12
Photosynthesis and the Application of Molecular Genetics

J. BARBER AND J.B. MARDER

AFRC Photosynthesis Research Group, Department of Pure and Applied Biology, Imperial College of Science and Technology, London SW7 2BB, UK

Introduction

Photosynthesis is the process by which solar energy is captured and converted into chemical energy. This chemical energy is used to construct and maintain living organisms such that without photosynthesis there would be no life on our planet in the form that we know at present. Moreover, it was the processes of photosynthesis which gave rise to the fossil fuels which have played such an important part in Man's technological advancement during the past two hundred years. There exists today a very wide range of pigmented organisms which carry out photosynthetic processes: these are known as phototrophs, whereas heterotrophic organisms not endowed with photosynthetic pigments, and ranging from bacteria to mammals, derive their free energy directly or indirectly, by consuming the organic materials synthesized by phototrophs. In this way Man is dependent on his agricultural crops and his grass- or grain-eating livestock for his day-to-day supply of metabolic energy.

The fact that photosynthesis established and maintains the biosphere is reason enough to study the molecular and cellular processes involved, but such investigations may also have important practical implications, not only leading to increased yields in agricultural crops, but also promising the discovery of new technologies for capturing and utilizing solar energy using man-made photoactive systems.

The simplest equation of photosynthesis is:

$$2H_2X + CO_2 \xrightarrow{\text{light}} (CH_2O) + H_2O + 2X \qquad (1)$$

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriophaeophytin; CF_0–CF_1, ATP synthase/coupling factor complex; Fd, ferredoxin; Fp, flavoprotein; kbp, kilobase pairs; LHC, light-harvesting complex; MQ, menaquinone; PC, plastocyanin; PQ, plastoquinone; PQH_2, plastoquinol; PS, photosystem; Q_A, Q_B, bound quinones; RPP, reductive pentose phosphate; RUBCase, ribulose-bisphosphate carboxylase

Biotechnology and Genetic Engineering Reviews—Vol. 4, September 1986

where H_2X is a source of hydrogen ions and electrons needed to reduce carbon dioxide to carbohydrate (CH_2O) and other organic molecules, and X is the oxidation product. In the case of photosynthetic bacteria, H_2X can be any one of a number of compounds with redox potentials below +400 mV, including H_2S and a wide range of organic acids. Only in higher plants, green, red and brown algae, and the cyanobacteria (blue-green algae) can the supply of hydrogen ions and electrons come from H_2O. In this latter case the oxidation product is molecular oxygen and it was the establishment of this ability about 3500 million years ago which heralded a major step in the evolutionary development of life on our planet. This was because H_2O represented an unlimited supply of reducing material which allowed the establishment of an oxygen-rich atmosphere necessary for the evolution of oxygenic heterotrophs. Today we can consider the Earth's surface and upper atmosphere as being boundaries of a 'macroscopic reaction vessel' in which the complex biophysical and biochemical reactions of life are continuously powered by incoming radiation from the sun. The potentially useful radiation incident on vegetation is equivalent to about 15×10^{23} J/year while the energy captured by photosynthesis is estimated to be equivalent to 3×10^{21} J/year, representing a conversion of about 0·2%. This calculation ignores seasonal factors and does not make allowances for the poorly vegetated areas on the Earth's surface (deserts, polar caps etc.) and, in fact, efficiencies as high as 2–5% are possible for tropical crops such as sugar cane (*see also* Coombs, 1984).

In this chapter, we first describe the basic biophysical and biochemical processes in a section on light and dark reactions of photosynthesis. This is followed by a section on the organization of the light-harvesting and electron-transport systems. We then go on to discuss the application of molecular genetics and the ways in which this valuable tool is enhancing our understanding of photosynthesis.

Light and dark reactions of photosynthesis

For all classes of photosynthetic organisms, equation 1 can be sub-divided into two parts where the symbol e^- denotes an electron:

(i) the light reactions

$$2H_2X \xrightarrow{\text{light}} 4H^+ + 4e^- + 2X \qquad (2)$$

(ii) the dark reactions

$$CO_2 + 4H^+ + 4e^- \rightarrow (CH_2O) + H_2O \qquad (3)$$

LIGHT REACTIONS

The light reactions are those processes which involve the capturing of light energy by pigments, followed by charge separation and electron transfer

(Barber, 1978). The resulting products are the oxidant X (O_2 in the case of higher plants and algae) and a reduced hydrogen carrier, nicotinamide adenine dinucleotide (NAD^+) for photosynthetic bacteria and nicotinamide adenine dinucleotide phosphate ($NADP^+$) for higher plants and algae. In addition to the production of NADH or NADPH, the electron transfer processes also drive the conversion of adenosine diphosphate (ADP) to the more energy-rich adenosine triphosphate (ATP). The pigments and electron transfer processes giving rise to these water-soluble products are sited in, or on, lipoprotein membranes referred to as thylakoids in higher plants, algae and cyanobacteria and as chromatophore membranes in photosynthetic bacteria. In both cases the membranes form closed vesicular structures so as to give an asymmetry to their topography. A remarkable feature of the photosynthetic pigments is that, when taken together, their absorption spectra cover the entire range of energetically useful radiation reaching the Earth's surface, that is 350–1100 nm (Barber, 1983a). The carotenoids, of which there are many different types, absorb long-wavelength ultraviolet and blue light (350–500 nm). This part of the spectrum is also covered by the short-wavelength maxima of various forms of chlorophylls, including bacteriochlorophyll. The phycobiliproteins, phycocyanin and phycoerythrin (which occur in the red algae and cyanobacteria) display absorption bands in the mid-region of the visible spectrum (500–620 nm). The absorption of red light (620–700 nm) is accomplished by the long-wavelength absorption bands of chlorophyll *a* (found in all oxygen-evolving organisms) and chlorophyll *b* (found almost entirely in higher plants and green algae). Beyond 700 nm and out to 1100 nm, the radiation can be absorbed by a range of different forms of bacteriochlorophyll, the precise region being unique to a particular class of organism. In addition to these pigments there are a number of less common classes, including chlorophylls *c* and *d* which are found in certain algal species.

Most of the photosynthetic pigments act as 'antenna' systems whereby an exciton produced by an absorption of a photon in any one of a number of pigment molecules is rapidly transferred, probably by inductive resonance, to an individual photochemical reaction centre. With such an antenna system, photosynthetic organisms are able to operate efficiently at relatively low light intensities and to avoid the unnecessary manufacture and maintenance of reaction centres and electron transport chains which will, at normal light intensities, be used only a few times per minute. The precise number of pigment molecules per reaction centre varies between different types of organisms and with different growth conditions, but there are usually several hundred. The photochemically active reaction centre also contains a few molecules of chlorophyll, which always include chlorophyll *a* in oxygen-evolving organisms and bacteriochlorophyll *a* or *b* in photosynthetic bacteria. As depicted in *Figure 1*, on reaching the reaction centre, the exciton brings about the removal of electrons from the photochemically active form of chlorophyll or bacteriochlorophyll (designated by the symbol P) to a primary acceptor A_1. The oxidized reaction centre chlorophyll P^+ is then reduced by a primary electron donor D_1. In this way the energy of the exciton is converted to, and stored as, the electrochemical potential energy of the

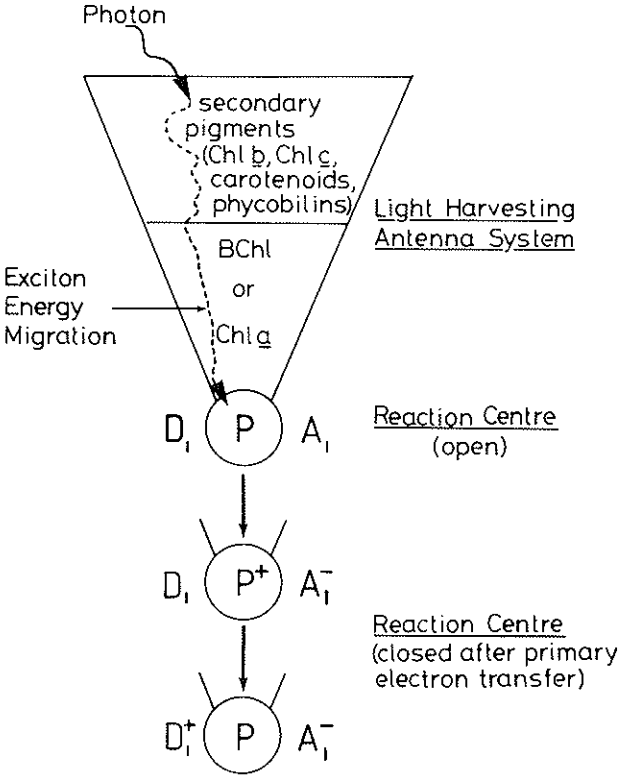

Figure 1. Diagrammatic representation of light interception and exciton transfer in the antenna systems of photosynthetic organisms leading to charge separation and stabilization within the reaction centre. P is the reaction-centre chlorophyll which is initially photo-oxidized and D_1 and A_1 are primary electron donor and acceptor, respectively.

charge transfer state $D_1^+PA_1^-$. These electron transfer steps are very fast, the reduction of A_1 taking place in a few picoseconds, while the subsequent reduction of P^+ occurs in the nanosecond–microsecond time domain. Once the $D_1^+PA_1^-$ state has been created, the possibility exists for further stabilization of the charge complex by electron transfers involving secondary donors (D_2, D_3 etc.) and acceptors (A_2, A_3 etc.). The nature of the various electron donors and acceptors varies between different classes of organisms and in some cases not all have been chemically identified.

There is a major difference between the light reactions of photosynthetic bacteria and those of the oxygen-evolving organisms. Because the former class of phototrophs use hydrogen donors with relatively low redox potentials, only one reaction centre suffices to generate a large enough redox span ($\Delta E_m \sim 1000$ mV) to drive the oxidation of the substrate and concomitant reduction of NAD^+. On the other hand, the oxygen-evolving organisms must create an oxidizing potential as high as +1000 mV to extract electrons and H^+ from water. To do this, and at the same time to reduce $NADP^+$

(ΔE_m~1600 mV), it has been necessary to link two different types of reaction centres in series. These centres are known as photosystem one (PS1) and photosystem two (PS2), each having its own light-harvesting system with slightly different absorption properties. As a consequence of the absorption of light, PS2 generates the strong oxidant necessary to split water chemically, while PS1 forms a strong reductant capable of reducing $NADP^+$. The weak reductant of PS2 and weak oxidant of PS1 interact via a series of electron/photon transfer processes (*see Figure 2*). The free energy available from this interaction is partially conserved by the synthesis of ATP from ADP. This phosphorylation process relies on a vectorial movement of electrons and H^+ across the thylakoid membrane (*see Figure 3*). As a result of this proton pumping, an electrochemical potential gradient of H^+ is established and acts as an energy reservoir for driving the phosphorylation of ADP to ATP at a membrane-bound ATP-synthase complex. A similar vectorial

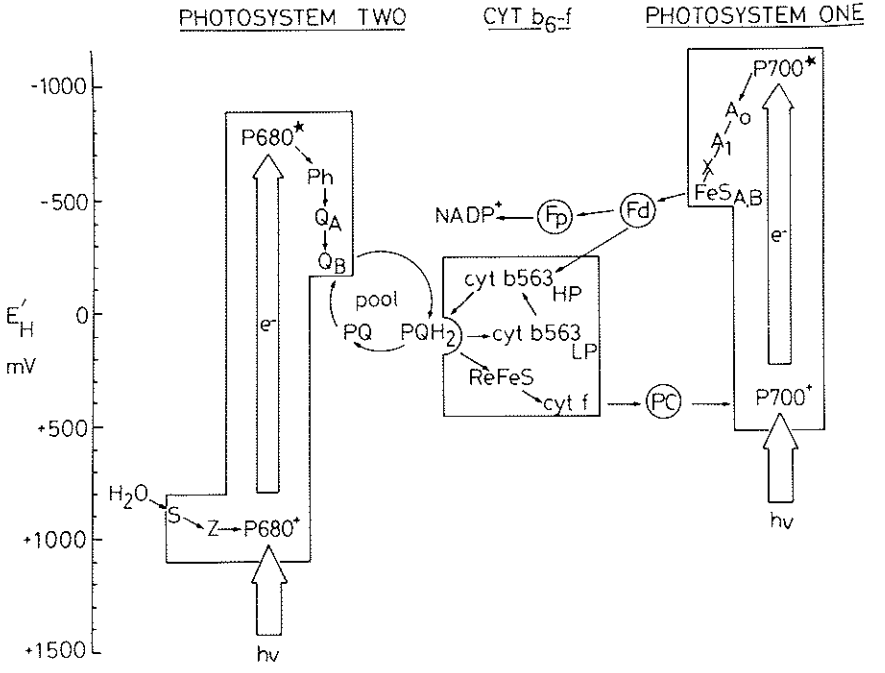

Figure 2. A scheme for electron transfer in oxygen-evolving photosynthetic organisms based on mid-point redox potentials at pH 7·0. The symbols are: S, components which can accumulate oxidizing potential for water splitting; Z, primary electron donor to P680, the reaction-centre chlorophyll of PS2; Ph, phaeophytin; Q_A, bound plastoquinone (one-electron acceptor); Q_B, plastoquinone (two-electron acceptor) able to exchange with plastoquinone (PQ) pool; ReFeS, Rieske iron–sulphur centre; cyt, cytochrome; PC, plastocyanin; P700, reaction-centre chlorophyll of PS1; A_0, A_1, X, primary electron acceptors of PS1; $FeS_{A,B}$, bound iron–sulphur centres A and B; Fd, soluble ferredoxin; F_p, flavoprotein with $NADP^+$–ferredoxin reductase activity; $NADP^+$, oxidized nicotinamide adenine dinucleotide phosphate. The boxed-in sections represent three distinct intrinsic membrane-protein complexes while other components are water soluble except the PQ pool which is soluble in membrane lipids.

transport of electrons and H^+ also exists in the chromatophore membranes of photosynthetic bacteria and other energy-converting membrane systems, such as those found in mitochondria.

