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Minireview

Photosystem I reaction center: past and future

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Abstract

Science has always been drawn to uncover fundamental life processes. Photosynthesis is one, if not the most fascinating, of them. Within it, the protein complexes that catalyze light-induced electron transport and photophosphorylation are enchanting creations of evolution. Plant Photosystem I (PS I) is not the largest protein complex in nature but it is the most elaborate in the number of prosthetic groups involved in its fabric. Thirty years ago, one of us (NN) developed a fascination for this complex and, despite the apparent neglect (lack of publications in the last few years), never let it go. Only a crystal structure at 2 Å resolution will satiate our curiosity. In this minireview, we trace the past, and end the article with a comment on future prospects. For the present situation, see Parag Chitnis (2001).

Past

Prologue

Much like scientists, historians pretend to be objective. But was there ever a historian whose objectivity was not influenced by his surroundings? So it is as a historian that one of us (NN) will now look at the history of the Photosystem I (PS I) reaction center with total subjectivity. [Since this article was written by two authors, the use of 'I' and 'we' may get confusing. Thus, we have decided that when 'I,' or 'me,' or 'my' will be used, it will refer to the first author (NN) throughout this text.] Hopefully, my subjectivity will be tempered by my co-author (AB), who has yet to endure any battle scars during his work. Description of the present has been left to my former postdoc Parag Chitnis (see Chitnis 2001). My 30-year relationship with PS I has convinced me that this is the most fascinating machine on earth, and that understanding it will diminish the need for irrational beliefs. The reader will be happy to know that we will not discuss the history of photosynthesis research, but will restrict ourselves to selected aspects of the PS I reaction center starting in the 1960s.

In the early 1960s, there was a thylakoid membrane that in the light was capable of water oxidation, NADP (nicotinamide adenine dinucleotide phosphate) reduction and ATP formation (Whatley et al. 1963), and it was good. Biochemical and biophysical studies revealed that these reactions are catalyzed by two separate Photosystems (PS I and PS II) and an ATP synthase that produces ATP at the expense of an elusive high-energy intermediate formed by the light reaction (see e.g., Hill and Bendall 1960; Avron and Shavit 1965; Vambutas and Racker 1965; McCarty and Racker 1966; Jagendorf 1967; Rabinowitch and Govindjee 1969; Jack Myers, Roderick Clayton, and Jan M. Anderson, this issue). Between the two photosystems, there was a cytochrome b_6f complex that mediated the electron transport between them, and converted the redox energy into a high-energy intermediate for ATP formation (Cramer and Butler 1967; Nelson and Neumann 1972). (See Figures 1 and 2 for photographs of the people involved. A 1973 group

Figure 1. Top left: Left to right are: Jeff Schatz, Efraim Racker, and Nathan Nelson (1983). *Top right*: Nathan Nelson (1973). *Bottom*: A group photograph at a Gordon Conference (1973). Left to right are: *First row:* D. Arnon; N. Bishop; R. Clayton; W. Butler; A. San Pietro; W. Arnold; C.S. French; H. Gaffron; A. Jagendorf; and B. Ke. *Second row:* S. Gairon; B. Chance; J. Brown; C. Sybesma; Govindjee; N. Nelson; B. Mayne; and H. Witt. *Third row:* J. Coombs; R. Jensen; H. Huzisige; R. Wang; F. Nostrand; T. Bannister; T. Punnet; H. Lyman; B.A. Melandri; J-M. Briantais; Bo C. Johansson; J. Michel; and K. Zankel. *Fourth row:* D. Walker; G. Hoch; M. Gibbs; J. Myers; E. Bamberger; L. Vernon; L. Anderson; A. Mitsui; J. Leigh; R. Fuller; and H. Gest.

photograph is shown in the bottom panel of Figure 1: see the legend for the location of Clayton, Govindjee, Jagendorf, Myers, and Nelson; it also shows Bacon Ke and Leo Vernon, mentioned later. Chitnis, mentioned above, is in the bottom left panel of Figure 2)

The 1970s started with a biochemical inquiry into the nature of the protein complexes that are able to perform partial reactions of photosynthesis, and it was good. The question was how to go about the resolution of the system into its defined four complexes? In my perspective, the clue was in volume X of *Methods in Enzymology*, where the Mitochondriacs (as we called the scientists who study mitochondria; see Schatz 2000) describe how they managed to resolve the defined complexes that catalyze partial reactions in oxidative phosphorylation. Moreover, they attempted not only to resolve the system but also (God forbid) to reconstitute it. The champion of this approach was Efraim Racker, who in 1970 took the risk of accepting me as a postdoctoral fellow (the top left photo in Figure 1 shows Racker in the middle; and top right photo is that of Nelson by himself).

Figure 2. Top left: Wolfgang Junge; Rachael Nechushtai; and Nathan Nelson (1990). *Top right:* Hannah and Nathan Nelson (1988). *Bottom left:* Parag Chitnis (1988). *Bottom middle:* Guenther Hauska (1989). *Bottom right:* Reinhold Herrmann (1987).

