chlorophyll-protein complexes and thylakoid formation. Non-pigmented, leaf plastids formed under these conditions are called 'etioplasts' (Figure 5). Such etioplasts represent an arrested stage in the normal development

of proplastids into chloroplasts. Starting with the pioneering investigations of Heitz (1954) and Leyon (1954), numerous electron microscopical studies have reported on the unique ultrastructure of etioplasts and the changes in architecture of their thylakoids during light-induced chloroplast development (reviewed in Tilney-Bassett 1978; Wellburn 1982). The most notable feature of etioplasts is a semi-crystalline tubular lattice, the 'prolamellar body,' whose walls are physically continuous with prothylakoids that extend from its surface (Gunning 1965; Figure 6). The primary chemical constituents of prolamellar bodies are mono- and digalactosyldiacylglycerol and phosphatidylglycerol lipids (Lütz 1986), protochlorophyllide and the enzyme protochlorophyllide oxidoreductase (Shaw et al. 1985). In contrast, the prothylakoids have been shown to contain ATP synthase (Wellburn 1977), cytochrome b_6f and small amounts of protochlorophyllide reductase (Shaw et al. 1985). The light-induced conversion of etioplasts into chloroplasts takes 24 to 48 h and involves the transformation of the prolamellar bodies into thylakoids within which all of the functional complexes of the photosynthetic electron transport chain accumulate (Wellburn 1982).

The study of chloroplast mutants has also played a major role in the elucidation of the roles of proteins, lipids and pigments in the formation of the 3-D architecture of thylakoid membranes. However, a discussion of this literature is beyond the scope of this review (see Henningsen and Stummann 1982; Falbel and Staehelin 1996).

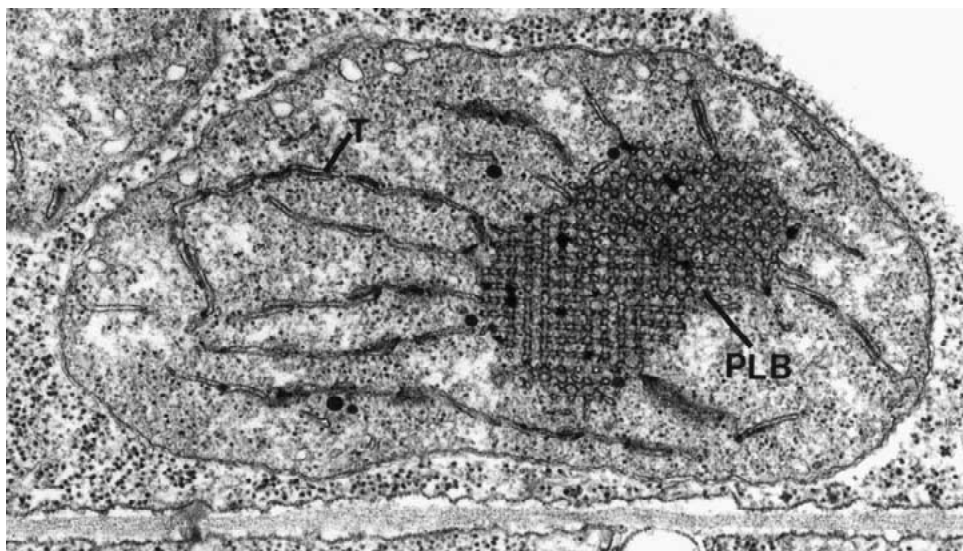


Figure 5. Thin section electron micrograph of a pea etioplast during a very early stage of light-induced conversion into a chloroplast. Thylakoid (T), prolamellar body (PLB).

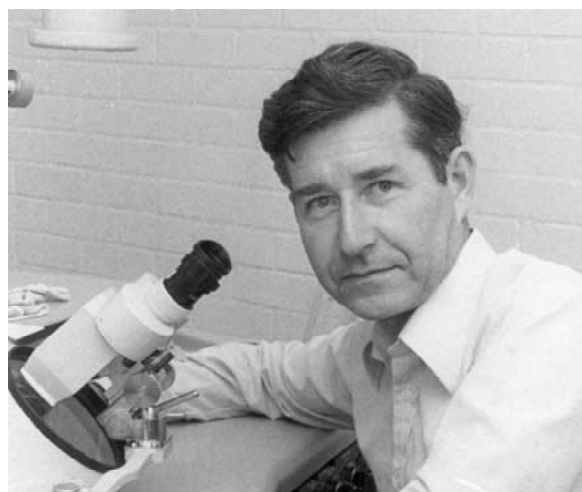


Figure 6. Brian Gunning, electron microscopist and plant cell biologist, whose studies of etioplasts provided important insights into the architectural principles of prolamellar bodies.