DARK REACTIONS (UTILIZATION OF ENERGY DERIVED FROM LIGHT)

The products of the light reactions are NADH or NADPH and ATP, and it is these compounds which provide the reducing power and free energy necessary for converting atmospheric CO_2 to carbohydrate, as indicated in equation 3. There are several different biochemical pathways involved, but in all cases they have no direct requirement for light and therefore have been termed the dark reactions. In actual fact the division between light and dark reactions is less sharp than indicated so far, as there are 'dark' processes during electron transport, while the dark reactions of carbon fixation require 'light' activation of several enzymes. Nevertheless, the division presented above is useful in that it clearly separates the oxidation processes of the hydrogen donor from the reduction processes of carbon fixation. The precise biochemical pathway by which carbon dioxide is converted to carbohydrate varies considerably between different classes of organisms and between different species. For example, in the case of temperate plants and algae, the carbon fixation processes follow the classical C_3 Benson–Calvin cycle or reductive pentose phosphate (RPP) pathway with its requirement of 3 ATP and 2 NADPH for every molecule of carbon dioxide fixed (Leegood, Walker and Foyer, 1985). On the other hand, many tropical and desert plants have modified pathways for carbon fixation. One common pathway is that which involves the initial conversion of atmospheric carbon dioxide into the C_4 malic and aspartic acids which act as 'concentrated sources' of carbon dioxide for the RPP pathway (Edwards, Ku and Monson, 1985). Desert plants have also devised biochemical processes similar to those found in C_4 plants. In this case, carbon dioxide is taken up during the night when the opening of stomata has a less dramatic effect on water loss. This carbon is converted via phospho*enol*pyruvate carboxylase (EC 4.1.1.31) to oxaloacetate and then to malic acid. As with C_4 metabolism, the malic acid acts as a cellular carbon source for conventional carbohydrate synthesis via the RPP pathway. This type of carbon fixation is known as crassulacean acid metabolism or CAM (Winter, 1985).

In spite of the various adaptations which have evolved depending on particular ecological niches, all photosynthetic organisms, including bacteria, contain large quantities of the key enzyme ribulose-bisphosphate carboxylase (RuBPCase; EC 4.1.1.39) (Gutteridge and Keys, 1985). It is this enzyme which provides the major point of entry of carbon into the Earth's biomass and is the most abundant protein in nature. It catalyses the transfer of carbon as carbon dioxide to ribulose 5-phosphate to yield two molecules of 3-phosphoglycerate which then enters a cycle of reactions with a net production of starch or sucrose. In addition to the carboxylation, the active site of the enzyme also catalyses a competitive reaction, the oxygenation of ribulose

bisphosphate to yield one molecule of 3-phosphoglycerate and one of 2-phosphoglycolate. The former product enters the RPP pathway in the normal way but the latter leaves the chloroplast as glycolate and is converted to 3-phosphoglycerate with the loss of one molecule of carbon dioxide. This process leads to the uptake of oxygen and the release of carbon dioxide with the net consumption of ATP and NADPH, and is in direct competition with the normal carbon fixation process of photosynthesis: it has been termed photorespiration and is considered to be a wasteful process which reduces the efficiency of the RPP pathway. It is often argued, therefore, that if the oxygenase activity of RuBPCase could be decreased with a concomitant increase in carboxylase activity, then photosynthesis would be more efficient. In fact, in the case of C_4 metabolism the problem of oxygenase activity is overcome by the production of C_4 acids and by locating the RPP cycle in bundle sheath chloroplasts devoid of the oxygen-evolving system. In this way the oxygenase activity is suppressed by the high carbon dioxide:oxygen ratio at the catalytic site within the RuBPCase. For C_3 plants, which include virtually all temperate crop plants, the oxygenase problem can be overcome at present only by carbon dioxide enrichment, a procedure which is frequently used in greenhouses. However, with the emerging techniques of molecular genetics it may be possible to manipulate the RuBPCase by *in vitro* methods in such a way that its affinity for carbon dioxide is increased, thus abolishing its oxygenase activity. Attempts in this direction have already been made and are summarized in a later section of this chapter.

Organization of the light-harvesting and electron-transport systems

MEMBRANE STRUCTURE

As already mentioned, all photosynthetic organisms have their light-harvesting and electron-transport chains intimately associated with membrane systems. A distinct feature of the chloroplasts of higher plants and green algae (those organisms which contain chlorophyll *b*) is that their thylakoid membranes are differentiated into stacked and unstacked regions. Such an organization is not observed with the membranes of red algae and cyanobacteria, which do not contain chlorophyll *b*, but possess phycobiliproteins located in structures known as phycobilisomes, attached to the membrane surface (Coombs and Greenwood, 1976). In the case of non-oxygenic bacteria, the organization of the photosynthetic membrane can be quite varied, from being only the cytoplasmic boundary membrane to an internal membrane system consisting of vesicles, and in some cases may have their membranes arranged in complex folds or stacks (Kuehlbrandt, 1986).

Freeze-fracture and chemical analyses of these membranes have revealed them to be composed of macroscopic multipeptide protein complexes embedded in a surrounding matrix of polar lipids. In the case of oxygen-evolving organisms, the polar lipids are dominated by monogalactosyldiacylglycerol (MGDG) and by digalactosyldiacylglycerol (DGDG) possessing acyl chains which usually have a very high level of unsaturation (mainly C18:3 linolenic

acid) (Quinn and Williams, 1985). The photosynthetic bacteria contain relatively high levels of phospholipids and therefore contrast with the lipid composition of the thylakoid membrane. The high degree of unsaturation seems to be necessary to maintain a fluid environment in which the protein complexes and other hydrophobic components (e.g. plastoquinone), can diffuse laterally (Barber, 1985).

PROTEIN COMPLEXES AND THEIR FUNCTIONAL INTERACTIONS

As detailed above, the light reactions of photosynthesis are those processes which use the energy of absorbed quanta to drive the transport of electrons and H^+ against the thermodynamic gradient in order to produce NADH or NADPH, and ATP. As shown in *Figure 3*, in the case of higher plants and green algae these reactions involve co-operation between five membrane protein complexes: the light-harvesting chlorophyll *a/b* complex (LHC-2); the photosystem two complex (PS2); the photosystem one complex (PS1); the cytochrome b_6–f complex and the ATP-synthase or coupling-factor complex (CF_0–CF_1) (*see also* Haehnel, 1984). In the case of red algae and cyanobacteria, the number and type of complexes are the same except that the LHC complex is replaced by the bilin-containing phycobilisomes. The membrane proteins of non-oxygenic photosynthetic bacteria are significantly different in that they only have one type of reaction centre complex (with its light-harvesting system) and various forms of dehydrogenases. Depending on the class of bacteria, the reaction centre complexes are similar either to PS2 (purple bacteria) or to PS1 (green bacteria). The bacteria also have cytochrome and ATP-synthase complexes which are very similar to those found in the membranes of the oxygen-evolving photosynthetic organisms. In most of this article we will be concerned mainly with the proteins of higher-plant thylakoids but, where appropriate, comparison with the bacterial and cyanobacterial systems will be made.

The LHC-2 complex contains no photochemical reaction centre, so its only function is to capture light energy. It is a major constituent of the thylakoids, representing as much as 50% of the membrane protein. Most of the energy it captures is efficiently transferred to the PS2 complex, although under some conditions it also directs energy to PS1 (*see* Barber, 1985). The phycobiliproteins of the red algae and cyanobacteria function in the same way as LHC-2 by passing energy preferentially to PS2 (Glazer, 1981). The PS2 complex, which functions as a water–plastoquinone-9 (PQ) oxidoreductase, also contains a considerable amount of antenna chlorophyll of its own. Charge separation occurs within the PS2 reaction centre which leads to the production of a strong oxidant and a weak reductant. As the production of an oxygen molecule from water is a four-electron process it is necessary for the PS2 reaction centre to turn over four times to create an oxidation state S^{4+} before a molecule of oxygen is released (Joliot and Kok, 1975).

$$2H_2O + S^{4+} \rightarrow O_2 + 4H^+ + S \qquad (4)$$

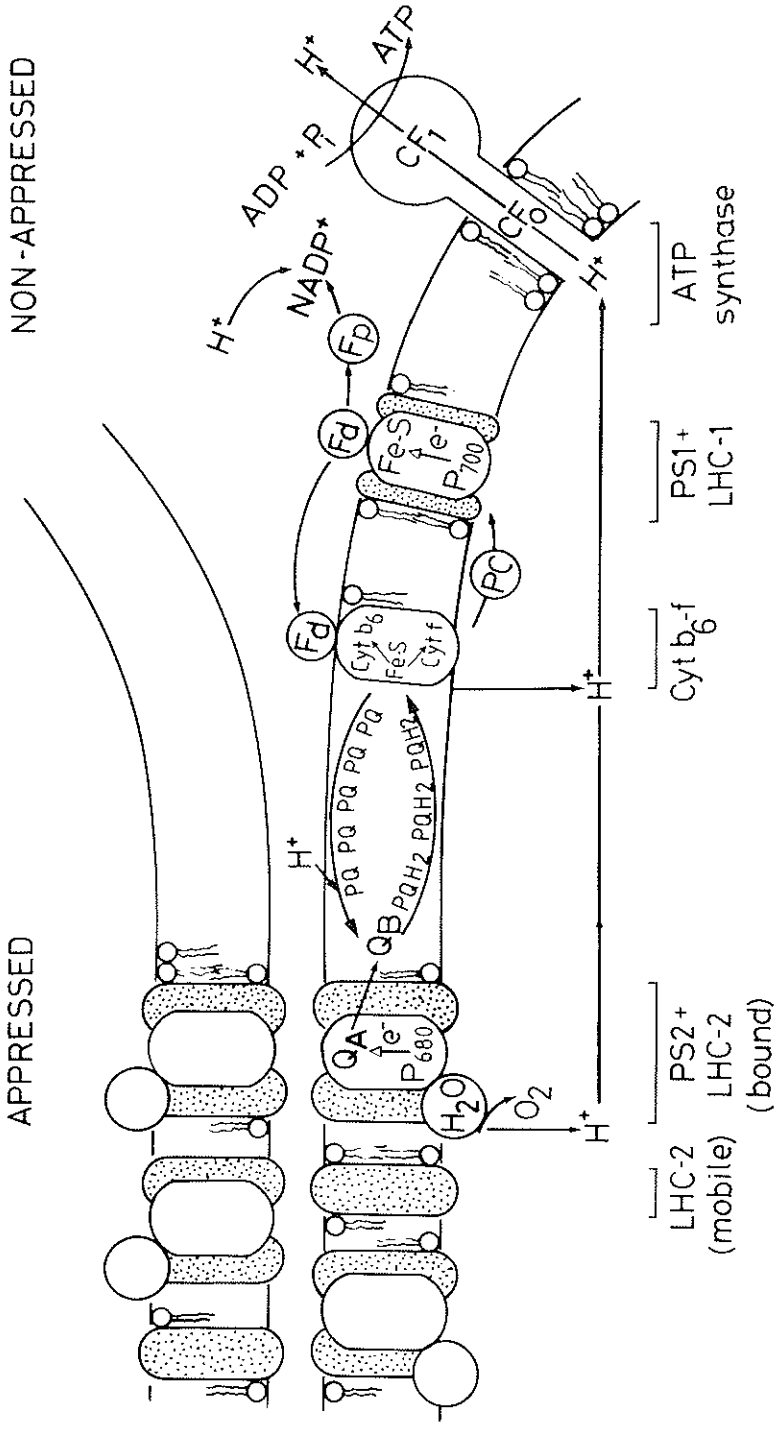

Figure 3. A diagram indicating the spatial and functional relationships between five types of intrinsic membrane complexes which co-operate to capture light energy, promote electron and proton transport and bring about the synthesis of ATP. The lateral separation of these complexes requires diffusion of mobile components (PQ/POH_2, PC and Fd) and gives rise to the vectorial pumping of protons across the membrane so that the electrochemical potential gradient created can be used to synthesize ATP. Symbols as for *Figure 2*, together with: LHC-2, light-harvesting complex containing a high level of chlorophyll-*b* and usually located close to PS2; LHC-1, light-harvesting complex with a low level of chlorophyll-*b* which forms a part of the PS1 complex; ADP or ATP, adenosine di- or tri-phosphate; CF_o and CF_1, intrinsic and extrinsic portions of ATP synthase complex; Fe–S, iron–sulphur centres; cyt b_6, two redox forms of cytochrome 563. In this and all subsequent figures showing the thylakoid membrane, the lumen is on the lower side.

The precise mechanism for the accumulation of four equivalents of oxidizing potential in the PS2 complex is not fully elucidated but probably involves multivalency states of manganese (Govindjee, Kambara and Coleman, 1985). The weak reductant is used to reduce one of a pool of PQ molecules to plastoquinol (PQH_2). These quinones act not only as carriers of electrons but also of H^+. The reduction of PQ to PQH_2 is a two-electron/2 H^+ process and occurs, as shown in *Figure 3*, at the outer surface of the membrane so that protons are taken up from the aqueous medium surrounding the thylakoids. The oxidation of PQH_2 occurs within the cyt b_6–f complex near to the inner surface so that the electron-exchange processes involved in the oxidation release protons from the quinol into the lumenal compartment enclosed by the thylakoids (*Figure 3*). The electrons pass to various redox carriers within the cyt b_6–f complex and then to the water-soluble copper-containing protein plastocyanin (PC), situated within the thylakoid lumen. The cyt b_6–f complex therefore acts as a plastoquinol–plastocyanin oxidoreductase.

Like PS2, the PS1 complex possesses antenna chlorophylls and a reaction centre. As explained earlier, the excitation of chlorophyll (P700) within the PS1 reaction centre, generates a strong reductant and a weak oxidant. The weak oxidant takes an electron from the reduced plastocyanin which is thought to be able to diffuse along the inner membrane surface from its site of reduction (*see Figure 3*). The strong reductant of PS1 reduces a soluble ferredoxin (Fd) which then reduces $NADP^+$: PS1 therefore acts as a plastocyanin–ferredoxin oxidoreductase. The reduction of $NADP^+$ by ferredoxin is catalysed by a flavoprotein (Fp) acting as a ferredoxin–$NADP^+$ reductase (EC 1.18.1.2) and involves the uptake of protons from the external aqueous phase. The overall uptake and release of protons, shown in *Figure 3*, leads to the establishment of an electrochemical potential gradient of H^+ necessary to drive ATP synthesis at the CF_0–CF_1 complex. According to *Figure 3*, for every electron transferred to $NADP^+$, two H^+ are pumped across the membrane. However, other cyclic processes may occur which do not bring about the reduction of $NADP^+$ but generate additional H^+ pumping activity and thus increase ATP synthesis. For example, under some circumstances it is possible for reduced ferredoxin to donate electrons to the cyt b_6–f complex to create cyclic electron transfer involving this complex and PS1 (*see Figure 3*). In addition, a process known as the Q-cycle may operate under some conditions whereby electron flow via the high- and low-potential forms of cytochrome b_6 can reduce plastoquinone at the outer surface so that its subsequent oxidation at the inner surface results in the net transport of H^+ across the membrane (*see below* and Mitchell, 1976; Bendall, 1982; Rich, 1984).