'Detergents' were the name of the game. All the commercial detergents at that time were highly pure in the catalog, but magically became dirty by the time they arrived at the lab. Cholic acid and deoxycholic acid, the favorite anionic detergents for mitochondrial solubilization, had to be purified by a charcoal treatment and recrystallization. However, they turned out to be less useful for solubilization of chloroplast membranes (thylakoids). Nonionic detergents became the favorites for that purpose. Digitonin and Triton X-100 competed for the title of top detergent. Though Sigma's catalog listed their Digitonin as pure, a pointed letter from Racker reduced the listing to 80% pure. Surely as a sign of posthumous respect, it is listed today as 50% pure. Triton X-100, a favorite soap detergent, was not a defined molecule and had a strong tendency to oxidize.

With all these obstacles, some impressive work was carried out in the attempt to resolve the four complexes that together mediate water oxidation, NADP reduction, and ATP formation (see Jagendorf 1967 on the acid-base transition leading to ATP formation in the dark). However the most important ingredient was missing. There was no convenient and precise way to analyze the protein (subunits) in various preparations. The renaissance came with the discovery of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis in 1969 (Weber and Osborn 1969; Laemmli 1970). Arguably, this invention was as important for membrane biochemistry as was polymerase chain reaction (PCR) for molecular biology. For the first time we were able to clearly see the polypeptide composition of membrane protein complexes. We, at that time the young generation, had a ball. At nights we used to analyze 'factors' that were 'purified' a thousand-fold by distinguished scientists to find out that they have a similar protein profile to submitochondrial particles. While we were celebrating Alan Senior came up with the real thing – a purified F1 (ATPase) with five distinct subunits of an SDS gel (Senior and Brooks 1970). The chloroplast CF1 came soon thereafter and demonstrated what a chloroplast protein complex should look like (Racker et al. 1972; Lien et al. 1972; Nelson et al. 1972). The

chlorophyll-containing complexes were more difficult to crack.

Initial steps

Spectroscopic evidence for the existence of Photosystem I (PS I) was obtained by Bessel Kok, who analyzed the absorption changes induced by the photochemical reaction of photosynthesis and discovered P700 (Kok 1957, 1961). The attempts to enrich the content of PS I following solubilization with nonionic detergents was divided into two camps: digitonin and Triton affiliates. JSC Wessels and his colleagues, Jan Anderson, Keith Boardman and colleagues, all treated thylakoids with digitonin and separated PS I, PS II, and cytochrome b_6f fractions on sucrose gradients (Wessels 1966; Anderson and Boardman 1966; Anderson et al. 1966; Wessels et al. 1973; see Anderson, this issue). Meanwhile, Leo Vernon and his colleagues achieved enrichment of PS I by Triton treatment and separation on sucrose gradients (Vernon et al. 1966, 1967; Briantais 1969). In parallel, several other groups separated chlorophyll–protein complexes in native polyacrylamide gels in the presence of various detergents (Kung and Thornber 1971; Nelson and Racker 1972; Markwell et al. 1978; Camm and Green 1982). These latter groups were able to demonstrate that most, if not all, the chlorophyll molecules are associated with proteins. These pioneering attempts were highly sophisticated, smart, and full of novel discoveries. The identity of the primary and secondary acceptors of PS I was studied by light and EPR spectroscopy to reveal a fascinating complexity (Malkin and Bearden 1971; Hiyama and Ke 1971, 1972; Bearden and Malkin 1972; Ke 1973; Ke et al. 1975; Evans et al. 1975; Sauer et al. 1978; see Bacon Ke, this issue, for a discussion of the electron acceptor P430 of PS I; Ke's photo is in Figure 1, bottom panel). This advanced not only the photosynthetic research to a new modern era but also set the standards for the study of membrane proteins in general.

There were a few obstacles that had to be solved before the defined photosynthetic protein complexes could be purified to reasonable homogeneity. Outside of the lack of SDS gel resolution, biochemical definitions of the protein complexes were missing. The salvation, and the confusion, for the chloroplast researchers came from the study of the bacterial photosynthetic reaction center. At the end of the 1960s and beginning of the 1970s, reaction centers from photosynthetic bacteria were isolated and