Figure 7. The author (Andrew Staehelin) in 1994.

Freeze-fracture (-etch) electron microscopy provides insights into the organization of integral and peripheral protein complexes of thylakoid membranes, respectively

The primary tools of this author's (Figure 7) investigations of thylakoid structure were freeze-fracture and freeze-etch electron microscopy and immuno electron microscopy. Freeze-fracture electron microscopy provides a means for visualizing the lateral distri-

bution of *integral* protein complexes in thylakoid membranes (Photosystems I and II [PS I and II], cytochrome b_6f [Cyt b_6f], light-harvesting complex II [LHC II] and the F_0 -subunit of the ATP synthase in thylakoid membranes; Figure 8), whereas freeze-etch electron microscopy provides information on the lateral distribution of *peripheral* protein complexes (F_1 -subunit of the ATPsynthase and the water splitting complex associated with PS II; Figure 9). (For a personal perspective on the lateral distribution of

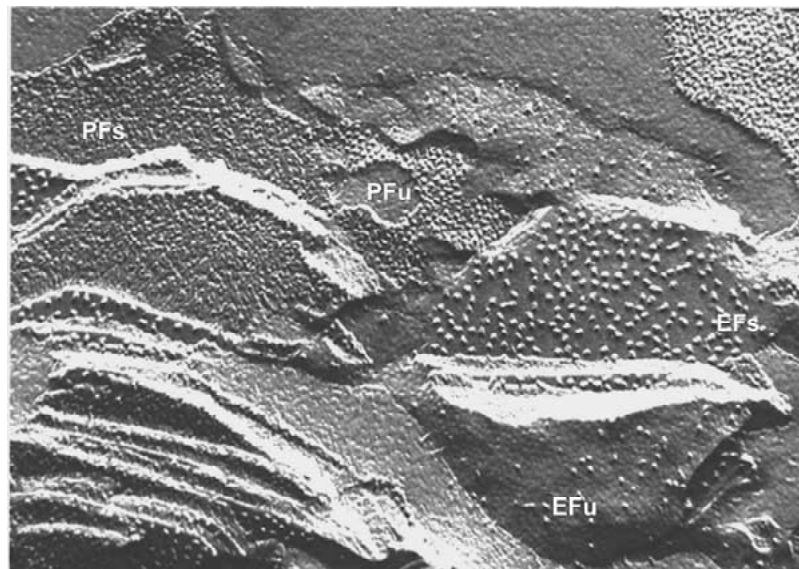


Figure 8. Freeze-fractured thylakoids of spinach. The flat, partly circular membranes of two grana stacks (left, right) appear interconnected by more tubular membranes of a stroma lamella. The complementary type fracture faces marked PFs and EFs are characteristic of stacked membrane regions, whereas the fracture faces PFu and EFu belong to unstacked membrane regions (see interpretive diagram Figure 5). Note the different sizes and densities of particles on the different types of fracture faces. Reproduced from Staehelin (1976).

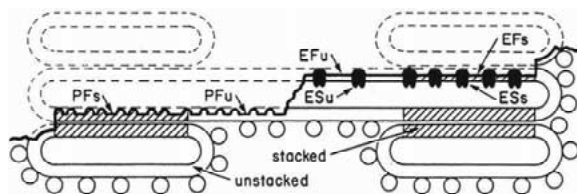


Figure 9. Schematic diagram illustrating the nomenclature used to label freeze-fractured and freeze-etched thylakoid membranes (see Figures 3 and 8 and explanation in the text).

thylakoid membrane complexes, see Jan Anderson 2002.)

As first recognized by Daniel Branton (1966; Figure 10), the process of freeze-fracturing of frozen biological specimens at -100°C causes membranes to be split along the central hydrophobic plane of their bilayer continuum to produce two complementary fracture faces. The structural details of these frozen fracture faces are preserved for viewing in the electron microscope by means of high resolution platinum/carbon replicas (Chapman and Staehelin 1986). Because the integral protein complexes are not split during the fracturing process, the individual complexes are seen as discrete particles that rise above the smooth fracture face of the bilayer (Figure 8). To observe true thylakoid surfaces in freeze-etch replicas (Figure 11), the freeze-fractured samples are subjected



Figure 10. Daniel Branton, cell biologist, who discovered that the process of freeze-fracturing splits bilayer membranes along their central plane.

to an additional 'etching' step to remove the frozen water from the membrane surfaces before replication. Together, the two complementary fracture faces and the etched membrane surfaces can provide remarkably detailed insights into the 2-D and 3-D organization of proteins and lipids in thylakoid membranes.