The diagrammatic representation of the interactions of the five complexes shown in *Figure 3* also presents another important general feature. The five complexes are not normally randomly distributed in the plane of the membrane: the LHC-2 and PS2 complexes are preferentially located in the appressed membranes of the thylakoid stacks whereas PS1 and CF_0–CF_1 are found only in the non-appressed regions (Barber, 1982; Anderson and

Andersson, 1982); the cyt b_6–f complexes are believed to be more evenly distributed between both membrane regions. It seems that this lateral separation of PS1 and PS2 allows regulation of their interactions at the level of energy transfer in response to different environmental conditions (Barber, 1985).

STRUCTURE AND COMPOSITION OF MEMBRANE COMPLEXES

Before discussing in detail how the techniques of molecular genetics are having an impact on the study and manipulation of photosynthetic processes, it is necessary to review our current knowledge of the structure and composition of the five intrinsic complexes which co-operate in the production of NADPH and ATP. In so doing it should be mentioned that for all eukaryotic photosynthetic organisms (higher plants and red, brown and green algae) the chloroplasts contain DNA and the necessary apparatus to synthesize many of their own proteins. Other proteins within the chloroplast are encoded by the DNA of the nuclear genome and enter the chloroplast after being synthesized in the cell cytoplasm. Thus the proteins which constitute the photosynthetic machinery can be divided into nucleus and chloroplast-encoded groups. In prokaryotic photosynthetic organisms (bacteria and cyanobacteria) there is no such division of genes and only one protein-synthesis system operates.

Light-harvesting chlorophyll a/b *(LHC-2) complex*

As mentioned above, this is a major constituent of the thylakoid membrane and is normally composed of at least two nucleus-encoded polypeptides having apparent molecular weights of approximately 27 kD and 25 kD (Thornber, 1975). The chlorophyll *a/b* ratio is in the region of 1·0–1·5 and the carotenoid content is mainly the xanthophylls, neoxanthin and lutein, rather than β-carotene. The structure of the LHC-2 complex *in vivo* is not certain but when isolated it forms trimers (Kuehlbrandt, 1984). The *in vivo* complex can be viewed by freeze-fracture electron microscopy which reveals it to be a particle of approximate diameter 7 nm which is located, together with PS2, mainly in the appressed membranes (Staehelin and Arntzen, 1983). Each one of these oligomeric complexes contains 40–60 chlorophyll molecules and has a molecular weight in the region of 300 kD. Under some conditions the LHC-2 polypeptides become phosphorylated, a process which leads to its lateral migration from the appressed to the non-appressed regions of the thylakoids (Barber, 1983b). As a consequence of this diffusion, the phosphorylated LHC-2 changes from being a light-harvesting system of PS2 to a light-harvesting system for PS1. Only a proportion of the total LHC-2 (that with high levels of 25 kD polypeptides) seems to be involved in this phosphorylation-induced 'mobile antenna' system, while the other LHC-2 complexes (with a high level of 27 kD polypeptide) remain tightly associated with PS2 within the appressed membrane regions (Larsson and Andersson, 1985). The phosphorylation occurs at threonine groups near the *N*-terminus

of the polypeptide which is exposed at the outer surface of the membrane (Bennett, 1982).

As yet our knowledge of the heterogeneity of the LHC-2 complex is far from satisfactory. The polypeptides which compose these chlorophyll-binding proteins are products of a light-activated multigene family in the nuclear genome. To date, amino-acid sequences for LHC-2 polypeptides have been obtained for two plants, pea (Cashmore, 1984) and *Lemna* (Tobin *et al.*, 1984), based on the analyses of isolated and cloned genes. The sequences for the two proteins (about 228 amino acids) show a high degree of homology except at the *N*-terminus. The *N*-terminus seems to be very important for the ability of LHC-2 to facilitate membrane stacking as well as being the site for phosphorylation. Although further studies of the LHC-2 genes and their expression is required to understand the heterogeneity of this protein, the work to date has allowed a folding model of one of the LHC-2 polypeptides to be drawn, based on hydropathy indices. Such a model is shown in *Figure 4b*, which suggests that the polypeptide has three helical transmembrane segments and that a significant proportion of amino acids are exposed on the outer surface compared with the inner. This suggested asymmetry between the inner and outer exposed surface is in line with the image-processing studies of Kuehlbrandt (1984) on electron micrographs of isolated LHC-2 (*see Figure 4a*). Clearly, these models are useful but unfortunately they give no hints as to how the chlorophylls are bound to the polypeptide backbone. To obtain this information it will be necessary to produce ordered crystals of isolated LHC-2 which can be subjected to high-resolution X-ray diffraction studies.

Photosystem two complex

This complex is composed of polypeptides which are either within the membrane (intrinsic) or attached to the inner surface (extrinsic) (*Figure 5*). In its entirety, it probably has a native molecular weight of at least 600 kD and a diameter of about 8 nm. Normally, however, it has four to six LHC-2 complexes closely associated with it, giving an overall diameter of 15–18 nm (Armond and Arntzen, 1977). The PS2 complex contains only chlorophyll *a* representing 10–15% of the total membrane chlorophyll; this corresponds to 50–60 chlorophylls per P680. These antenna chlorophylls are bound to polypeptides with apparent molecular weights of 43 kD and 47 kD and which also bind β-carotene. As will be discussed later, it seems unlikely that the reaction centre photo-oxidizable chlorophyll (P680) is bound to either of these polypeptides, as was generally thought until recently (Nakatani *et al.*, 1984): rather, it is probably associated with two polypeptides having molecular weights of about 32 kD designated D1 and D2 in this article. These two polypeptides may also bind other components involved in primary charge separation. The PS2 antenna chlorophylls absorb maximally at 673 nm in the red and have room temperature fluorescence which peaks at 685 nm. The other major intrinsic polypeptides in the PS2 complex have molecular weights of 10 kD and 4 kD, as determined by SDS gel electrophoresis, and

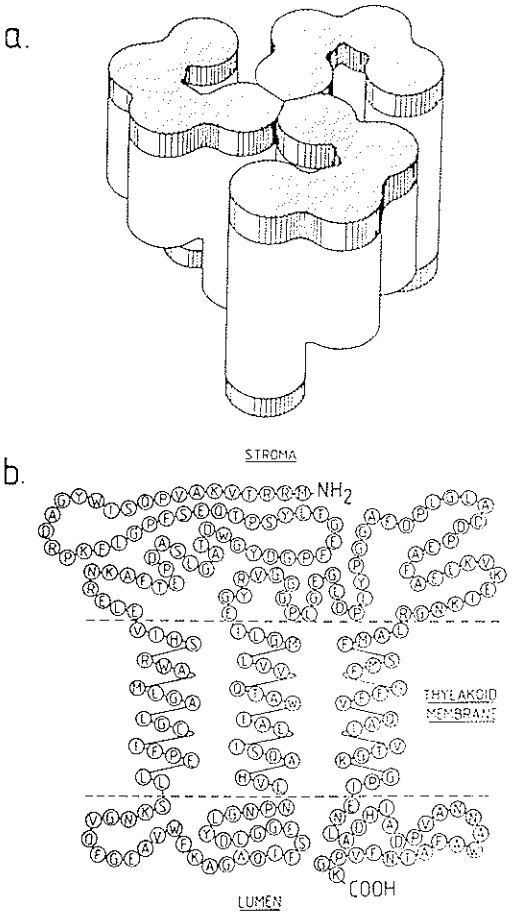

Figure 4. (a) A representation of the trimeric structure of isolated LHC-2 as elucidated from the image analysis of electron micrographs of two-dimensional crystals (Kuehlbrandt, 1984). The resolution is 16Å (1·6 nm) and the shaded areas show those parts of the trimers that probably protrude from the membrane. (b) A folding model of a single polypeptide of LHC-2 based on the amino-acid sequence obtained from the gene of *Lemna* chromosome (Tobin *et al.*, 1984).

bind the haems of cytochrome *b*-559. The precise function of cyt *b*-559 is unknown but may be involved in a regulatory cyclic electron pathway process around PS2. Associated with the PS2 core proteins are a number of extrinsic polypeptides which have an important role in the water-oxidation process (Govindjee, Kambara and Coleman, 1985). Of those studied to date, all seem to be nucleus-encoded. One of them has a molecular weight of 33 kD and is normally an absolute requirement for oxygen evolution to occur. Its binding to the membrane is related to the binding of manganese but it is not itself a mangano-protein: indeed, after certain treatments it can be removed without loss of manganese and in this case oxygen evolution can

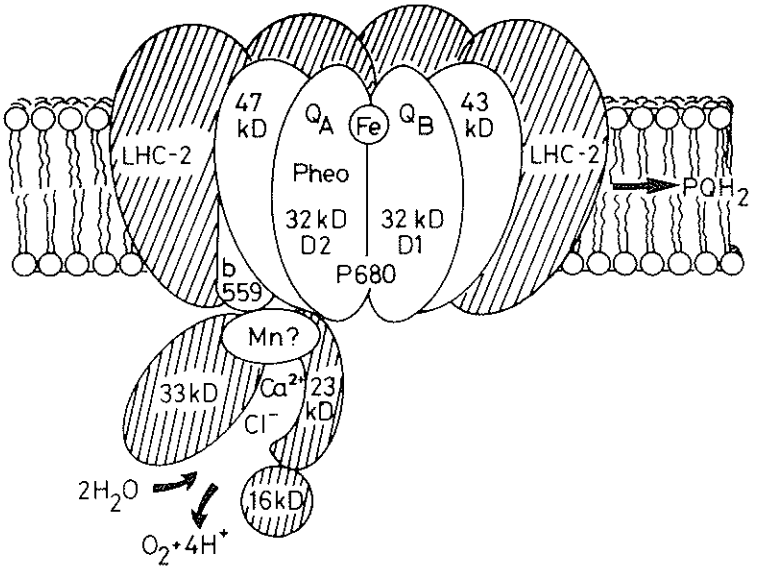

Figure 5. A schematic model of the composition and organization of the photosystem-two complex surrounded by LHC-2 complexes. Shaded components are probably nucleus encoded while unshaded are chloroplast encoded.

still occur, as long as the Cl^- level in the reaction medium is high (*see* Murata and Miyao, 1985). Recently, a PS2 core preparation has been isolated which evolves oxygen and has associated with it the 33 kD extrinsic protein and a full complement of bound manganese (Ikeuchi, Yuasa and Inoue, 1985). The other extrinsic proteins of apparent molecular weights of about 23 kD and 16 kD also seem to play a part in maintaining an ionic environment for the water-splitting process to occur, but are themselves not directly involved in redox reactions. The inhibition of oxygen evolution brought about by their removal can be overcome by elevating the levels of Ca^{2+} and Cl^- in the suspending medium (Ghanotakis and Yocum, 1985). In addition to the three extrinsic proteins mentioned, there are probably others with lower molecular weights. The existence of further hydrophobic polypeptides in the PS2 core complex is also possible, so that *Figure 5* must be viewed only as a simple and incomplete cartoon of the organization of the PS2 complex which will improve as our knowledge of the water-splitting process increases. As it is possible to re-establish oxygen evolution by adding back extrinsic proteins to washed thylakoid fragments, the *in vitro* technique of site-directed mutagenesis could be extremely useful for defining more precisely the interactions and mechanisms involved.

Photosystem one complex

Freeze-fracture electron microscopy suggests that, *in vivo*, this complex has a diameter of about 10·6 nm, indicative of a molecular weight of 800 kD or

more (Mullet, Burke and Arntzen, 1980). For higher plants grown under normal lighting conditions, 30% of the total chlorophyll is contained in the PS1 complexes with an estimated 200 chlorophyll molecules per complex (Malkin, 1986). Each complex contains a P700 reaction-centre chlorophyll which probably exists as a dimer or special pair and is thought to be associated with a core polypeptide of about 60–70 kD; this core is often referred to as CP1 and is ubiquitous among a wide range of photosynthetic organisms. It contains, in addition to P700, primary electron acceptors of unknown chemical nature, A_0 and A_1 (one being possibly monomeric chlorophyll *a* and the other, menaquinone). About 50 light-harvesting chlorophylls are bound to the core polypeptide but, in addition, closely associated with this core may be another light-harvesting chlorophyll *a* complex consisting of a polypeptide, also of 60–70 kD, containing a further 50 chlorophylls. Both of these core chlorophyll-binding proteins are encoded on the chloroplast chromosome. Thus it is possible to isolate from the thylakoid membrane a complex of molecular weight greater than 120 kD which binds 100 chlorophyll molecules per P700 and which may or may not, depending on treatment, contain the electron acceptors A_2 and iron–sulphur centres A and B ($Fe–S_A$ and $Fe–S_B$). These secondary electron acceptors are bound to proteins of 20 kD and less.

The complex with 100 chlorophyll *a* molecules per P700 is obtained by removal of an outer light-harvesting system which has associated with it a low level of chlorophyll *b* (chlorophyll *a*/*b* ratio of about 4) (Haworth, Watson and Arntzen, 1983). This peripheral light-harvesting system is often called LHC-1 to distinguish it from the major chlorophyll *b*-containing complex, LHC-2, which is more closely associated with PS2. Taken as a whole, LHC-1 adds an additional 100 chlorophylls per P700 and can be split into two forms, LHC-1a and LHC-1b, the former being composed of two polypeptides of molecular weight 22 and 23 kD and the latter of one polypeptide of 20 kD (Malkin, 1986). Athough this has not yet been confirmed, the LHC-1 polypeptides are probably nucleus encoded, as are the polypeptides containing $Fe–S_A$ and $Fe–S_B$.

A diagrammatic representation of the structure of the native PS1 complex is given in *Figure 6* but, as already stated for PS2, this diagram is tentative and incomplete. It does, however, emphasize that PS1 acts as a plastocyanin/ferredoxin oxidoreductase and suggests that a non-chlorophyll-binding 20 kD polypeptide, which occurs in the complex, possibly functions to bind plastocyanin close to the P700 reaction centre.

Cytochrome b_6–f *complex*

This complex functions as the intermediate between PS2 and PS1 by having plastoquinol-plastocyanin oxidoreductase activity. As mentioned earlier, it is also required for cyclic electron flow supported by PS1 and is probably capable of operating the Q-cycle electron/H^+ exchange process. Both in structure and function it resembles the ubiquinol/cytochrome *c* reductase (EC 1.10.2.2) complexes of mitochondria and photosynthetic bacteria. This complex has been well characterized after isolation from spinach thylakoids

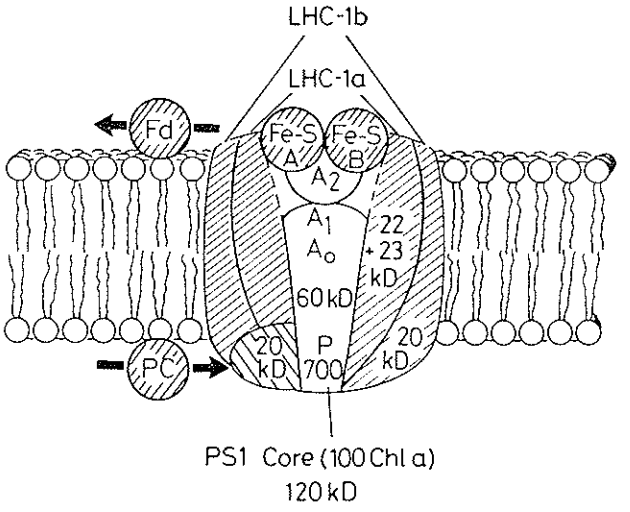

Figure 6. A schematic model of the composition and organization of the photosystem-one complex. Shaded components are probably nucleus encoded while unshaded are chloroplast encoded.