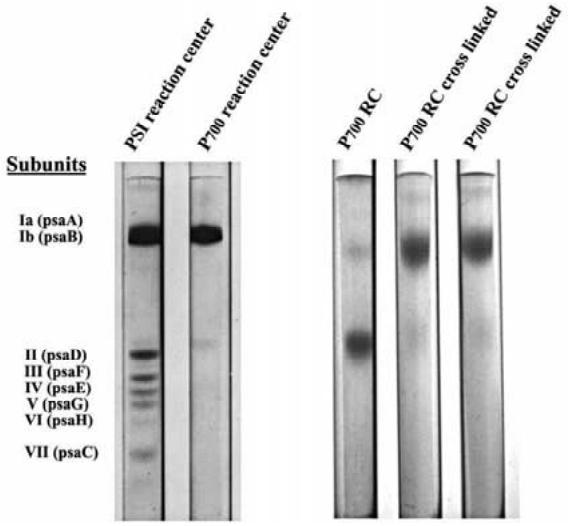

Figure 3. SDS gel electrophoresis pattern of the purified PS I reaction center and P700 reaction center. The reaction centers were purified from Swiss chard chloroplasts as described by Bengis and Nelson (1975). The preparations were dissociated (at room temperature – we observed that subunit I polymerized at high temperatures) and electrophoresed in cylinder gels (Weber and Osborn 1969). Seven subunits (I – VII) were detected in PS I reaction center. Later it was demonstrated that the band denoted as subunit VII contains several polypeptides (Chitnis and Nelson 1991). The right panel depicts a cross-linking experiment with P700 reaction center (Nelson and Notsani 1997). Cross-linking with two hexamethylene diisocyanate concentrations yielded subunit I dimer with at better then 95% efficiency. Later it was shown that subunit I is composed of two gene products psaA and psaB that form heterodimeric structure (Fish and Bogorad 1986).

purified extensively (Reed 1969; Feher 1971; Clayton and Haselkorn 1972; Clayton 1973; Okamura et al. 1974; Steiner et al. 1974; for a historical account, see Roderick Clayton, this issue). The preparations consisted of only three polypeptides with molecular weights of 27 000, 22 000, and 19 000 as determined by SDS gels (Okamura et al. 1974; Steiner et al. 1974). Moreover, a limited number of cofactors were present in those preparations that exhibited all the photochemical reactions that defined a photosynthetic reaction center (Reed 1969; Clayton 1973). The purified reaction centers contained only four bacteriochlorophyll and two bacteriopheophytin molecules as well as a nonheme iron (Feher 1971; Clayton and Haselkorn 1972). Attempts to get similar preparations of chloroplast reaction centers hampered the progress in our field.

When a multiprotein complex is defined by its biochemical activity, a polypeptide is considered an authentic subunit by virtue of its physical association

and its necessity for the function of the unit. Therefore, we defined the chloroplast PS I reaction center as the minimal structure within a photosynthetic membrane that catalyzed the photo-oxidation of plastocyanin and photo-reduction of ferredoxin (Bengis and Nelson 1975). Using a Triton X-100 treatment, DEAE (diethylaminoethyl)-cellulose chromatography, and a sucrose gradient we were able to purify, for the first time, a well-defined PS I reaction center. Apparently, it contained five distinct bands on SDS gels plus an additional broad band containing several polypeptides and pigments at the bottom of the gel (Bengis and Nelson 1975). Figure 3 shows one of the original preparations that was run on a cylindrical gel containing SDS. The purified preparation exhibited a relatively high NADP photo-reduction activity that was dependent on the addition of ascorbic acid, plastocyanin, ferredoxin, and ferredoxin-NADP-reductase. This work was done in the Technion-Israel Institute of Technology where George Feher served on the Board of Trustees. During his annual visit in 1973, George looked at the gel, saw the data, and gave it a stamp of approval, albeit with a poker face. I would guess that he was unhappy with the presence of about 100 chlorophyll (Chl) molecules per reaction center. This number haunted me for a few years until in the early 1980s it became widely known to be a conservative number.

Thus, PS I preparations isolated from chloroplasts of higher plants were composed of 8–10 different polypeptides, approximately 100 Chl *a* molecules, several β-carotenes, and a pair of vitamin K1 molecules (Hauska 1988; see a photograph of Hauska in Figure 2, bottom middle). This was in contrast to the bacterial reaction center that contains only three polypeptides, four bacteriochlorophyll, and two bacteriopheophytin molecules (Feher 1971; Clayton and Haselkorn 1972). Like everybody else, we also attempted the depletion of the PS I reaction center from its excess chlorophylls. Extensive washing of the reaction center, bound on DEAE-cellulose, with Triton X-100 only resulted in a minor change in the chlorophyll/protein ratio. Further washing inactivated the NADP photoreduction activity of the PS I preparation. In looking for highly resolved PS I preparations, we treated the purified preparation with a 0.5% SDS on ice and separated a green band containing only subunit I (psaA + psaB) on a sucrose gradient. Because this preparation appeared to be the minimal structure that catalyzes the photo-oxidation of P700, we called it the P700 reaction center (Bengis and Nelson 1975). All the other subunits of the PS I reaction center were located in lighter fractions of the sucrose gradient and appeared to be free of chlorophyll, suggesting that subunit I harbored most, if not all, the chlorophyll molecules in the PS I reaction center.

PS I reaction center comes to light

Armed with these observations, I participated in the Third International Congress on Photosynthesis Research that was held in 1974 in Rehovot, Israel (Nelson and Bengis 1975). At the end of my lecture, I was attacked by everybody for calling the PS I preparation a reaction center. Since it came from the photosynthetic community, it was gentlemanly and mild in comparison with the discussions by the Mitochondriacs in Racker's laboratory. During the Plant Physiology meeting in 1977, I was still under attack and some of my friends told me that none of the big scientists of PS I believed in my reaction center. It was 'hell' to publish my two *Journal of Biological Chemistry* (JBC) papers, but they were finally published because the editor ruled in my favor over one of the referees (Bengis and Nelson 1975, 1977).