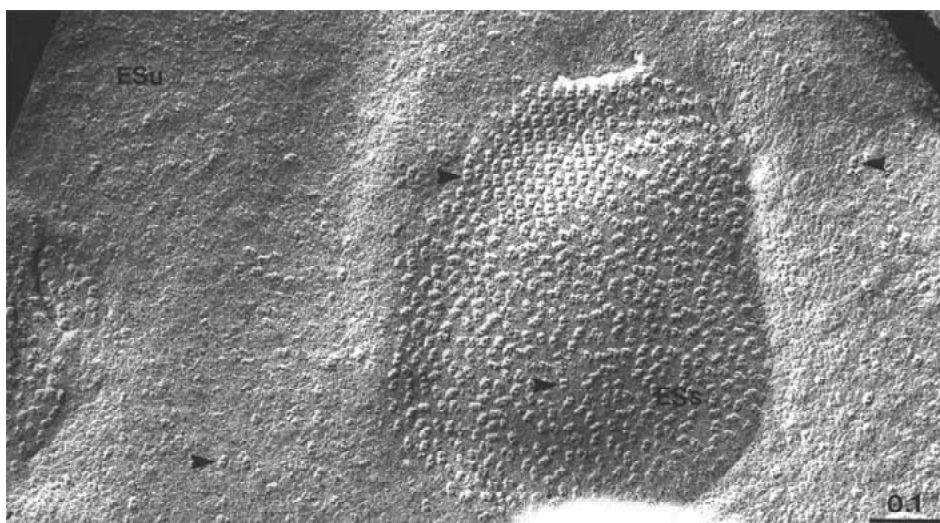


Figure 11. Lumenal surface of a spinach thylakoid exposed by freeze-etching. The stacked, grana membrane domain (ESs) in the center of the micrograph can be clearly distinguished from the surrounding unstacked, stroma membrane region (ESu) due to the high density of large protruding particles that exhibit two sub-domains (arrowheads). A small number of similar particles are seen in the surrounding stroma membrane area (arrowheads). The large dimeric particles in the ESs region correspond to the luminal domains of the dimeric PS II complexes that protrude into the thylakoid lumen. Each dimeric particle lobe contains a set of the 17, 23, and 33 kD water-splitting enzymes. Reproduced from Staehelin (1976).

Development of a common freeze-etch nomenclature

The nomenclature for the fracture faces and the true surfaces of all types of biological membranes was defined in the paper 'Freeze-etching nomenclature' (Branton et al. 1975). Because I was the instigator of this nomenclature, I will provide a brief account of its history here. At the time that Branton (1965) proposed that freeze-fracturing split biological membranes along the central plane of their bilayer, there was no commonly accepted model of biological membranes. Indeed, in my first review, with M.C. Probine, on structural aspects of cell membranes (Staehelin and Probine 1970), I reproduced diagrams of 16 membrane models, 15 of which had been published between 1956 and 1967! The 'fluid mosaic membrane model' of Jon Singer and Garth Nicolson that dominates today's thinking on this subject was published only in 1972. This lack of consensus on membrane structure helped spawn a bewildering assortment of nomenclatures in the freeze-fracture/-etch literature, which made communication difficult. The problem came to a head at the 1974 annual meeting of the American Society of Cell Biology, where in one short-talk session, four different nomenclatures were used by different speakers. At the end of that session I proposed that those

interested in developing a new common nomenclature meet in the corridor to discuss how to develop such a nomenclature. Peter Satir (Albert Einstein University, New York) and I agreed to organize this undertaking. Daniel Branton was recruited to the effort and came up with the idea of the EF/PF and ES/PS (vide infra)-nomenclature, and while he wrote the paper, I prepared the diagram explaining the nomenclature. Finally, we got 12 leaders in the field to sign on to the new nomenclature and persuaded Science to publish the paper (Branton et al. 1975). The nomenclature builds on the fact that each membrane has two halves, one of which is in contact with the protoplasm, the P-leaflet, the other with the *ex*oplasmic or *ex*tracellular space (E-leaflet). The corresponding fracture faces were designated PF and EF, respectively, and the true surfaces of these leaflets, PS and ES. In the case of chloroplasts, the suffixes 'u' for *un*stacked (stroma) membranes and 's' for *s*tacked (grana) membranes were added as shown in Figure 9 (Branton et al. 1975).