(Hauska *et al.*, 1983) and was found to consist of at least five polypeptides (34, 33, 23, 20 and 17·5 kD) which contain one cytochrome *f* (cyt *f*), two cytochrome *b*-563 (cyt b_6), an Fe–S protein (differing from that in photosystem one) which is known as a Rieske centre, two non-haem irons and some bound plastoquinone. The 20 kD component is nucleus-encoded and harbours the Rieske Fe–S centre while the two cyt *b*-563 haems (low- and high-potential forms) are bound to the 23 kD polypeptide which is chloroplast encoded. Cyt *f* is contained in a 34 kD polypeptide which again is encoded for by the chloroplast genome. The function of the 17·5 kD chloroplast-encoded polypeptide (subunit IV) is unknown but may be a part of the *in vivo* cyt b_6 polypeptide organization (Widger *et al.*, 1984) and involved in the binding of a quinone/ferredoxin reductase necessary for cyclic electron flow (Vallejos, Ceccarelli and Chan, 1984). This latter enzyme could be the same as the 37 kD nucleus-encoded flavoprotein which functions as a ferredoxin/NADP reductase (EC 1.18.1.2). As stated previously, the type of diagrammatic representation given in *Figure 7* is only tentative and the complex could contain other low-molecular-weight polypeptides which have not yet been defined.

As indicated in *Figure 7*, the full oxidation of PQH_2 gives rise to the release of $2H^+$ which is obviously a $2e^-$ process. Although the precise details of the electron/proton exchange reactions involved are not known, it is certain that the first electron is passed to the Rieske centre (Rich, 1984). The Rieske centre then reduces cyt *f* which is oxidized by plastocyanin (PC). The second electron could either be passed to the reoxidized Rieske centre or donated to cyt b_6. If the latter occurs, then the electron on the cyt b_6 is probably passed to the other cyt b_6, which has a higher redox potential, and

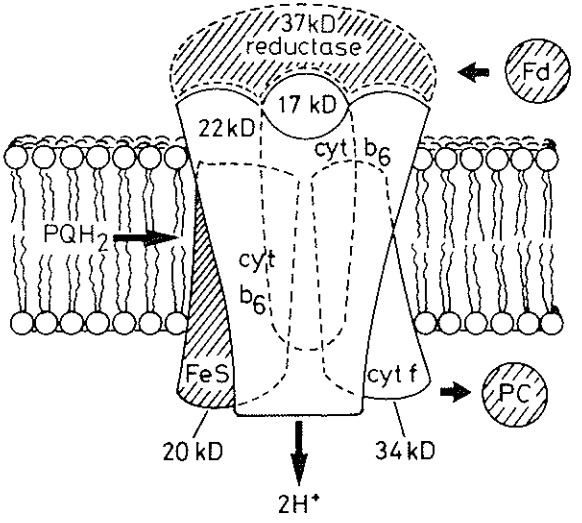

Figure 7. A schematic model of the cytochrome b_6–f complex where cyt b_6 indicates cytochrome b-563 in its high- or low-potential forms. Under some conditions the complex may bind the 37 kD ferredoxin–NADP reductase (Clark and Hind, 1983) at the 17 kD polypeptide (Vallejos, Ceccarelli and Chan, 1984). The shaded components are probably nucleus encoded; unshaded, chloroplast encoded.

is then used to reduce a bound plastoquinone to a semi-plastoquinone. It is thought that further reduction of this semi-quinone to plastoquinol can be accomplished by several routes, including electron donation by ferredoxin. If this reduction of PQ occurs near the outer surface, then $2H^+$ will be consumed from the external medium and released at the inner surface when the quinol is subsequently oxidized at the Rieske Fe–S site. As stated above, this sequence of events is known as the Q-cycle and shifts the H^+/e^- stoichiometry from 1 to 2 for quinol oxidation. Variations on the basic Q-cycle mechanism have been postulated and it is possible that it operates only under certain conditions, such as low light intensities (Bendall, 1982).

ATP-synthase (CF_0–CF_1) complex

This complex consists of the CF_0 portion, which is an intrinsic component of the membrane having hydrophobic polypeptides, and of the CF_1 extrinsic portion held to the outer thylakoid surface. CF_0 has never been isolated in an intact form but is known to contain three polypeptides of apparent molecular weights 15, 13 and 8 kD, which are called subunits I, II and III, respectively (Nelson, 1982). Subunit I and III are chloroplast genomic products whereas subunit II may be nucleus-encoded. It seems that six copies of subunit III form a hexagonal proton-conducting channel across the membrane while subunit II may function to help maintain this structural organization. It has been suggested that subunit I acts to anchor CF_1 to the CF_0 complex. CF_1 is a spherical complex which appears on the outer surface as

10 nm diameter knobs. Its native molecular weight is in excess of 400 kD and can be resolved into five subunits, usually designated α, β, γ, δ and ε with approximate molecular weights of 60, 56, 39, 19 and 14 kD, respectively (Nelson, 1982). It is believed that these subunits have the relative stoichiometries 3, 3, 1, 1 and 1, respectively. The phosphorylation of ADP to ATP occurs in CF_1 but the precise chemical processes involved are not known. It has, however, been shown that the β-subunit is the site of adenylate binding. A diagrammatic representation of the CF_0–CF_1 complex is shown in *Figure 8*, which also emphasizes that not only subunit II, but also CF_1 subunits γ and δ are probably nucleus encoded.

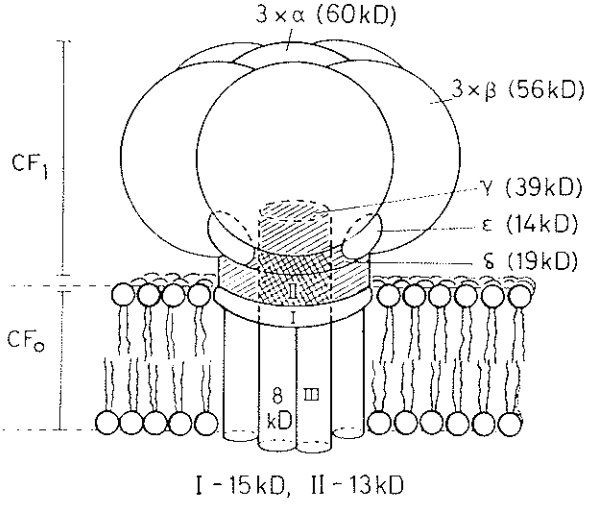

Figure 8. A schematic model of the composition and organization of the ATP-synthase (CF_0–CF_1) complex. The shaded components are probably nucleus encoded, unshaded chloroplast encoded.

ORGANIZATION AND STRUCTURE OF COMPLEXES INVOLVED IN PHOTOSYNTHESIS IN PURPLE BACTERIA

In general, the electron transport systems of the photosynthetic bacteria are simpler than those found in higher plants and algae. As stated earlier, this is because the photosynthetic bacteria contain a single type of reaction centre and relatively simple cyclic electron transport pathway leading to ATP production. In all except the green sulphur photosynthetic bacteria, the reduction of NAD^+ is achieved by reversed electron flow from added electron/H^+ donors such as organic acids and sulphur compounds. The energy required to drive this endothermic reaction is supplied by the ATP produced from light-induced cyclic electron flow. Although there are many different electron transfer pathways occurring in photosynthetic bacteria, some of which are a part of the respiratory system, the primary photosynthetic processes are usually restricted to a reaction centre complex with its light-

harvesting system and a cytochrome *b–c* complex. These complexes are found in chromatophore membranes and are similar to the functionally related complexes which occur in higher plants. They do, however, differ in detail, not only from the higher plant systems but also between different species of bacteria. Of the various classes of photosynthetic bacteria, more is known about the structural and functional properties of the purple non-sulphur bacteria, particularly *Rhodopseudomonas sphaeroides*, *Rhodopseudomonas capsulata*, *Rhodospirillum rubrum* and *Rhodopseudomonas viridis*, which are very briefly reviewed below.

Reaction centre complex

Reaction centre complexes can be isolated, free of their light-harvesting system, from non-sulphur photosynthetic bacteria using detergent treatments of chromatophore membranes (*see* review by Thornber *et al.*, 1983). As yet, no similar preparation has been obtained from higher plants, algae or cyanobacteria. The *Rps. sphaeroides* reaction centre has a molecular weight of about 100 kD and contains four molecules of bacteriochlorophyll (BChl), two of bacteriophaeophytin (BPh), a high-spin ferrous iron and bound ubiquinone. Two of the bacteriochlorophylls are associated in the form of a dimer, or 'special pair' $(BChl)_2$, which act as an initial photochemical species involved in primary charge separation and designated P870. When P870 is photo-oxidized (E_m = +450 mV) the electron is passed very rapidly to BPh (E_m = −700mV) via one of the BChl molecules. From there the electron passes to the bound quinones Q_A and Q_B (E_m = −180 mV). Q_A is reduced first in a single-electron process whereas the reduction of Q_B is a two-electron process involving the uptake of $2H^+$. This series of reactions seems to be common to all the non-sulphur bacteria although the precise optical and redox properties vary slightly between different species. Moreover, there is convincing evidence to suggest that a similar series of electron transfers occur in the PS2 reaction centre (Rutherford, 1985), as already indicated in the model given in *Figure 5*.

The *Rps. sphaeroides* reaction centre consists of three polypeptide subunits, denoted L (21 kD), M (24 kD) and H (28 kD). The L and M subunits span the membrane and contain all the pigments of the reaction centre; however, Q_A is bound to the M subunit while Q_B is associated with the L subunit. The H subunit also spans the membrane but does not seem to be directly involved in the primary charge separation process. The reduction of $P870^+$ is brought about in most cases by electron donation from a soluble cytochrome *c*. However, with *Rps. viridis* the primary donor to the photo-oxidized reaction centre ($P960^+$) is a bound cytochrome with a molecular weight of 38 kD containing four haems. Furthermore, in this bacteriochlorophyll *b*-containing organism the apparent molecular weights of the L, M and H subunits are 24, 28 and 35 kD.

A diagrammatic representation of a reaction centre of *Rps. viridis* is shown in *Figure 9*, together with the positioning of the chromophores as determined by X-ray crystallography by Deisenhofer *et al.* (1984). Very recently the

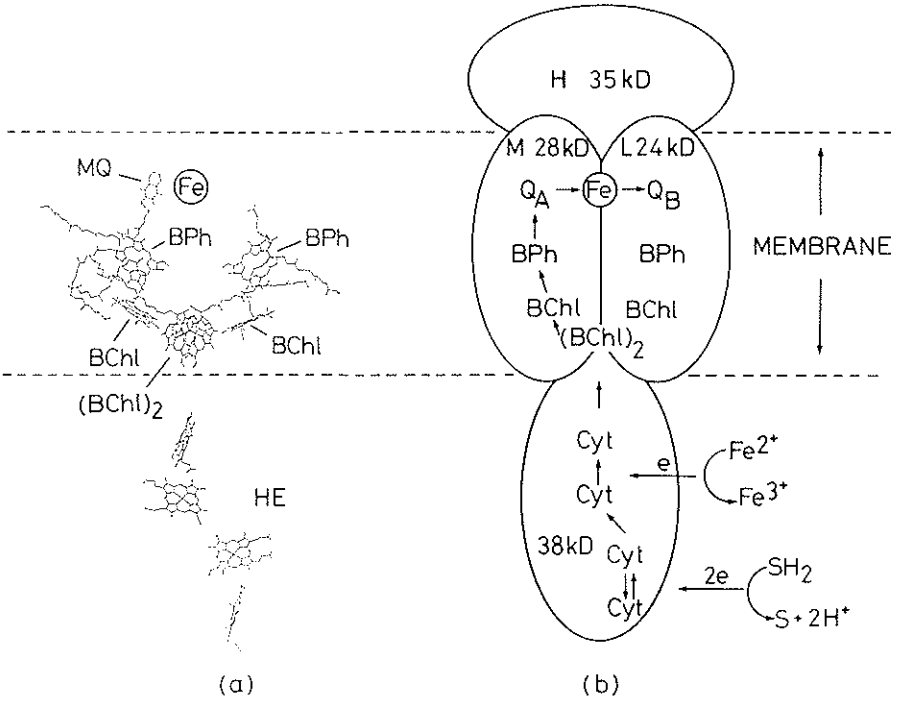

Figure 9. (a) Positioning of the chromophores within the reaction centre of *Rps. viridis* as determined by X-ray crystallographic analysis (Deisenhofer *et al.*, 1984). (b) A diagrammatic representation of the composition and organization of the reaction centre of *Rps. viridis*. The outer side is at the top, and the lumenal side at the bottom of the diagram. BChl: bacteriochlorophyll; BPh: bacteriophaeophytin; Q_A, Q_B: bound quinones; HE, haems; MQ: menaquinone.

structural analysis of the polypeptides within the reaction centre crystals has allowed an understanding of the amino-acid residues involved in chromophore and quinone binding (Deisenhofer *et al.*, 1985). This work has also shown that the L and M subunits have a symmetrical relationship with each other, both having five transmembrane helices. On the other hand the H-subunit has only one transmembrane segment, with the bulk of the polypeptide on one side of the L and M core. These very important findings confirm the folding models based on hydropathy analyses of primary amino-acid sequences obtained from cloning genes of *Rps. capsulata* (Youvan *et al.*, 1984a,b) and *Rps. sphaeroides* (Williams *et al.*, 1983, 1984). As *Figure 9* shows, the cytochrome complex is bound on the opposite side to the H-subunit in such a way that at least one of the haems is very close to the 'special pair' which constitutes P960. The twofold symmetrical character of the complex is particularly striking for the positioning of the chromophores, with BChl and BPh located in both L and M subunits. Even so, all evidence to date suggests that primary electron transfer occurs only via the BChl and BPh molecules bound to the M subunit.