With the improvement in the SDS gel system, the same PS I preparation showed more subunits, and within a few years 13 polypeptides were identified in the purified reaction center (Chitnis and Nelson 1991; Chitnis 2001). Mild treatment of PS I reaction center with SDS followed by sucrose gradient centrifugation resulted in the isolation of P700 reaction center composed of subunit I only (Bengis and Nelson 1975 and Figure 3). Cross-linking of this preparation revealed the presence of subunit I heterodimer composed of subunits Ia and Ib, which were later identified as psaA and psaB gene products (Figure 3). Thus, subunit I, consisting of two of the polypeptides (psaA + psaB), was shown to function in the primary photochemical reaction, and it was likely to harbor the special chlorophyll pair of P700 and the primary electron acceptors A_0 [chlorophyll *a* (Chl *a*)], A_1 (phylloquinone), and the iron–sulfur center Fx (Shiozawa et al. 1974; Bengis and Nelson 1975, 1977; Golbeck 1987; Chitnis and Nelson 1991). What is the function of the other subunits? The time was before molecular biology (BMB), and there were two main approaches available: biochemistry and immunology. The attempt to deplete the isolated PS I reaction center from excess chlorophylls produced a bonus in the form of a reaction center preparation lacking subunit III (psaF) (Bengis and Nelson 1977). This preparation was deficient in the partial reaction of plastocyanin reduction of P700

or cytochrome *c*552 (a plastocyanin analog from green algae) photo-oxidation. It was suggested that subunit III (psaF) provided the plastocyanin-binding site in the PS I reaction center (Bengis and Nelson 1977; Hippler et al. 1996). Biochemical studies also revealed the function of subunit VII (psaC) as the subunit that functions as iron–sulfur centers FA and FB (Lagoutte et al. 1984; Golbeck 1987; Fischer et al. 1998). These studies were good examples of the power of resolution and reconstitution of membrane proteins.

While we were busy looking for the minimal structure that defines PS I reaction center, John Mullet, John Burke, and Charlie Arntzen looked for the maximal PS I complex (a photograph of Arntzen and Mullet is in John Allen's contribution, this issue). In a landmark study, they treated chloroplast membranes with low Triton X-100 concentrations or glycosidic surfactants and isolated a PS I particle that contained not only the PS I reaction center subunits but also the light-harvesting chlorophyll-proteins (LHC) of PS I (Mullet et al. 1980a, b). Less than 5 years after claiming 100 chlorophylls per reaction center was considered blasphemy, over 200 became gospel. This was a fine example of psychological effects on scientific perception. Charlie's PS I particle was going to play an important role in our future studies.

In my view, the scientific community is the most wonderful community on Earth. Indeed it is very critical and competitive, but it provides the opportunity to make friends all over the world. In 1975, soon after I convinced myself that I have a genuine PS I reaction center in hand, I went to Berlin (Germany) to work with Wolfgang Junge on the orientation of pigments in the PS I reaction center (see a photograph of Junge in Figure 2, top left panel). The orientation was evaluated by polarized photochemistry and in order for us to do that we had to immobilize the reaction center. We came up with a simple solution for immobilization on DEAE-Sephadex (Junge et al. 1977). We did not come up with earth-shaking observations, but this method was used for the immobilization of F-ATPase and the detection of its ATP-dependent rotation of γ subunit (Sabbert et al. 1996). Much more important was the life-long friendship that has developed following our mutual experiments. I have had a similar experience with Guenther Hauska (see bottom middle panel of Figure 2). I went to Regensburg (Germany) for three days with a reconstitutable chloroplast F-ATPase (CF1-CFo). While having a good time, we reconstituted the F-ATPase together with the PS I reaction center, and in the presence of ascorbate and phenazine methosulfate (PMS) we measured appreciable amounts of light-dependent ATP formation (Hauska et al. 1980). Thus we reconstituted photophosphorylation by using two purified thylakoid protein complexes. To my delight, the pleasant collaboration and mutual visits repeated themselves several times.

Antibodies

Antibodies were very useful in proving the mechanism of biochemical reactions that contain multiple enzymes and factors. They were used in the study of ferredoxin-NADP-reductase, the function of plastocyanin in the chloroplast electron transfer chain, and the mitochondrial and chloroplast coupling factor (Racker 1976). When Steve Lien (Lien et al. 1972) demonstrated the subunit structure of the chloroplast coupling factor CF1, we realized that subunit-specific antibodies may be useful for the study of the function of individual subunits in protein complexes (Nelson et al. 1972, 1973). We developed a system for electro-elution of individual subunits from SDS gels and raising subunit-specific antibodies against denatured subunits. Once again, most of the photosynthesis community did not approve of our use of denatured subunits and asked questions that I could not grasp. Nevertheless, it became a habit in my laboratory to raise such subunit-specific antibodies to every complex that we purified. The PS I reaction center was no exception. It did not significantly advance our understanding of the function of individual subunits, but the availability of these antibodies paid off handsomely in our future studies of the biogenesis of the protein complexes and in the cloning of cDNAs encoding the individual subunits. Once again a technological breakthrough of electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets prevailed (Towbin et al. 1979). Today we cannot imagine membrane biochemistry without electrotransfer and decoration with antibodies raised against denatured subunits.