Functional insights gained from the analysis of freeze-fractured and freeze-etched thylakoid membranes

As seen in Figure 8, striking differences in the distribution of freeze-fracture particles are seen both



Figure 12. Diter von Wettstein, geneticist, who produced a large library of photosynthetic barley mutants at the Carlsberg Laboratory in Copenhagen, Denmark. Many of these mutants provided important insights into structure-function relationships of thylakoid membranes.



Figure 13. David Simpson, electron microscopist, whose freeze-fracture studies of thylakoid membranes of von Wettstein's barley mutants contributed to the elucidation of the identity and composition of the particles seen on thylakoid fracture faces (see Figure 8).

between the EF and PF faces as well as between the stacked (EFs and PFs) and the non-stacked or unstacked (EFu and PFu) thylakoid membrane domains. The first correct interpretation of the four fracture faces was provided in a study of wild-type and mutant *Chlamydomonas* thylakoids (Goodenough and Staehelin 1971). The two principle findings of this investigation were that the particles associated with each of the four fracture faces contained particles that

fell into characteristic size classes, and that the non-random lateral distribution of the particles between grana and stroma membranes was dependent on membrane stacking. This set the stage for two decades of investigations devoted to the correlation of specific types of freeze-fracture/-etch particles with the five types of functional complexes identified by other means. The principle research groups involved in this effort were located in East Lansing, Michigan (C. Arntzen, P. Armond, J. Burke, P. Haworth, D. Kyle, J. Mullet), in Copenhagen, Denmark (D. von Wettstein [Figure 12], G. Høyer-Hansen, B. Møller, D. Simpson [Figure 13]), in Paris, France (P. Bennoun, J. Olive, M. Recouvreur, F. Wollman), in Canberra, Australia (J. Anderson, N. Fuad, D. Goodchild, I. Ryrie), and my research group in Boulder, Colorado (A. Staehelin, K. Allen, D. Allred, D. Carter, T. Dunahay, T. Falbel, T. Giddings, A. McDonnell, K. Miller, E. Mörschel, M. Sigrist, S. Sprague, G. van der Staay, A. Varga). These investigations involved correlative structural and biochemical studies of thylakoids of plants grown under different light conditions (Armond et al. 1977), of barley and *Chlamydomonas* chloroplast mutants (reviewed in Simpson et al. 1986; Olive and Vallon 1991), and of purified and reconstituted protein complexes (reviewed in Staehelin 1986). A diagrammatic summary of the principle findings of these studies is illustrated in Figure 14. Most notably, every type of protein complex was found to partition either with the EF or the PF fracture face. Only Photosystem (PS) II-related complexes partition with the EF faces; all of the other complexes are seen on PF faces. Whereas the LHC II, Cyt b_6f , and CF_0 complexes each fall into one size class, the size of the PS I and PS II complexes varies depending on the amount of bound antenna proteins. Finally, these studies provided information to suggest that PS II forms dimeric complexes in thylakoid membranes (Seibert et al. 1987).

Freeze-fracture/-etch, thin section immunolabeling and biochemical fractionation studies all contributed significantly to the elucidation of the lateral distribution of the different functional complexes between stacked and non-stacked membrane domains (Figures 8, 11, 14, Table 1). The first definitive study of this kind demonstrated that the ATP synthase complex was limited to non-stacked stroma thylakoids and that the density of those complexes was about 700 per μm^2 of stroma membrane (Miller and Staehelin 1976). In contrast, about 85% of the PS II complexes are concentrated in the grana and 15% in the