Light-harvesting system

The non-sulphur photosynthetic bacteria contain a wide range of different complexes for capturing light energy, dependent on the particular class of organism. All, however, follow the same general principle already summarized for higher plants. In the case of *Rps. sphaeroides*, two main complexes exist, known as B800–850 and B870. The numbers correspond to the long wavelength maxima of the bacteriochlorophyll molecules contained in these structures. Analyses of the composition and organization of these complexes is an active area of research (Thornber *et al.*, 1983; Zuber, 1987). Like all the light-harvesting systems of purple bacteria, the B800–850 exists in the membrane as supermolecular aggregates of heterodimers of low-molecular-weight (~10 kD) polypeptides designated α and β. As shown in *Figure 10*, the basic unit of B800–850 is $\alpha_2\beta_2$ binding six bacteriochlorophylls; this model is based on sequencing data (Zuber, 1987) and spectroscopic

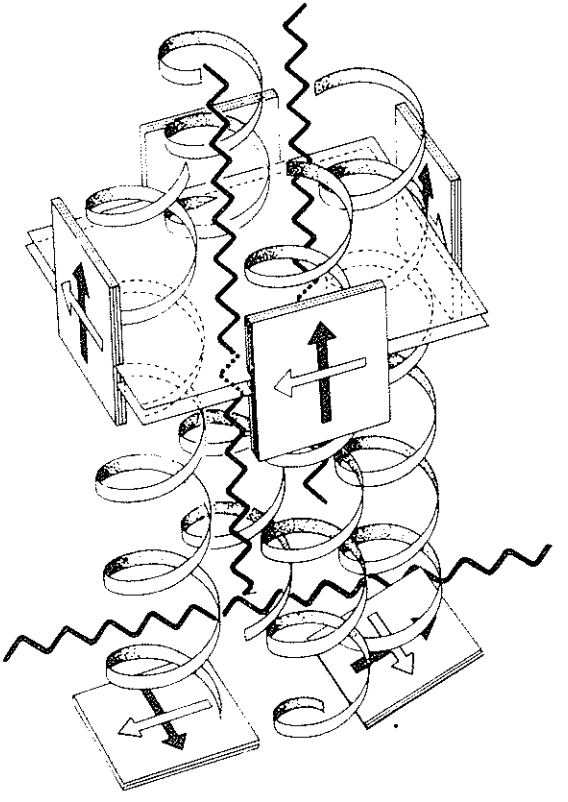

Figure 10. Model of the B800–850 complex of *Rps. sphaeroides* as proposed by Kramer *et al.* (1984). The basic unit consists of four transmembrane helices ($\alpha_2\beta_2$), four BChl 850 (upper boxes), two BChl 800 (lower boxes) and three carotenoids (zigzag lines). Open and closed arrows show Q_y and Q_x absorption transitions, respectively.

analyses (Kramer *et al.*, 1984). The longer-wave-absorbing antenna complex has been better characterized in *Rhodospirillum rubrum* (Zuber, 1987): in this case it is known as B890 and each αβ heterodimer binds two bacteriochlorophylls. Zuber and colleagues (Zuber, 1987) have obtained the sequences of many of the bacterial α and β polypeptides, including those of B1015 of *Rps. viridis*, using conventional chemical means, but sequence information has also become available from analyses of genes, particularly from the organism *Rps. capsulata* (Youvan *et al.*, 1984a,b). The expression of these genes is under the control of metabolism (light and oxygen supply) so that the purple bacteria are ideal candidates for a thorough study of the molecular genetics that controls the synthesis and assembly of light-harvesting complexes (Drews, 1985).

It should be mentioned that, side by side with the work on purple bacteria, complementary information is being obtained about the composition and organization of reaction centres and light-harvesting systems of green bacteria and cyanobacteria (*see* Zuber, 1987). This work is also making important contributions to our general understanding of the mode and regulation of light interception and storage in photosynthesis.

Cytochrome b–c *complex*

As mentioned earlier, in non-sulphur purple bacteria this complex interacts closely with the reaction centre complex in catalysing cyclic electron flow. It can be directly compared with the cyt *b–f* complex of oxygen-evolving photosynthetic organisms in that it contains a Rieske Fe–S centre (in a 25 kD polypeptide), two *b*-type high- and low-redox-potential cytochromes bound to a 40 kD polypeptide, and a 33 kD cytochrome *c*-containing polypeptide. The complex also has quinone-binding sites and a 10 kD polypeptide of unknown function. The complex not only acts as a quinol/cytochrome *c* oxidoreductase but also can support a Q-cycle.

Application of molecular genetics

Recent advances in molecular genetics are having a significant impact on attempts to understand the photosynthetic machinery. A major area has been the elucidation of the genetic control of key proteins during chloroplast development. In this review we wish to concentrate on the structures of the photosynthetic complexes once they are assembled and functioning. The prolific appearance of published DNA sequences for many of the constituent polypeptides has provided accurate information about their primary structures. Direct amino-acid sequencing of the proteins by modern chemical techniques would have taken much longer to produce this volume of information. It should be remembered, however, that the sequence of mature proteins depends not only on the gene sequence, but also on possible post-transcriptional and post-translational modifications, and this must be allowed for in making structural predictions.

Perhaps the most impressive use of sequence information has been in elucidating the structure of the *Rps. viridis* reaction-centre complex (Deisenhofer *et al.*, 1985). The deconvolution of an X-ray diffraction pattern from ordered crystals of the complex had initially made it possible to discern (as shown in *Figure 9a*) the relative locations and orientations of the chromophores (Deisenhofer *et al.*, 1984). By including in the analysis the complete amino-acid sequences of three of the four polypeptide components, it became possible to solve the structure of the entire complex almost completely at atomic resolution. It is also worth pointing out that this is the first membrane complex in any biological system to be characterized to such a high level of spatial resolution.

Clearly, similar resolution of other complexes would be a great asset in understanding the photosynthetic apparatus, and would be a firm basis for applying recombinant DNA techniques to construct new mutants, including new crop varieties. Molecular genetics thus provides not only knowledge in the form of sequence information, which can be used in defining specific molecular objectives for plant improvement, but also the technical means of realizing those objectives through gene reconstruction.

CHLOROPLAST GENOME

The chloroplast DNA probably exists in a supercoiled circular configuration (*see* Dyer, 1984, 1985). Its size varies considerably, depending on species, but is usually in the region of 150 kilobase pairs (kbp). There are numerous copies of this DNA per chloroplast (e.g. 300 for mature wheat) and these are located in discrete regions known as nucleoids. The genome is large enough to code for at least 100 different polypeptides. The chloroplast genome composition is rather conservative between species. In higher plants, the content of guanine–cytosine pairs in the chloroplast DNA is a remarkably constant 37–44%. The organization is also highly conserved, the most common arrangement being a pair of inverted repeats each containing 22–27 kbp linked by a large (80–100 kbp) and a small (13–30 kbp) single-copy region to form a closed circle (*see Figure 11*). The inverted repeats carry the ribosomal operons coding for the ribosomal RNA. There are many exceptions to the general arrangement. Among the higher plants, certain legumes, most notably pea and broad bean, lack the inverted repeat and have only the one ribosomal operon. The algae show much more variation: for example, the chloroplast genome of various strains of *Euglena gracilis* contains up to five ribosomal operons in tandem repeats. High homology is also shown in the actual sequences, as revealed by interspecific hybridization of the DNA. In higher plants, this has been used to show conservation of sequence arrangement (Fluhr and Edelman, 1981) indicating similar gene locations. In some cases, apparent gross differences are best explained as the inversion of a large portion of the genome (Palmer and Thompson, 1982).

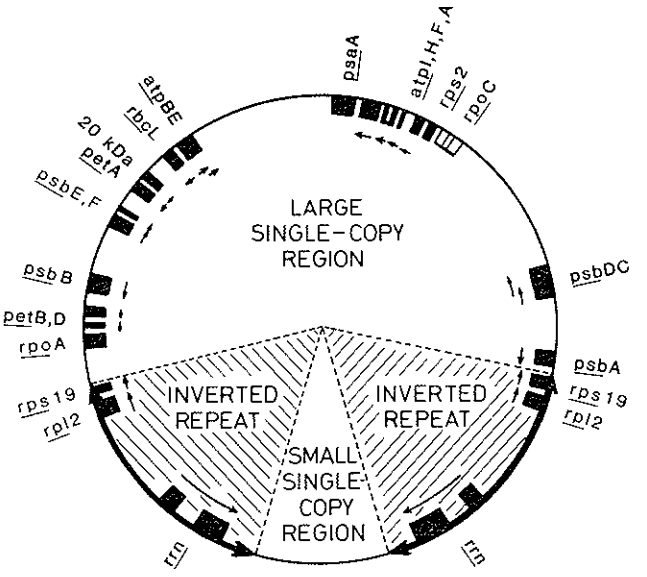

Figure 11. Physical map of the chloroplast genome of wheat, supplied by T.A. Dyer. The circular double-stranded molecule consists of 135 kbp and contains a pair of inverted repeats linked by large and small single-copy regions. The positions of the genes for several proteins are indicated (*atp*, ATP synthase subunits; *pet*, cytochrome b_6–*f* subunits; *psa*, photosystem 1 subunits; *psb*, photosystem-2 subunits; *rbc*L, RuBPCase large subunit; *rps* and *rpl*, proteins of the large and small ribosomal subunits; *rpo*, RNA polymerase). The positions of the ribosomal RNA genes are also shown (*rrn*).

The mapping of the chloroplast genome of many different species has progressed rapidly during the past three or four years. This progress has been aided by the high degree of sequence conservation, allowing DNA probes containing a particular gene to be used to locate the gene in several species; thus, the procedure described below is necessary only the first time a gene is mapped. The mapping primarily requires a means of identification of the polypeptide product from cell-free translation of the mRNA. This is complicated by the fact that this polypeptide may be a different size from the mature protein, for example because of the presence of precursor extension sequences. One possibility for identification is to raise an antibody to the isolated mature protein and use it to immunoprecipitate the related product specifically from cell-free translation reactions. The other part of the mapping is the production of DNA clones of the chloroplast genome and identification of the ones associated with the relevant polypeptide product. This association may be revealed by several experimental methods: the desired clone can sometimes be successfully introduced to a linked transcription–translation system provided that the DNA contains a competent promoter for the transcription step; otherwise, the clones are screened by hybridizing to extracts of chloroplast or total cell RNA. Hybridizations are revealed either as specific arrest of translation of the relevant mRNA ('hybrid arrest'), or

alternatively the bound RNA can be released and then translated to show the encoded polypeptide products ('hybrid selection').

Hybrid selection or arrest may be used to identify a series of overlapping clones which all contain at least a part of the mRNA sequence. In this way, the region of interest can be reduced to a subclone which contains little additional flanking DNA and is small enough to be sequenced completely. A typical map of the positions of the thylakoid membrane protein genes for wheat is shown in *Figure 11* and the gene designation follows the recommendation of Hallick and Bottomley (1983). More detailed maps are available for the chloroplast genomes of spinach, *Euglena* and *Chlamydomonas* (Dyer, 1985).

PHOTOSYSTEM TWO COMPLEX

As indicated in *Figure 5*, only some of the polypeptides which constitute the complete PS2 complex are encoded by the chloroplast genome. In the past two or three years the chloroplast genes for these PS2 polypeptides have been located and sequenced.

47 kD Chlorophyll-binding protein

In spinach, the gene for this polypeptide, referred to as *psb*B, occurs in the large single-copy region close to the genes for cyt b_6 (*pet* B) and the 17 kD (*pet* D) subunit of the cyt *b–f* complex, and all three may be translated from a polycistronic mRNA. Morris and Herrmann (1984) have shown that the open reading frame consists of 1524 nucleotides corresponding to 508 amino acids and an unprocessed molecular mass of 56 246. However, initiation may in fact occur with a methionine 60 codons downstream which is preceded by a Shine–Dalgarno-type ribosome-binding sequence (GGAG). This would give a polypeptide product with a molecular weight of 49 865. Ignoring this possibility, the amino-acid sequence has a hydrophobic character of 48%, indicative of a membrane-spanning protein. A hydropathy plot based on the procedure of Kyte and Doolittle (1982) suggests seven transmembrane regions (*see Figure 12a*). The striking feature of this plot, however, is the very large hydrophilic domain of about 200 amino-acid residues between helices VI and VII which, according to *Figure 12b*, would be on the lumenal side of the membrane. It has been suggested that this large hydrophilic segment, which contains four arginines, may play a part in binding the extrinsic water-splitting enzyme to the PS2 core complex (Morris and Herrmann, 1984). Also worth noting is the existence of histidine residues separated by 13 or 14 amino acids which seem to be strategically located in transmembrane segments III, IV, V and VII, and may have a role in the binding of chlorophyll (*see Figure 12b*).

43 kD Chlorophyll-binding protein

The gene for this polypeptide, denoted as *psb* C, has been isolated and sequenced for spinach (Holschuh, Bottomley and Whitfeld, 1984; Alt *et al.*,

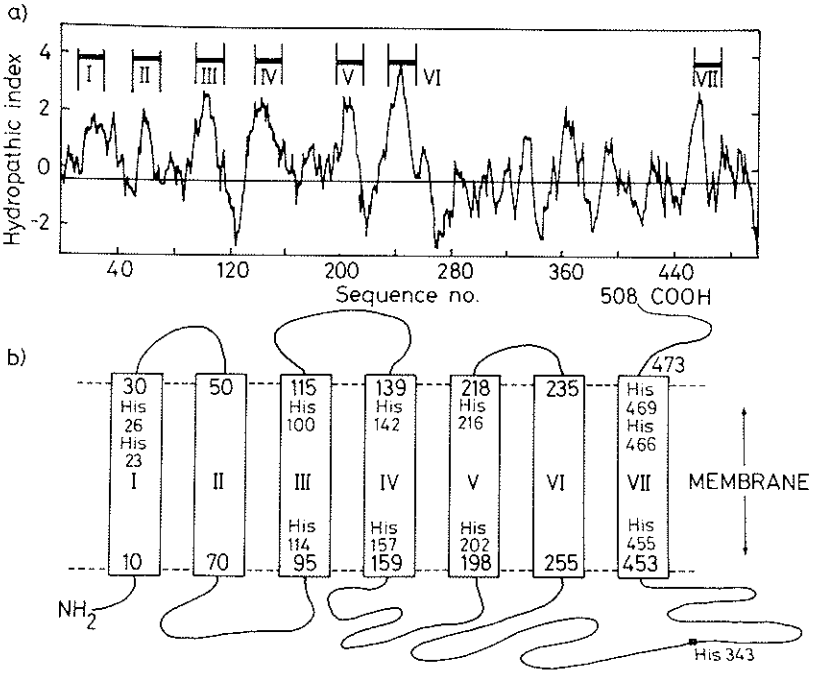

Figure 12. (a) A plot of hydropathy distribution for the *psb* B gene product (47 kD protein) as presented by Alt *et al.* (1984) using Kyte and Doolittle indices and an amino-acid window of eleven units. (b) A folding model based on the hydropathy profile and the strategic placing of histidine residues in transmembrane segments. This model has been drawn in order to have symmetry with the model for the *psb* C product shown in *Figure 13b*.