Molecular biology

After molecular biology (AMB), nothing looked the same anymore. In the summer of 1978, I took a sabbatical and joined the laboratory of Jeff Schatz (see a photo of Schatz in Figure 1, top left) in the Biocenter in Basel (Switzerland). This was effectively

the primordial time for membrane molecular biology. It was apparent that the gallery of subunit-specific antibodies that we collected in the last ten years became extremely valuable, and it was also apparent that having an annual budget of approximately $5000 in my laboratory would not permit serious research with molecular-biology tools. In one of Jeff's trips, he met a 'young, bright and enthusiastic' scientist, who wanted to clone all the genes encoding polypeptides involved in photosynthesis of higher plants. Jeff recommended that Reinhold Herrmann and I should collaborate and utilize the specific antibodies for the cloning of those genes (see Herrman in bottom right panel of Figure 2). In 1980, we initiated a fruitful, pleasant and exciting collaboration that left us friends for life (Herrmann et al. 1985). Reinhold made me a moderately famous plant molecular biologist without the performance of a single cloning experiment in my laboratory (Westhoff et al. 1981). Before we published our first paper, we learned lesson number one in molecular biology – *if you do it you better do it fast.* Pulse-label experiments revealed that all the major protein complexes contain subunits that are encoded by chloroplast and nuclear DNA (Chua and Gillham 1977; Gurevitz et al. 1977; Nechushtai and Nelson 1981; Nechushtai et al. 1981) (Rachael Nechushtai can be seen in the top left panel of Figure 2). Sequencing of the entire chloroplast chromosome, which was achieved for *Marchantia* in 1988 (Ohyama et al. 1988) and for rice in 1989 (Hiratsuka et al. 1989), was not even a dream in 1980. However, libraries of chloroplast DNA, and later on of nuclear cDNA, were constructed and the genes were there for the fishing (Bedbrook et al. 1979, 1980).

The genes and transcripts for the P700 Chl *a* apoprotein and subunit II of the PS I reaction center from spinach chloroplasts were identified (Westhoff et al. 1983), but their sequencing took too much time. Laurie Bogorad and his colleagues reported for the first time on the sequence of two (psaA and psaB) partially homologous adjacent light-inducible maize chloroplast genes encoding the two polypeptides that comprise subunit I of the PS I reaction center (Fish et al. 1985; Fish and Bogorad 1986). cDNA clones of nuclear origin came soon thereafter (Tittgen et al. 1986). Today all the chloroplast and nuclear genes encoding the PS I reaction center are known due to the effort of several laboratories and the global sequencing of the *Arabidopsis thaliana* genome. In 1985, lack of research money and good fortune prompted me to move to the Roche Institute of Molecular Biology (New Jersey, USA). The Institute was a scientist's paradise. I was able for the first time to afford experiments in molecular biology and much more. Looking back, I probably acted like a deprived child in an FAO-Schwartz toy store. I expanded my scope from photosynthesis to neurobiology, and had the luxury of succumbing to my attention deficit disorder. Even still, my love affair with PS I prevailed. Our previous biochemical experiments revealed that PS I reaction centers from higher plants, green algae and cyanobacteria are very similar (Nechushtai et al. 1981, 1983; Nechushtai and Nelson 1981; Schuster et al. 1985). Consequently, I decided to utilize the newly developed system of *Synechocystis* sp. PCC 6803 as the tool for the study of the molecular biology of the PS I reaction center (see Shestakov, this issue, for the historical aspects of the molecular biology of *Synechocystis* sp. PCC 6803). This organism was ideal for molecular biology studies due to its nearly perfect homologous recombination and its ability to circumvent its dependence on photosynthesis for growth (Debus et al. 1988). Since *psaA* and *psaB* were cloned from another cyanobacterium, we elected to start with the cloning of *psaD*, which encodes subunit II of PS I reaction center. Lee McIntosh was generous to provide us not only with his bacterial strain but also with its genomic DNA library. We purified the reaction center, cut subunit II from the gel, used the method that was just published by Matsudaira (1987) to obtain subunit-specific amino acid sequences, synthesized oligonucleotides and fished the gene from the genomic library (Reilly et al. 1998). A similar approach was used in my other research subjects, and I turned into a jockey in the horse race for genes. Parag Chitnis (see bottom left panel of Figure 2) joined my laboratory to clone more of these genes, and we started looking for phenotypes of *Synechocystis* mutants in which genes encoding subunits of the PS I reaction center were inactivated by insertional mutagenesis (Chitnis et al. 1989a, b, 1991). When he left my lab, Parag continued this approach with spectacular results (Chitnis et al. 1993, 1997; Chitnis 2001).