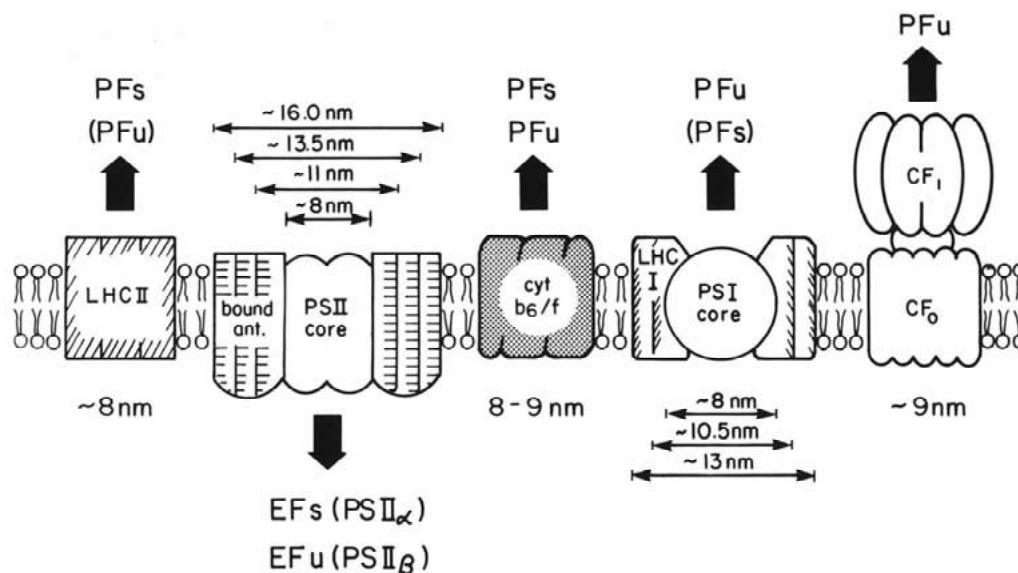


Figure 14. Schematic diagram relating the five functional complexes of thylakoid membranes to different categories of freeze-fracture particles. Reproduced from Staehelin and van der Staay (1996).

Table 1. Distribution of chloroplast membrane components between grana and stroma thylakoids under state 1 and state 2 conditions. The numbers indicate the percentage of each component found associated with the grana and stroma membranes in state 1 and state 2 (LHC II phosphorylation). Reproduced from Staehelin and van der Staay (1996)

Component	State 1		State 2	
	Grana	Stroma	Grana	Stroma
PS II	85	15	85	15
PS I	10	90	10	90
CytB ₆ f	50	50	30	70
LHC II	90	10	70	30
Plastocyanin	40	60	55	45
ATP synthase	0	100	0	100

stroma thylakoid regions (Andersson and Anderson 1980). Interestingly, while the PS II complexes in the stacked regions were shown to be of the larger (dimeric) type, those in the non-stacked domains were of the smaller (monomeric) type (Staehelin 1976). Most likely, these differences in size relate to the D1 damage and refurbishing cycle during which the dimeric PS II complexes with a damaged D1 protein first fall apart to allow only the damaged complex to transfer to the stroma membrane regions (Barbato et al. 1992). The PS I complexes show an opposite distribution between grana and stroma membrane

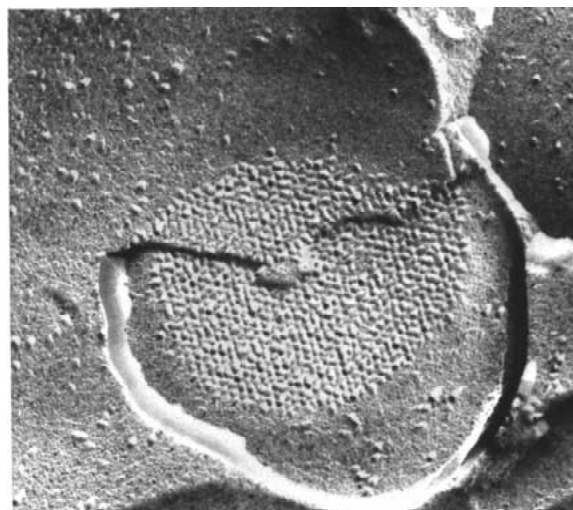


Figure 15. Freeze-fracture micrograph of reconstituted lipid vesicles containing purified LHC II complexes in the presence of 100 mM NaCl. The ~80 Å particles correspond to individual trimeric LHC II complexes, and they are seen to be concentrated in the central adhesion zone between the two vesicles. These types of experiments proved that LHC II is the principle membrane adhesion factor of thylakoid membranes. Reproduced from McDonnell and Staehelin (1980).

domains with over 90% of the complexes associated with the stroma thylakoids (Vallon et al. 1986). The localization of between 70 and 90% of the light harvesting complex (LHC) II proteins and particles to the stacked regions (Andersson and Anderson 1980;



Figure 16. From left to right (in the front row): Anne Joliot, Wolfgang Junge, Andrew Staehelin and Jacques Breton at the 1978 CIBA Symposium on Chlorophyll Organization and Energy Transfer in Photosynthesis held at the CIBA Foundation (now Novartis Foundation) in London. Pierre Joliot and Jim Barber are seen in the background.

Kyle et al. 1983) is consistent with the finding that liposomes with reconstituted LHC II complexes can be induced to stack under the same ionic conditions that produce thylakoid stacking (Figure 15; McDonnell and Staehelin 1980). The Cyt b_6f complexes, finally, are distributed nearly equally between grana and stroma thylakoid domains (Cox and Andersson 1981; Allred and Staehelin 1986).