1984). An unusual feature is that its 5′ end overlaps with the 3′ end of the *psb* D gene (*see below*) by 50 kbp with the reading frames out of phase. Both genes are positioned almost centrally in the large single-copy segment of the chromosome adjacent to the two genes coding for the chlorophyll-binding proteins of PS1 (*see below*). The amino-acid sequence obtained from the gene reveals a hydrophobic protein consisting of 473 amino-acid residues with a predicted molecular weight of about 51 800. As shown in *Figure 13*, hydrophobic amino-acid clusters can be identified in the sequence from a Kyte and Doolittle analysis, suggesting that there are several transmembrane segments. We have chosen seven such segments and ignored the short hydrophobic chain between residues 195–212, which was considered by Alt *et al.* (1984) to be a transmembrane span. The resulting model shown in *Figure 13b* is remarkably similar to that given for the 47 kD protein in *Figure 12b*: thus, not only is there a comparable long hydrophilic segment towards the *C*-terminal end between segments VI and VII, but there is also a striking homology between the two proteins with regard to the positioning of histidine residues in segments I, III, V and VII. The conservation of the histidine

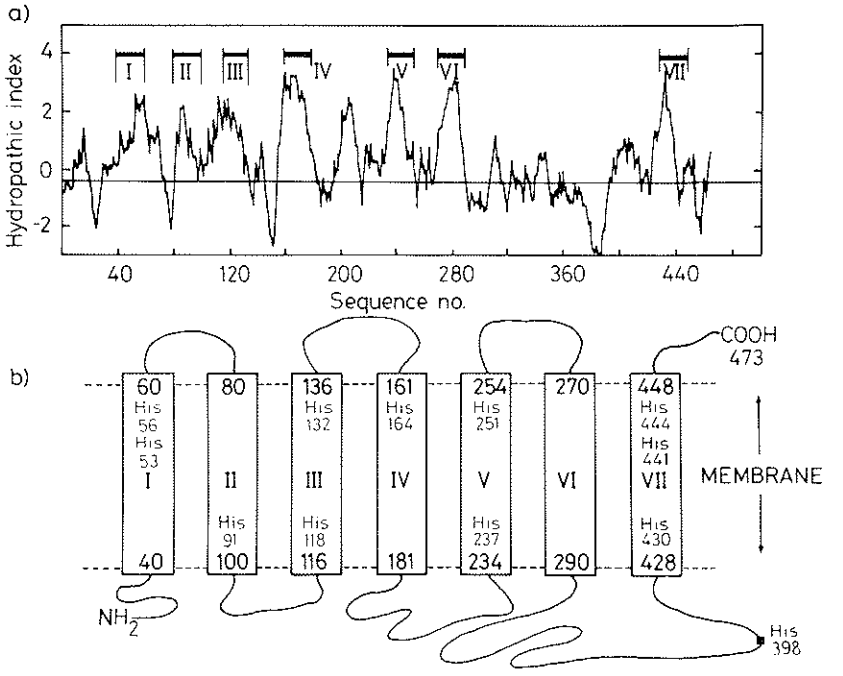

Figure 13. (a) A plot of hydropathy distribution for the *psb* C gene product (43 kD protein) presented by Alt *et al.* (1984) using Kyte and Doolittle indices and an amino-acid window of eleven units. (b) A folding model based on the hydropathy profile and the strategic placing of histidine residues in transmembrane segments. This model has been drawn in order to have symmetry with the model for the psb B gene product shown in *Figure 12b*.

positions and spacings raises the strong possibility that these residues are involved in the non-covalent binding of chlorophyll and that both proteins, in their native state, give rise to two similar light-harvesting systems serving the PS2 reaction centre as depicted in *Figure 5*.

D1 Protein

This 32 kD protein was first identified as 'peak D' among the proteins synthesized by intact chloroplasts (Eaglesham and Ellis, 1974). It is often characterized by its high turnover rate (Edelman, Mattoo and Marder, 1984) and its ability to bind herbicides such as the triazines (e.g. atrazine) and substituted ureas (e.g. diuron or DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea). These herbicides inhibit the Q_A to Q_B electron transfer, probably by blocking the plastoquinone- (Q_B)-binding process which is assumed to occur within the D1 protein (Vermaas *et al.*, 1983). The reason for the high turnover rate of the D1 protein is not fully understood but may be its susceptibility to photodamage: for example, it has been shown that the rates

of both synthesis and degradation are light-intensity dependent (Mattoo *et al.*, 1984) and suggested that, under very high illumination, destruction may exceed resynthesis, leading to an inhibition of photosynthetic activity (Kyle, Ohad and Arntzen, 1984). The mRNA for the D1 protein is induced by light (Bedbrook *et al.*, 1978), although this reflects developmental changes occurring during greening: mature chloroplasts maintain consistently high levels of this mRNA in the light and the dark (Fromm *et al.*, 1985). The protein is coded for by the *psb* A gene which, in most cases, is located in the large single-copy region of the chloroplast genome, immediately adjacent to one of the inverted repeat regions. In *Chlamydomonas*, however, the *psb* A gene lies within the inverted repeat and thus exists in two identical copies per genome. The nucleotide sequence of genes isolated from a wide variety of oxygen-evolving photosynthetic organisms, including algae and cyanobacteria, has shown a remarkable conservation of amino-acid composition and order, predicting 352–360 residues (353 in higher plants) and a molecular weight of about 39 kD (Zurawski *et al.*, 1982; Marder, 1985). There are arguments, however, that translation actually starts at 37 triplets downstream, giving a translation product of 317 residues and molecular weight 34·6 kD (Hirschberg and McIntosh, 1983; Cohen *et al.*, 1984). A precursor with an electrophoretic mobility corresponding to 33·5 kD has been observed and is processed to the mature size by removal of about 15 residues from the carboxyl terminus (Marder, Goloubinoff and Edelman, 1984). Studies with triazine-resistant mutants indicate that a single base change in the *psb* A gene can inhibit the action of herbicides to block the Q_A to Q_B electron transfer. In *Amaranthus hybridus* (Hirschberg and McIntosh, 1983) and *Solanum nigrum* (Goloubinoff, Edelman and Hallick, 1984) conversion of a serine at residue 264 to a glycine (using the numbering system of Zurawski *et al.*, 1982) results in triazine resistance. With *Chlamydomonas*, resistance occurs when the same serine is converted to alanine and similar point mutations can also give rise to herbicide resistance, e.g. valine at 219 to isoleucine and phenylalanine at 255 to tyrosine (Erickson *et al.*, 1985). A notable feature of this protein is that it is lysine-free in all higher plants so far sequenced. Despite the fact that an earlier analysis of the hydrophobicity properties of this protein suggested seven transmembrane segments (Rao, Hargrave and Argos, 1983), in *Figure 14* we have chosen to identify only five membrane-spanning regions. As will be discussed below, this selection is based on analogies with the L and M subunits of the reaction centres of purple bacteria and its homology with the D2 protein. The experimental data of Marder, Goloubinoff and Edelman (1984) suggest that the connection between helices IV and V is exposed on the stromal side of the membrane, as is the carboxy terminus. The inconsistency between the model presented in this article and the experimental data with regard to the position of the carboxy terminal could possibly be resolved by invoking a sixth helical span near the carboxy end. However, this would not affect the arrangement of the principal regions of homology to the L subunit, which lie in helix IV.

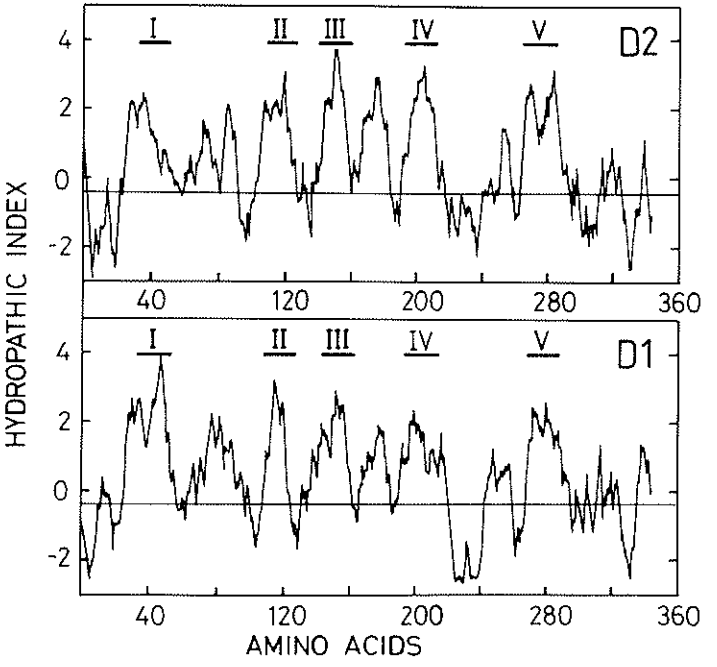

Figure 14. Hydropathy plots of D1 and D2 polypeptides presented by Alt *et al.* (1984) based on Kyte and Doolittle indices and an amino acid window of eleven units. The five hydrophobic stretches identified as transmembrane segments have been used to construct the folding model shown in *Figure 15*.

D2 Protein

This protein is encoded by a chloroplast gene denoted as *psb* D. It was first described in *Chlamydomonas* (Chua and Gillham, 1977) and has a molecular weight on SDS–PAGE of about 32 kD. Its sequence has been obtained for *Chlamydomonas* (Rochaix *et al.*, 1984), pea (Rasmussen *et al.*, 1984) and spinach (Holschuh, Bottomley and Whitfeld, 1984; Alt *et al.*, 1984), and indicates a highly conserved protein with a non-processed molecular weight of 39·5 kD. As mentioned above, the 3′ end of the D2 gene overlaps the 5′ gene of the *psb* C gene by 50 nucleotides. The *psb* D gene shows sequence homology with the *psb* A gene, but is distinguished by the presence of several codons for lysine. The hydropathy plot, using Kyte and Doolittle indices of the D2 primary structure, is shown in *Figure 14* and reveals its similarity to the D1 protein. Again, we have chosen to identify only five transmembrane regions.

A model for D1 and D2 organization

Although the overall sequence homology between the D1 and D2 proteins is only 27%, hydropathy plots (*Figure 14*) and close comparison with the L

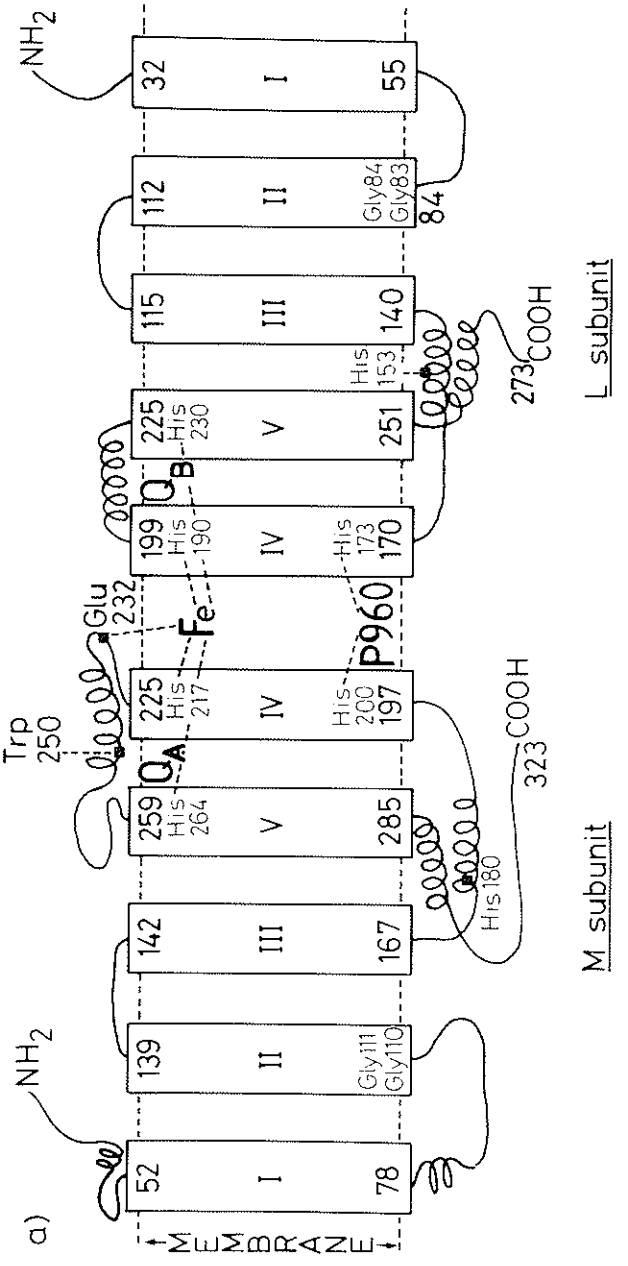

Figure 15a. A diagrammatic representation of the organization of the L and M polypeptides of the reaction centre of *Rhodopseudomonas viridis* based on details obtained by X-ray crystallography (Deisenhofer *et al.*, 1985). The Figure indicates how the helical transmembrane segments IV and V of both L and M polypeptides are involved in the binding of Fe and the special pair of bacteriochlorophyll-*b* molecules which form P960.