As mentioned above, a membrane protein complex is defined as the minimal structure that catalyzes a specified biochemical reaction. Analysis of the first isolated membrane complexes by SDS gels revealed that they contained a 'shocking' number of subunits. Although these preparations were what the biochemist called 'reasonably pure,' biochemical methods always faced difficulties in the positive identification of a polypeptide as an integral subunit of a membrane

protein. A subunit must be present in stoichiometric amounts and must be necessary for the complex's activity as well as the assembly or stability of the holoenzyme. Purified biochemical preparations may contain irrelevant polypeptides or lack genuine subunits that were dissociated during the preparation of the complex without detectable effect on its assayed activity. The fact that the PS I reaction center is almost identical in its subunit composition in eukaryotes and prokaryotes enabled the utilization of *Synechocystis* genetics for the determination of the subunit structure and function of the PS I reaction center. *Synechocystis* genetics also allowed the identification of special properties of individual subunits (Chitnis 2001). The *Synechocystis* genome has been sequenced, and this has ended the race for the genes at least in this cyanobacterium and opened up the postgenomic era (Johnson et al. 2000; Zybailov et al. 2000; Semenov et al. 2000; Chitnis, 2001). In parallel, Don Bryant, John Golbeck and their colleagues conducted highly advanced studies in *Synechococcus* sp. PCC 7002 and achieved enormous progress in our understanding of PS I (Zhao et al. 1990, 1992; Rhiel et al. 1992; Yu et al. 1993; Jung et al. 1996). The specific function of each individual subunit in the PS I reaction center will continue to inspire many scientists in the years to come.

Evolution of the PS I reaction center

The discovery of almost identical structures of PS I reaction centers from cyanobacteria and higher plants refocused the attention on the evolution of this system. It was obvious that the PS I reaction center is very ancient, and it may have been operative at the dawn of life on Earth. Oxygenic photosynthesis may have existed as early as 3.5 billion years ago, and, if so, the PS I reaction center was there as well (Brocks et al. 1999). Moreover, PS I may have preceded oxygenic photosynthesis using the weak hydrogen donors such as hydrazine and hydroxylamine, which presumably were abundant in the environment of the early pre-Cambrian era (Olson 1981; Catling et al. 2001). Alternatively, photosynthesis may have been invented by bacteria after their divergence from the Archea (Olson 2001; Xiong and Bauer 2002). The evolution of hydrogenase enzymes, which reduce protons to hydrogen gas, facilitated electron transport in PS I and, with a cytochrome complex and a reversible ATPase, enabled the system to function in photophosphorylation. The capacity of PS I to utilize sulfide as an electron donor through sulfide-quinone reductase may be metabolic relics of the evolutionary history of photosynthesis (Shahak et al. 1992; Arieli et al. 1994; Bronstein et al. 2000). Regardless of the presence or the absence of oxygen at the beginning of photosynthesis on Earth, PS I was primordial in the evolution of photosynthesis (Olson 2001). Sequencing of the PS I reaction center revealed some steps in the evolution of the system. From the moment that two partially homologous adjacent chloroplast genes encoding polypeptides of the P700 Chl *a*-protein complex of PS I (psaA and psaB) were discovered, it was apparent that the core of PS I was evolved by gene duplication of an ancestral gene (Fish et al. 1985; Blankenship 2001). Thus subunit I of the PS I reaction center is composed of heterodimeric structure that is supposed to have evolved from a homodimeric core. This pattern is very common in the evolution of multisubunit complexes, but very few ancient relicts have been preserved to provide direct evidence for this pattern (Nelson 1992). In 1991, Guenther Hauska and his student Michael Büttner came to my laboratory with a purified reaction center from the green photosynthetic bacterium *Chlorobium lemicula* that was known to be structurally related to PS I reaction center (Hurt and Hauska 1984; Blankenship 1985). We embarked on our molecular biology routine: preparation of polypeptides from the different subunits, amino acid sequencing, generating a genomic library, and cloning the genes encoding the various subunits. We obtained enough polypeptide sequences of the large subunit not only to clone the gene but also to conclude that only one gene encodes the dimer of subunit I (Büttner et al. 1992a, b). Thus, we demonstrated that the core of the *Chlorobium* reaction center is encoded by a single gene, and it represents an ancestral homodimeric reaction center from which PS I evolved. A similar conclusion was reached for *Heliobacillus mobilis* (Liebl et al. 1993), and this left little doubt that there exist bacterial photosynthetic reaction centers that represent the relict of the PS I reaction center from higher plants.