Stacking affects thylakoid organization and function, and is regulated

Thylakoid stacking is responsible for the non-random distribution of the different functional complexes between grana and stroma thylakoids. This was shown by unstacking thylakoids in low ionic strength buffers developed in the laboratory of Norman Good (Izawa and Good 1966) and by restacking them with ~ 5 mM Mg^{++} or ~ 150 mM Na^+ ions (Staehelin 1976). While unstacking led to the complete randomization of the particles, restacking led to the same re-segregation of complexes as in controls. Barber (1980) has postulated that the distribution of protein complexes in membranes is dependent on the surface charge distribution of the proteins and that stacking is mediated by proteins with a low charge density. The primary function of the stacking-mediated lateral separation of PS II and PS I complexes appears to be to physically separate a slow exciton trapping system, PS II, from a $\sim 3 \times$ faster and thus more efficient system, PS I (Staehelin and Arntzen 1983; Trissl and Wilhelm 1993).

State transitions (see Allen 2002) also lead to changes in membrane architecture. In particular, when PSII receives too much excitation energy, up to 25% of the grana LHC II complexes become phosphorylated (Larsson et al. 1983), which causes them to translocate to stroma thylakoids in a reversible manner (Kyle et al. 1983). This movement of LHC II complexes helps balance the distribution of excitation energy between PS II and PS I (Staehelin and Arntzen 1983). Other thylakoid components that redistribute during state transitions and thereby help regulate the electron transport chain include the Cyt b_6f complex (Vallon et al. 1991) and plastocyanin (Haehnel et al. 1989).

Final thoughts

Looking back, I feel very privileged to have had the opportunity to be a member of and to have contributed to the field of photosynthesis research at a very exciting time of its development. During the many photosynthesis meetings that I attended I also met many great scientists who were also wonderful people, and the memories of the many stimulating hours spent discussing photosynthesis with those colleagues are what I treasure most from those very exciting years of my research career (Figure 16).

Acknowledgments

I would like to thank Golnar Vazirabadi for her help in the preparation of this manuscript and Professor Brian

Gunning for providing the picture of Hugo von Mohl. This manuscript was edited by Govindjee.