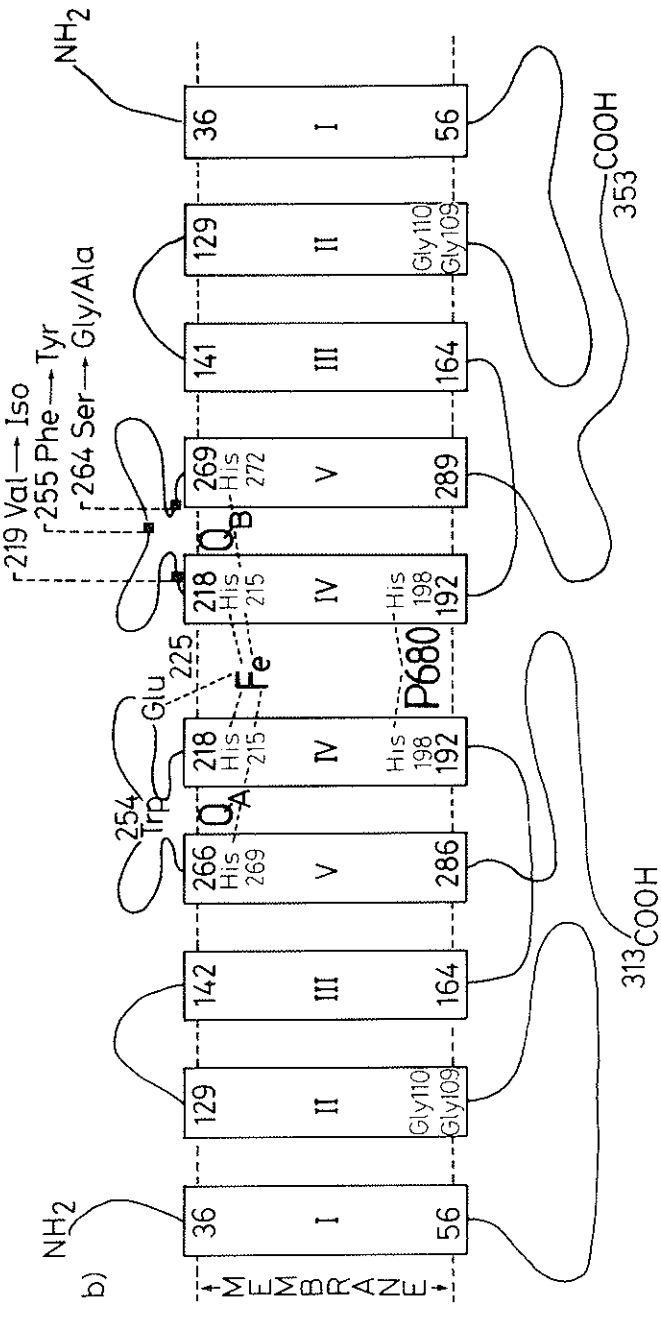

Figure 15b. A diagrammatic representation of the organization of D2 and D1 polypeptides of PS2 based on the transmembrane segments identified in *Figure 14* and on analogies with the bacterial reaction-centre structure shown in *Figure 15a*. The model has been contrived especially to emphasize homologies of segment IV and V with the bacterial system so as to suggest possible binding arrangements for Fe and P680. Other key conserved amino acids between the PS2 and bacterial polypeptides are also shown, together with residue positions 219, 255 and 264 in D1 which are mutation sites for conferring herbicide resistance (*see* text).

and M subunits of the reaction centres of purple bacteria, indicate the structural relationship shown in *Figures 15a* and *15b*. When the genes of the L and M subunits from *Rps. sphaeroides* and *Rps. capsulata* were located and sequenced (Williams *et al.*, 1983, 1984; Youvan *et al.*, 1984a,b) it was noted that they had a more than casual homology with the D1 protein, even though at that time there was no suggestion that D1 was a reaction-centre polypeptide of PS2 (Hearst and Sauer, 1984). The apparent functional similarity between them was that all three could bind quinones and, for this reason, emphasis was placed on histidines at 173 and 200 in the L and M subunits, respectively, and at 198 in the D1 protein. As *Figure 16* shows, a histidine at 198 also occurs in the D2 protein which was therefore also suggested to be a quinone-binding protein. However, because of the recent outstanding work of Deisenhofer *et al.* (1985), it is now feasible to predict the structure of the D1 and D2 proteins and to suggest that they are the reaction-centre polypeptides of PS2, as assumed in *Figure 5*. Deisenhofer *et al.* (1985) have succeeded in elucidating the structure of crystallized reaction centres isolated from the *Rps. viridis*, by X-ray diffraction analysis, to a resolution of atomic distances: it has been possible, therefore, to define the precise positions of the various chromophores and quinones in relation to amino-acid residues. The X-ray analysis indicates the L and M subunits to be highly symmetrical and to have five transmembrane helices each, with segments IV and V playing an important part in binding the special pair (P960) and the iron atom positioned between Q_A and Q_B. In particular, the crystallography has shown that histidines 173 L and 200 M are not involved in quinone binding, as had been speculated earlier (Hearst and Sauer, 1984), but act as ligands for the special pair of bacteriochlorophyll-*b* molecules which form the primary donor P960. Some of the residues involved in quinone, Fe and chromophore binding are indicated diagrammatically in *Figure 15a*. As can be seen, many of the key residues are histidines, which can also be identified in similar positions in the D1 and D2 polypeptides shown in *Figures 15b* and *16*. Thus, several features indicate that D1 corresponds to the L subunit and D2 to the M subunit. The crystal structure indicates that the binding of Q_A (i.e. menaquinone) is likely to be due to an interaction with the ring of the tryptophan at 250 M (on the M subunit) and the identical residue seems to be conserved in approximately the same position, trp 254, in the D2 protein. The comparison of D1 and L polypeptides is based on the fact that both are the site for Q_B binding. Another interesting feature is that the L and M subunits start transmembrane segment II with a glycine pair (110–111 M and 83–84 L) and the occurrence of a glycine pair in a similar position in the D1 and D2 proteins (109–110, may be significant (*see Figure 15a,b*). It is highly probable that other homologies will emerge in due course as more details are extracted from crystallographic data.

The consequences of the structural details of the *Rps. viridis* reaction centre are far-reaching but for the purpose of this article it allows a hypothetical model to be conceived for the PS2 reaction centre involving the D1 and D2 proteins. There is no direct experimental proof as yet for this model

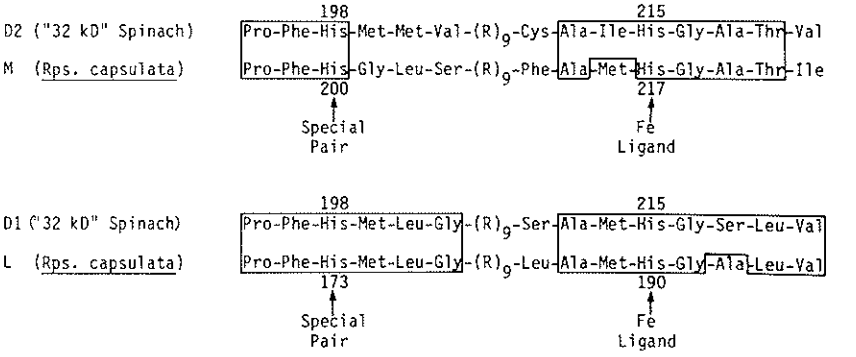

Figure 16. Sequence homologies between the PS2 and bacterial polypeptides which correspond to transmembrane helices IV in *Figures 15a* and *15b*.

but the diagrammatic representation given in *Figure 15a,b* shows how the amino-acid changes involved in herbicide resistance are clustered around the proposed site for Q_B binding. Such a conclusion is in line with the fact that herbicides, like DCMU (diuron) and atrazine, block electron flow by interacting with, or disturbing, the Q_B-binding site (Vermaas *et al.*, 1983). Recently Wolber, Eilmann and Steinback (1986), using azido-atrazine, have shown that this type of herbicide binds to methionine residue 214 of the D1 protein which is adjacent to a histidine proposed to stabilize the binding of Q_B. Moreover, it is known that, in herbicide-resistant strains, point mutations (e.g. serine 264 to glycine) reduce the efficiency of electron transfer between Q_A and Q_B (Arntzen and Duesing, 1983; Bowes, Crofts and Arntzen, 1980).

From the above discussion it can be concluded that a combination of the application of *in vitro* molecular genetics applied to a wide variety of plants and of X-ray diffraction studies on a crystallized bacterial reaction-centre complex, has given more than a hint of the mechanism of sensitivity and resistance of plants to commercially important herbicides which act on PS2. In fact there is now sufficient information to attempt to engineer plants artificially in order to obtain herbicide-resistant strains and also to design new types of herbicides.

Cytochrome b-*559*

It seems likely that this cytochrome consists of two polypeptides with molecular weights of about 9 and 4 kD and designated α and β subunits, respectively (Cramer, Theg and Widger, 1986). Their genes (*psb* E and *psb* F) are located side by side on the spinach chloroplast chromosome and their sequences indicate 83 and 39 amino acids, respectively, with the notable absence of lysine residues (Herrmann *et al.*, 1984; Westhoff *et al.*, 1985). Hydropathy considerations of the two sequences indicate that each polypeptide has only one transmembrane segment. Each segment contains one

histidine (at residues 23 α and 18 β) and as there is spectroscopic evidence to suggest that the haem co-ordination must be bis-histidine (Babcock *et al.*, 1985), then it seems likely that the cytochrome has the structure depicted in *Figure 17*. Although there is some controversy about this in the literature, there is evidence for two cytochrome *b*-559 per PS2 complex, suggesting that the organization shown in *Figure 17* is a monomer of a dimeric arrangement (Cramer, Theg and Widger, 1986).

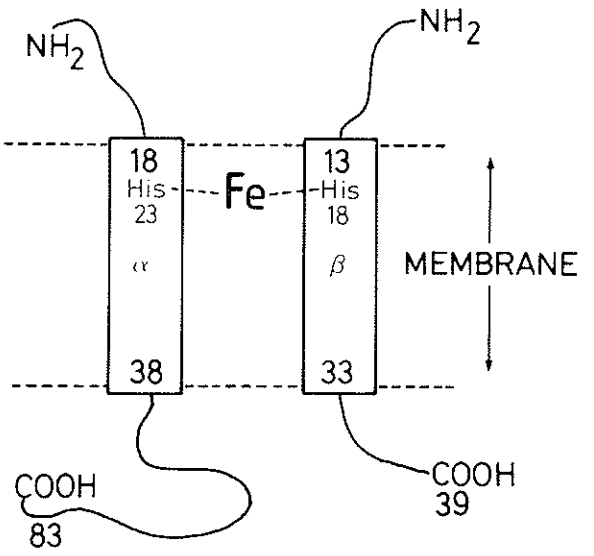

Figure 17. Diagrammatic representation of the cytochrome *b*-559 α (9 kD) and β (4 kD) polypeptide organization as a heterodimer binding one haem via histidine residues. The hydropathy indices of the two polypeptides indicate that they each have one transmembrane segment as shown (*see* Cramer, Theg and Widger, 1986).

PHOTOSYSTEM ONE COMPLEX

60 kD Chlorophyll-binding proteins

Of the several polypeptides which make up the PS1 complex, only the genes for the chlorophyll-binding core polypeptide have been located and sequenced. These genes, of which there are two (*psa* A and *psa* B), are located side by side in the maize chloroplast genome (Fish, Kuck and Bogorad, 1985) and code for polypeptides consisting of 752 (M. Wt. 83·2 kD) and 736 (M. Wt. 82·2 kD) amino acids, respectively. They are light-activated and have sequence homologies of 45% with possibly 11 membrane-spanning helices as indicated from hydropathy plots. The *psa* A and *psa* B proteins contain 42 and 38 histidines, respectively, with over 70% of them conserved between the two genes. The majority of these histidines are probably located

in membrane-buried segments and, as with the PS2 polypeptides, are probably involved in binding chlorophyll. In *Figures 5* and *15* it is speculated that the 47 kD and 43 kD chlorophyll-binding polypeptides of PS2 make up a light-harvesting system which is tightly coupled to the reaction centre consisting of the D-1 and D-2 proteins. Therefore, by analogy, it is conceivable (although not proved) that the two PS1 chlorophyll-binding proteins serve only as a light-harvesting system and that there exist other reaction-centre polypeptides which bind P700 and the primary electron acceptors. For the time being, however, the generally accepted working model is that one of the 60 kD chlorophyll-binding proteins contains P700 while the other acts as a light-harvesting antenna only (Malkin, 1986).

CYTOCHROME b_6–*f* COMPLEX

Three of the four major polypeptides which comprise this complex are chloroplast encoded and their genes have been located and sequenced. The remaining polypeptide contains the Rieske iron–sulphur centre and is almost certainly nucleus-encoded, although the gene has not yet been located.

Cytochrome f

The *pet* A gene codes for the cytochrome *f* protein and has been located and sequenced for pea (Willey, Auffret and Gray, 1984), wheat (Willey *et al.*, 1984) and spinach (Alt and Herrmann, 1984). Analysis of the genes reveals an open reading frame of 320 codons, of which 285 comprise the coding sequence for the mature polypeptide. Hydropathy plots indicate only one transmembrane segment near the *C*-terminus with a large hydrophilic portion (250 amino acids) exposed on the lumenal side of the membrane (*see Figure 18*). A histidine residue at position 25 is probably one of the ligands binding the haem, while lysine residue 145 or 222 may act as the other. It has been speculated that the ten highly conserved basic amino acids give rise to a positively charged region between residues 58 to 154, which may be important for binding plastocyanin—the oxidant of cytochrome *f* (Cramer *et al.*, 1985). Evidence that most of the protein is exposed at the inner surface and that the haem is located in this region has come from proteolytic digestion and immunological studies with isolated thylakoid membranes (Willey, Auffret and Gray, 1984; Mansfield and Bendall, 1984; Mansfield and Anderson, 1985).

Although the functionally analogous membrane-bound cytochrome *c* of mitochondria does not have a strong sequence homology with cytochrome *f*, it is interesting that it, too, has a haem-binding site near the *N*-terminus and has a hydrophobic region near the *C*-terminus.