Cloning of the other subunits of the reaction center and the cytochrome *b–c* complex revealed several unique features of the photosynthetic electron transfer chain (Illinger et al. 1993; Hager-Braun et al. 1995; Schutz et al. 2000). Why did the primitive structure survive through this long period of evolution, and what is the selective pressure that prompted this structural conservation? Figure 4 depicts a schematic proposal that Guenther and I drafted in 1992 dur-

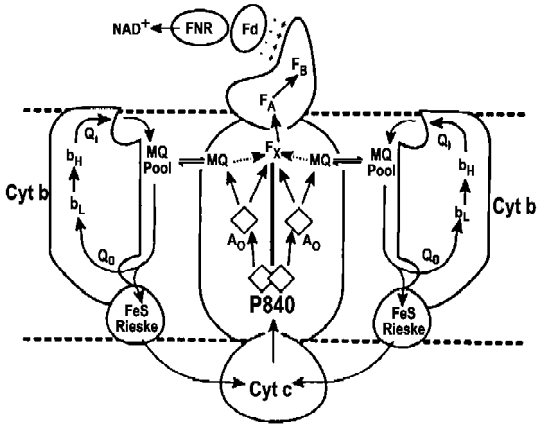

Figure 4. Schematic proposal for the function of the *chlorobium* homodimeric reaction center and cytochrome *b–c* complex in NAD photoreduction and cyclic photophosphorylation. P840 is the primary electron donor. Ao is the primary electron acceptor; MQ, menaquinone; Fx – FeS center 'X'; FA and FB – FeS centers 'A' and 'B'; Fd – ferredoxin; FNR – ferredoxin-NAD-reductase; Cyt – cytochrome.

ing a train journey from Regensburg to Frankfurt. It is proposed that the homodimeric reaction center is more suitable for the specific metabolic requirements of *Chlorobium*. The reduced environment in which *Chlorobium* grows provides abundant electron donors and low redox potential. Therefore, the *Chlorobium* metabolism requires much more ATP than reducing power in the form of NADH. The *Chlorobium* reaction center may be adapted for this by maintaining the menaquinones loosely bound in comparison with the PS I reaction center. This arrangement enables purple bacteria-like cyclic photophosphorylation, driven by PS I-like reaction center. If correct, this may suggest the PS I-like reaction center preceded the PS II-like reaction center of purple bacteria and that PS II evolved from PS I by losing the iron–sulfur clusters that mediate the reduction of NAD(P). Regardless of the arguments that improve this proposal (G. Hauska, in preparation), I still love the original one.

Future

Structure of cyanobacterial PS I reaction center

What is the future? For biologist in this field, it is spelled 'crystals.' This 'future' began relatively early in the development of the field. Biophysical studies showed incredible features of the PS I reaction center. For example, regardless of the number of pigments that the exciton visits, the quantum yield of the system remained close to one. It was apparent that revealing the special relationship between the pigments and the redox components is necessary to understand the mechanism of the reaction center. Therefore, in the beginning of the 1980s, several laboratories embarked on a quest to crystallize PS I reaction centers from various sources. Around 1987, several laboratories reported crystals of purified reaction centers that appeared dark green and beautiful but showed little or no X-ray diffraction (Ford et al. 1987, 1990; Witt et al. 1987; Reilly and Nelson 1988; Almog et al. 1991). Among all of these laboratories, only one in Berlin, that of Horst Witt, Petra Fromme, and Wolfram Seaenger, undertook the Sisyphean work of obtaining a diffracting crystal at very high resolution of 2.5 Å (Jordan et al. 2001), and the spark of comprehension started to shine through. The Berlin group realized that the key to ordered crystals was a homogenous population of PS I trimers devoid of other forms. Even a single monomer for every 10 000 trimers significantly impaired the crystal's quality (Fromme and Witt 1998). By repeatedly performing the crystallization procedure, large, dark-green and well-ordered crystals could be produced. Initially, the structure of PS I isolated from the thermophilic bacterium *Synechococcus elongatus* was reported at medium resolutions of 6Å and then 4.5–4 Å (Krauss et al. 1993, 1996; Fromme et al. 1996; Schubert et al. 1997). Further improvements in protein purification and crystallization allowed them to present, in all its glory, the high-resolution (2.5 Å) molecular architecture of the largest and most complex membrane protein thus far unraveled (Jordan et al. 2001). The structure reveals the exact location and orientation of 12 subunits, more than 2000 amino acids, 127 cofactors comprising 96 chlorophylls, 2 phylloquinones, 3 Fe_4S_4 clusters, 22 carotenoids (β-carotene), 4 lipids, a putative Ca^{2+} ion and 201 water molecules.

A Chl *a* molecule coordinated to a sulfur atom revealed that the 'special pair' is actually a heterodimer consisting of Chl *a* and an epimer (optical isomer) of Chl *a*, are only a few illustrative examples of the endless bewildering wealth of information that such a detailed structure offers its beholder.

PS I from higher plants

The PS I of green plants is a 660-kDa complex composed of two moieties: a 380 kDa reaction center core, which is essentially homologous to the entire

cyanobacterial PS I, and a 280 kDa peripheral light harvesting apparatus, LHC I (Chitnis 2001). The core lacks the cyanobacterial 3-kDa subunit M, but contains three additional subunits (H,G,N ∼10kDa each). Subunit G might be involved in LHC I binding (Jansson et al. 1996). *Arabidopsis* mutants lacking PS I-N were impaired in electron transfer from plastocyanin (Haldrup et al. 1999). PS I-H may function as a high-affinity anchoring site for phosphorylated LHC II (Lunde et al. 2000).