References

- Allen JF (2002) Plastoquinone redox control of chloroplast thylakoid protein phosphorylation and distribution of excitation energy between photosystems; discovery, background, implications. *Photosynth Res* 73: 139–148
- Allred DR and Staehelin LA (1986) Spatial-organization of the cytochrome b_6 -f complex within chloroplast thylakoid membranes. *Biochim Biophys Acta* 849: 94–103
- Anderson JM (2002) Changing concepts about the distribution of Photosystems I and II between grana-appressed and stroma-exposed thylakoid membranes. *Photosynth Res* 73: 157–164
- Anderson B and Anderson JM (1980) Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach-chloroplasts. *Biochim Biophys Acta* 593: 427–440
- Armond PA, Staehelin LA and Arntzen CJ (1977) Spatial relationship of Photosystem-I, Photosystem-II, and light-harvesting complex in chloroplast membranes. *J Cell Biol* 73: 400–418
- Barbato R, Friso G, Rigoni F, Vecchia FD and Giacometti GM (1992) Structural changes and lateral distribution of Photosystem-II during donor side photoinhibition of thylakoids. *J Cell Biol* 119: 325–335
- Barber J (1986) Surface electrical charges and protein phosphorylation. In: Staehelin LA and Arntzen CJ (eds) *Photosynthesis III: Photosynthetic Membranes and Light Harvesting Systems*, Vol 19, pp 653–664. Springer-Verlag, Berlin
- Brangeon J and Mustardy L (1979) The ontogenetic assembly of intra-chloroplastic lamellae viewed in 3-dimensions. *Biol Cell* 36: 71–80
- Branton D (1966) Fracture faces of frozen membranes. *Proc Natl Acad Sci USA* 55: 1048–1056
- Branton D, Bullivant S, Gilula NB, Karnovsky MJ, Moor H, Mühlethaler K, Northcote DH, Packer L, Satir B, Satir P, Speth V, Staehelin LA, Steere RL and Weinstein RS (1975) Freeze-etching nomenclature. *Science* 190: 54–56
- Chapman RL and Staehelin LA (1986) Freeze-fracture (-etch) electron microscopy. In: Todd WJ and Aldrich HC (eds) *Ultrastructure Techniques for Microorganisms*, pp 213–240. Plenum Publishing Co, New York
- Cox RP and Anderson B (1981) Lateral and transverse organization of cytochromes in the chloroplast thylakoid membrane. *Biochem Biophys Res Commun* 103: 1336–1342
- Deisenhofer J, Epp O, Sinning I and Michel H (1995) Crystallographic refinement at 2.3 Å resolution and refined model of the photosynthetic reaction centre from *Rhodospseudomonas viridis*. *J Mol Biol* 246: 429–457
- Falbel TG, Meehl JB and Staehelin LA (1996) Severity of mutant phenotype in a series of chlorophyll-deficient wheat mutants depends on light intensity and the severity of the block in chlorophyll synthesis. *Plant Physiol* 112: 821–832
- Goodchild DJ, Bjorkman O and Pyliotis NA (1972) Chloroplast ultrastructure, leaf anatomy, and content of chlorophyll and soluble protein in rainforest species. *Carnegie Inst Washington Yearb* 71: 102–107
- Goodenough UW and Staehelin LA (1971) Structural differentiation of stacked and unstacked chloroplast membranes. Freeze-etch electron microscopy of wild-type and mutant strains of *Chlamydomonas*. *J Cell Biol* 48: 594–619
- Gunning B (1965) The greening process in plastids. The structure of the prolamellar body. *Protoplasma* 60: 111–130
- Haehnel W, Ratajczak R and Robenek H (1989) Lateral distribution and diffusion of plastocyanin in chloroplast thylakoids. *J Cell Biol* 108: 1397–1405
- Heitz E (1954) Kristallgitterstruktur des Granum junger Chloroplasten von *Chlorophytum*. *Exp Cell Res* 7:606–615
- Henningsen KW and Stummann BM (1982) Use of mutants in the study of chloroplast biogenesis. In: Parthier B and Boulter D (eds) *Nucleic Acids and Proteins in Plants II*, Vol 14B, pp 597–644. Springer-Verlag, Heidelberg
- Heslop-Harrison J (1963) Structure and morphogenesis of lamellar systems in grana-containing chloroplasts. I. Membrane structure and lamellar architecture. *Planta* 60: 243–260
- Izawa S and Good NE (1966) Effects of salts and electron transport on the conformation of isolated chloroplasts. II. Electron microscopy. *Plant Physiol* 41: 544–553
- Jordan P, Fromme P, Witt HT, Klukas O, Saenger W and Krauss N (2001) Three-dimensional structure of cyanobacterial Photosystem I at 2.5 Å resolution. *Nature* 411: 909–917
- Kausche GA and Ruska H (1940) Zur Frage der Chloroplastenstruktur. *Naturwissenschaften* 28: 303–304
- Kirk JTO and Tilney-Bassett RAE (1967) *The Plastids. Their Chemistry, Structure, Growth and Inheritance*. Freeman, London.
- Kyle DJ, Staehelin LA and Arntzen CJ (1983) Lateral mobility of the light-harvesting complex in chloroplast membranes controls excitation-energy distribution in higher plants. *Arch Biochem Biophys* 222: 527–541
- Larsson UK, Jergil B, and Andersson B (1983) Changes in the lateral distribution of the light-harvesting chlorophyll-a/b-protein complex induced by its phosphorylation. *Eur J Biochem* 136: 25–29
- Leyon H (1954) The structure of chloroplasts. IV. The development and structure of the *Aspidistra* chloroplast. *Exp Cell Res* 7: 265–266
- Lütz C (1986) Prolamellar bodies. In: Staehelin LA and Arntzen CJ (eds) *Photosynthesis III: Photosynthetic Membranes and Light-Harvesting Systems*, Vol 19, pp 683–692. Springer-Verlag, Berlin.
- McDonnell A and Staehelin LA (1980) Adhesion between liposomes mediated by the chlorophyll a-b light-harvesting complex isolated from chloroplast membranes. *J Cell Biol* 84: 40–56
- McIntosh JR (2001) Electron microscopy of cells: a new beginning for a new century. *J Cell Biol* 153: F25–F32
- Menke W (1962) Structure and chemistry of plastids. *Annu Rev Plant Physiol* 13: 27–44
- Menke W (1990) Retrospective of a botanist. *Photosynth Res* 25: 77–82
- Miller KR and Staehelin LA (1976) Analysis of thylakoid outer surface: coupling factor is limited to unstacked membrane regions. *J Cell Biol* 68: 30–47
- Mustardy L (1996) Development of thylakoid membrane stacking. In: Ort DR and Yocum CF (eds) *Oxygenic Photosynthesis: the Light Reactions*, Vol 4, pp 59–68. Kluwer Academic Publishers, Dordrecht, The Netherlands
- Olive J and Vallon O (1991) Structural organization of the thylakoid membrane – freeze-fracture and immunocytochemical analysis. *J Elec Microsc Tech* 18: 360–374
- Paolillo DJ (1970) The three dimensional arrangement of intergranal lamellae in chloroplasts. *J Cell Sci* 6: 243–255
- Schimper A (1885). Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde. *Jahrbücher Wiss Bot* 16: 1–247