LHC I consists of four 20–24 kDa membrane proteins (Lhca1–4) encoded by the nuclear genome (Jansson 1994). Under most conditions, LHC I comprises equimolar amounts of these proteins, with probably two copies each. It thus harbors about 100 Chl *a/b* molecules and 25–35 carotenoids (xantophylls). However, it should be noted that this composition might vary with light intensity and properties. We note that most of the red pigments in plant PS I are coordinated by LHC I and not by the core antenna (Croce et al. 1998). LHC I proteins show sequence similarity to LHC II and may associate in hetero- or homodimers that attach to the RC independently of each other (Jansson et al. 1996). Lhca1 and Lhca4 form a heterodimer designated LHC I-730, since it coordinates 'red chlorophylls,' which are characterized with a fluorescence emission maxima at 730 nm (at 77 K). Lhca2 and Lhca3 form either homo- or heterodimers, which may also bind chlorophylls with absorption at wavelength longer than P700 (Ganeteg et al. 2001). The exact composition of LHC I remains vague.

With the aim of unraveling the protein architecture underlying unique features of plant PS I, we have recently obtained highly ordered crystals of this giant complex which contain all four light harvesting proteins (Figure 5). The crystals currently diffract to ∼4 Å with the synchrotron X-ray beam at the European Synchrotron Radiation Facility, Grenoble. A complete data set could be collected from a single crystal (A. Ben Shem, F. Frolow and N. Nelson, unpublished). A typical diffraction pattern is presented in Figure 6.

What details could be observed at medium resolution (4.5–5 Å) electron density maps? Judging from the 4.5 Å and 6 Å structures of cyanobacterial PS I reaction center (Krauss et al. 1993; Fromme et al. 1996) and the 6 Å one of LHC II (Kuhlbrandt and Wang 1991) we should be able to locate the trans membrane α-helices, the porphyrin headgroup of most of the chlorophylls, and components of the electron transfer chain. By exploiting the known cyanobacterial structure, we hope to determine the structural organization of the plant complex: LHC I composition and location, the position of core subunit that are missing in the cyanobacterial counterpart, the identity of core subunits that bind LHC I, and the interaction between different LHC I dimers and more. This structural knowledge should facilitate the addressing of questions such as whether excitation energy travels from one type of LHC dimer to the other, or is it transferred directly to the core from each type? Is it spectrally equilibrated among all or some LHC proteins before transfer to the core? Furthermore, we hope to shed light on

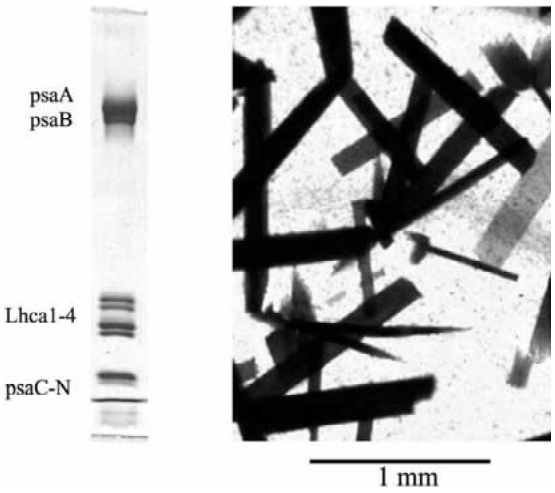

Figure 5. Left: SDS gel electrophoresis pattern of the purified PS I and crystals. The crystals of PS I were dissolved in SDS at room temperature and electrophoresed on 10% acrylamide gel. The low molecular weight subunits were not separated in this system. *Right*: Crystals of PS I. For a color version of this figure, see section in the front of the issue.

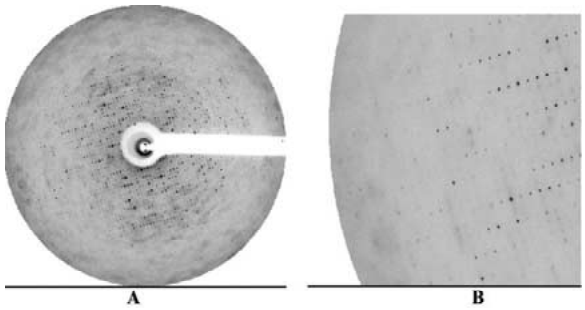

Figure 6. X-ray diffraction pattern of PS I crystals. The crystals were similar to those shown in Figure 5. (A) Typical PS I diffraction pattern as collected on MAR165 detector at ESRF ID14-1 station (30 s exposure/ 1 degree oscillation). The resolution at the edge of the detector is 4.66 Å. Best crystals diffract to ∼4 Å (A. Ben-Shem, F. Frolow and N. Nelson, unpublished). (B) Magnification of the central left section of the same diffraction picture.

the evolutionary forces that shaped the higher plant complex.

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