- Seibert M, Dewit M and Staehelin LA (1987) Structural localization of the O₂-evolving apparatus to multimeric (tetrameric) particles on the luminal surface of freeze-etched photosynthetic membranes. *J Cell Biol* 105: 2257–2265
- Shaw P, Henwood J, Oliver R and Griffiths T (1985) Immunogold localization of protochlorophyllide oxidoreductase in barley etioplasts. *Eur J Cell Biol* 39: 50–55
- Simpson D (1986) Freeze-fracture studies of mutant barley chloroplast membrane. In: Staehelin LA and Arntzen CJ (eds) *Photosynthesis III: Photosynthetic Membranes and Light-Harvesting Systems*, Vol 19, pp 665–674. Springer-Verlag, Berlin
- Singer SJ and Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes. *Science* 175: 720–731
- Staehelin LA (1976) Reversible particle movements associated with unstacking and restacking of chloroplast membranes *in vitro*. *J Cell Biol* 71: 136–158
- Staehelin LA (1986) Chloroplast structure and supramolecular organization of photosynthetic membranes. In: Staehelin LA and Arntzen CJ (eds) *Photosynthesis III: Photosynthetic Membranes and Light-Harvesting Systems*, Vol 19, pp 1–84. Springer-Verlag, Berlin
- Staehelin LA and Arntzen CJ (1983) Regulation of chloroplast membrane-function – protein-phosphorylation changes the spatial-organization of membrane-components. *J Cell Biol* 97: 1327–1337
- Staehelin LA and Probine MC (1970) Structural aspects of cell membranes. In: Preston RD (ed) *Advances in Botanical Research*, Vol 3, pp 1–52. Academic Press, London
- Staehelin LA and van der Staay GWM (1996) Structure, composition, functional organization and dynamic properties of thylakoid membranes. In: Ort DR and Yocum CF (eds) *Oxygenic Photosynthesis: the Light Reactions*, Vol 4, pp 11–30. Kluwer Academic Publishers, Dordrecht, The Netherlands
- Trissl HW and Wilhelm C (1993) Why do thylakoid membranes from higher plants form grana stacks? *Trends Biochem Sci* 18: 415–419
- Vallon O, Wollman FA and Olive J (1986) Lateral distribution of the main protein complexes of the photosynthetic apparatus in *Chlamydomonas reinhardtii* and in spinach. An immunocytochemical study using intact thylakoid membranes and a PS II-enriched membrane preparation. *Photobiochem Photobiophys* 12: 203–220
- Vallon O, Bulte L, Dainese P, Olive J, Bassi R and Wollman FA (1991) Lateral redistribution of cytochrome b₆/f complexes along thylakoid membranes upon state transitions. *Proc Natl Acad Sci USA* 88: 8262–8266
- von Mohl H (1837) *Untersuchungen über anatomische Verhältnisse des Chlorophylls*. Dissertation, W. Michler, University of Tübingen, Germany
- Wehrmeyer W (1964a) Zur Klärung der strukturellen Variabilität der Chloroplastengrana des Spinats in Profil und Aufsicht. *Planta* 62: 272–293
- Wehrmeyer W (1964b) Über Membranbildungsprozesse in Chloroplasten. II. Zur Entstehung der Grana durch Membranüberschiebung. *Planta* 63: 13–30
- Wellburn AR (1977) Distribution of chloroplast coupling factor (CF₁) particles on plastid membranes during development. *Planta* 135: 191–198
- Wellburn AR (1982) Bioenergetic and ultrastructural changes associated with chloroplast development. *Int Rev Cytol* 80: 133–191
- Zirkle C (1927) The growth and development of plastids in *Lunaria vulgaris*, *Elodea canadensis*, and *Zea mays*. *Am J Bot* 14: 429–445
- Zouni A, Witt HT, Kern J, Fromme P, Krauss N, Saenger W and Orth P (2001) Crystal structure of Photosystem II from *Synechococcus elongatus* at 3.8 Å resolution. *Nature* 409: 739–743