Cytochrome b_6

In higher-plant chloroplasts the gene for a protein which binds the two haems of this cytochrome is designated the *pet* B gene. In spinach, the gene is

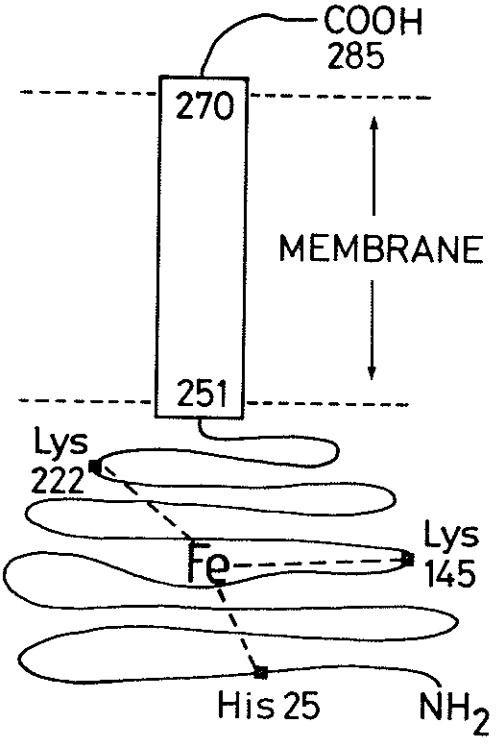

Figure 18. Diagrammatic representation of the organization of the cytochrome *f* polypeptide as deduced from hydropathy indices and proteolytic digestion studies (Willey, Auffret and Gray, 1984) indicating possible ligands for binding the haem.

immediately adjacent to the *pet* D gene (*see below*) in the large single-copy region between *rbc* L and the inverted repeat opposite the *psb* A gene (Widger *et al.*, 1984; Heinemeyer, Alt and Herrmann, 1984). The translated protein contains 211 amino-acid residues corresponding to a molecular weight of 23·4 kD, which is considerably smaller than the related cytochrome *b*-containing protein of mitochondria. Hydropathy plots indicate five transmembrane segments with four particular histidines which may be strategically located for binding the haems on opposite sides of the membrane (*see Figure 19*). The suggestion (Widger *et al.*, 1984) that, of the several histidines in the polypeptide, the four particular residues at 82 and 96 in segment II and 183 and 196 in segment V are involved in haem binding, has important implications for the related cytochrome *b*-containing proteins of the cytochrome *b–c* complexes of mitochondria and bacteria (Widger *et al.*, 1984; *see also* Barber, 1984). In the case of the mitochondrial polypeptide, the same four histidines are conserved despite the fact that the protein is larger, containing 380–385 residues and with a molecular weight of about 40 kD. Even though this larger protein probably has nine transmembrane segments,

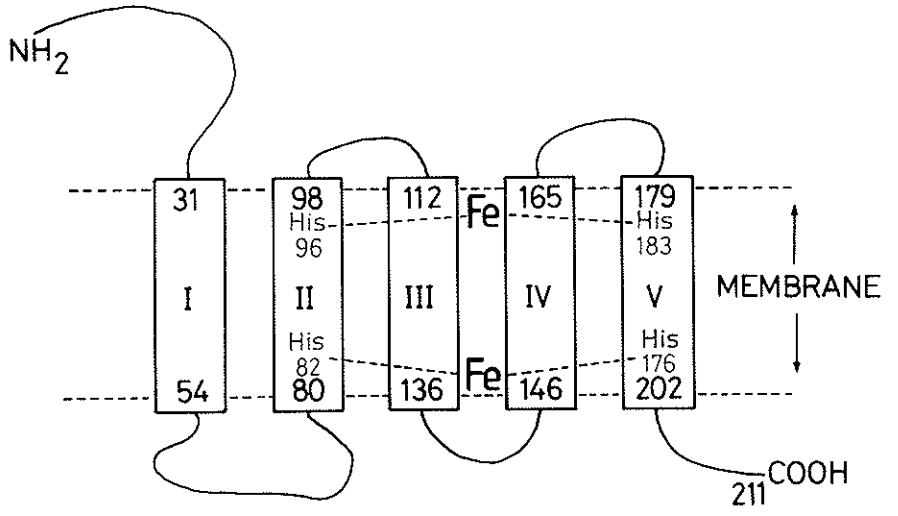

Figure 19. Diagrammatic representation of the organization of the cytochrome *b*-563 (cyt b_6) polypeptide based on hydropathy indices and on the arguments of Widger *et al.* (1984) regarding the conservation of the four histidines shown with other cyt *b*-binding proteins. These four histidines are proposed to bind two haems in the transmembrane configuration shown.

as compared with five for its chloroplast counterpart, the conserved histidines occur in the same transmembrane positions (i.e. segments II and V). Cramer *et al.* (1985) have therefore proposed that the haem binding depicted in *Figure 19* is a common arrangement for cytochrome *b* polypeptides of all types of *b*–*c* complex. The model predicts that the haems are orientated perpendicular to the plane of the membrane with the iron centres spaced at 20 Å (2 nm). The deduced positioning of the two haems on opposite sides of the membrane is in line with the functioning of the cyt b_6–*f* complex in transmembrane electron flow, including reduced-ferredoxin-mediated cyclic electron flow and Q-cycling.

Subunit IV

This is coded for by the *pet* D gene which lies close to the *pet* B gene on the chloroplast genome (*see Figure 11*). The gene has been sequenced and codes for a 139-residue translation product (Phillips and Gray, 1984; Heinemeyer, Alt and Herrmann, 1984): its processed molecular weight is about 17 kD but its function is unknown; it could be the component, suggested by Vallejos, Ceccarelli and Chan (1984), involved in the binding of the 37 kD NADP–ferredoxin oxidoreductase to the membrane. Interestingly, its amino-acid sequence and hydropathy indices closely match those of the haem-less C-terminus (residues 260–353) of the mitochondrial cytochrome *b* polypeptide (Widger *et al.*, 1984). It could be, therefore, that the single

cytochrome *b* gene of mitochondria is replaced by two genes in the chloroplast genome.

ATP SYNTHASE (CF_0–CF_1) COMPLEX

Molecular studies on this chloroplast complex (*Figure 8*), like those on the cytochrome b_6–*f* complex, show both functional and structural similarities to the ATP synthases of mitochondrial and microbial systems (Walker, Saraste and Gay, 1984). Of the eight known different components which make up the complete CF_0–CF_1 unit, five are encoded in chloroplast DNA. The α-subunit is the product of the *atp* A gene and has been located on the chloroplast genome of several different plants including tobacco (Deno, Shinozaki and Sugiura, 1983; Fluhr, Fromm and Edelman, 1983) and wheat (Howe *et al.*, 1985). In addition, using a variety of different plants, the chloroplast genes for the β-subunit (*atp* B) and ε-subunit (*atp* E) have been located and sequenced, for example barley (Zurawski and Clegg, 1984), maize (Krebbers *et al.*, 1982), wheat (Howe *et al.*, 1985), tobacco (Shinozaki *et al.*, 1983) and pea (Howe *et al.*, 1985). The stop codon of the *atp* B gene overlaps the initiation codon of the *atp* E, and the genes are cotranscribed to give a single polycistronic mRNA. The genes for the γ (*atp* C) and δ (*atp* D) subunits are probably located in the nucleus (Bouthyette and Jagendorf, 1978; Nechushtai *et al.*, 1981), as is the gene for subunit II (*atp* G) of the CF_0 complex. Subunits I and III are, however, chloroplast encoded and, again, their genes—*atp* F and *atp* H respectively (particularly the latter)—have been located and sequenced for several different plants, ranging from spinach (Alt *et al.*, 1983), wheat (Howe *et al.*, 1982; Bird *et al.*, 1985) and tobacco (Deno, Shinozaki and Sugiura, 1984; Shinozaki *et al.*, 1986) to *Euglena* (Passavant and Hallick, 1985). The *atp* A, *atp* F and *atp* H genes are arranged in a single cluster (*see Figure 11*). The cluster has a fourth open reading frame which has been designated *atp* I. This gene has recently been located and sequenced in pea and is homologous to the gene for subunit a in the proton-translocating portion of the mitochondrial and *E. coli* ATPase (Cozens *et al.*, 1986).

A comparison of the amino-acid sequences of the α- and β-subunits reveals a high degree of homology, especially at sites which may be involved in nucleotide binding (Deno and Sugiura, 1984; Howe *et al.*, 1985). There seem to be four critical regions where this homology is particularly strong, not only between the chloroplast polypeptides but also when compared with the comparable proteins of mitochondria and bacteria. In tobacco the α- and β-subunits consist of 507 and 498 amino acids, respectively, and some key residues on the β-polypeptide, glutamic acids 203, 209 and tyrosine 385, may be intimately involved in ATP synthase/ATPase activity (Deno and Sugiura, 1984).

The gene for ε-subunit (*atp* E) in maize consists of 138 amino-acid residues (Krebbers *et al.*, 1982). In this case the sequence shows a lower homology with the ε-subunit of *E. coli* ATPase complex (23%).

The sequencing of the gene for subunit III (*atp* H) indicates that the unprocessed polypeptide contains 81 amino acids corresponding to a mol-

ecular weight of about 8 kD (Howe *et al.*, 1982; Deno, Shinozaki and Sugiura, 1984). The polypeptide is very hydrophobic and has about 30% homology with the corresponding subunit of the *E. coli* ATP synthase (subunit c) (Walker, Saraste and Gay, 1984). Hydropathy plots of both sequences, however, are very similar and indicate that there are two membrane-spanning segments. A conserved glutamate residue at position 62 in subunit III and at position 66 in the subunit of *E. coli* and the related subunit in mitochondrial ATP synthase, is thought to be crucial for H^+ translocation and the site for the binding of the inhibitor dicyclohexylcarbodiimide (DCCD) (Walker, Saraste and Gay, 1984).

Nucleotide sequencing and analyses of RNA–DNA hybrids have indicated that the gene of the CF_0 subunit I (*atp* F) is very unusual for higher-plant plastid DNA as it is interrupted by an 823 bp intron (Bird *et al.*, 1985; Shinozaki *et al.*, 1986). The deduced amino-acid sequence of CF_0 subunit I indicates a polypeptide of 183 residues. This polypeptide is probably processed to 166 amino acids from its *N*-terminus (Bird *et al.*, 1985) so that its native molecular weight is about 19 kD. A hydropathy profile of the amino-acid sequence of the processed polypeptide indicates a short hydrophobic region at the *N*-terminus which spans the membrane from residue 9 to 28. The remaining 138 amino acids are hydrophilic (33% charged residues) and are likely to be exposed at the outer membrane surface and function to bind CF_1 to the CF_0 complex. Although the amino-acid sequence for CF_0 subunit I is not highly homologous with the related subunit b of *E. coli* ATP synthase, the hydropathy plots are, as for subunits II and c, very similar (Walker, Saraste and Gay, 1984; Bird *et al.*, 1985).

Ribulose bisphosphate carboxylase (RuBPCase)

As explained earlier, this enzyme has a central role in the fixation of atmospheric CO_2 by all photosynthetic organisms. In plants, algae and cyanobacteria, the enzyme is a complex containing eight copies each of a large (55 kD) and small (14 kD) subunit. However, in photosynthetic bacteria either the L_8S_8 aggregate or an active L_2 form of the enzyme may exist, depending on species and physiological state. Gutteridge and Keys (1985) have recently reviewed what is known about various forms of RuBPCase and their enzymology, including detailed discussion of the oxygenase/carboxylase activities.

RuBPCase large subunit

In higher plants, the large subunit gene (*rbc* L) is located in the large single-copy region of the chloroplast genome (*see Figure 11*) while several nuclear genes code for the small subunit. In cyanobacteria the genes for both subunits are close together on the genome (Shinozaki and Sugiura, 1983). This gene cluster has recently been introduced into the non-photosynthetic bacterium *E. coli*, resulting in the expression of an active enzyme complex (Gatenby, van der Vies and Bradley, 1985).

The *rbc* L gene has been sequenced in a large number of organisms (*see* Dyer, 1985 for references) and shows a high degree of interspecific homology. A point mutation in the *rbc* L gene of *Chlamydomonas* results in the production of a protein which lacks enzyme activity (Dron *et al.*, 1983). In higher plants the *rbc* L gene codes for about 476 amino acids and there may be some processing to a slightly smaller mature polypeptide (Langridge, 1981).

The sequence of the RuBPCase gene from *Rhodospirillum rubrum* is only 25% homologous to the higher plant *rbc* L (Nargang, McIntosh and Somerville, 1984). This gene has been cloned and expressed in *E. coli* to give an active L_2 dimer (Somerville and Somerville, 1984). Despite the low overall homology with higher plants, there are some regions of very high homology which probably correspond to active sites. Gutteridge *et al.* (1984) have applied site-specific mutagenesis to one such region of the *R. rubrum* RuBPCase gene which may be important for activation of the enzyme by the binding of divalent metal ions. This region contains a lysine residue, which may be carbamylated, and is surrounded by several acidic residues. One of these was altered from aspartate to glutamate and the modified gene expressed in *E. coli*. The mutated protein showed reduced carboxylase and oxygenase activities, confirming the functional importance of the aspartate residue concerned. Although this particular study did not result in an improved carboxylase/oxygenase ratio, the wide interspecific variation found does hold out hope of genetic improvement of this important parameter.

RUBPCASE SMALL SUBUNIT

The small subunit gene (*rbc* S) is nucleus-encoded in plants and algae. Broglie *et al.* (1984) have transferred a pea *rbc* S gene to *Petunia* using a vector based on the Ti plasmid of *Agrobacterium tumefaciens*. A hybrid L_8S_8 complex was found in the transformed *Petunia* plants. The exact role of the small subunit in RuBPCase function is not understood, especially as it is absent in active bacterial L_2 complexes. However, combined expression of the two subunits from the *Anacystis nidulans* genes in *E. coli* was necessary to produce active aggregates (*see above*). It is thus possible that site-directed mutagenesis of the *rbc* S gene could also alter the enzymic activity beneficially.

Concluding remarks

The location, isolation and sequencing of genes gives, among other things, information about the primary structure of functionally important proteins. Such information is particularly valuable for large hydrophobic proteins which are integral parts of biological membranes. Primary structures are useful for helping to pin-point likely catalytic or binding centres, for homology comparison with related polypeptides and for predicting secondary, tertiary and quaternary structures. In this chapter we have outlined how the application of molecular genetics is providing a better understanding of the

processes of photosynthesis and of the organization of the apparatus involved. We have presented a classic example of the importance of sequencing genes for elucidating the structure of a very large membrane-protein complex. Without the cloning and sequencing of the L and M subunits of the purple bacterial reaction centre, it would have been extremely difficult, or impossible, to have obtained the complete structure of the *Rps. viridis* reaction centre to a resolution of atomic distances from the X-ray diffraction pattern. We have emphasized how this outstanding crystallographic work with a bacterial system also has important implications for understanding higher-plant photosynthesis and the action of herbicides. The knowledge which is emerging from this comparative study and from identification of point mutations for herbicide resistance represents basic information necessary for a well-conceived programme of genetic engineering for new crop plants. For example, we now know precisely which nucleotides to alter in the *psb* A gene in order artificially to produce mutants resistant to triazine herbicides. *In vitro* manipulation of genes for proteins involved in photosynthesis is under way, but for technical reasons is usually carried out with prokaryotic organisms such as cyanobacteria and purple bacteria. An interesting study has begun with the cyanobacterium *Synechocystis* 6803, directed by Dr Charles Arntzen in the research laboratories at E.I. DuPont de Nemours at Wilmington, USA (Pakrasi *et al.*, 1985). In this work, the deletion of the 10 kD cytochrome *b*-559 gene (*psb* E) from the genome did not inhibit photosynthesis, indicating that this component is not an absolute requirement but is probably involved in regulation of PS2 activity. However, deletion of *psb* A, *psb* C and *psb* D genes resulted in mutants which were not photosynthetically active. This work is laying the foundations for site-directed mutagenesis studies on the PS2 complex, especially now that the importance of specific amino acids in the D2 and D1 polypeptides are being identified by comparison with the *Rps. viridis* reaction-centre structure. Using gene deletion and insertion techniques, site-directed mutagenesis studies have been successfully carried out with *Rps. capsulata* where the target amino acid was the histidine residue 173 in the L-subunit of the reaction centre (D.C. Youvan, personal communication). Modification of this residue, which was originally proposed to be involved in secondary quinone binding (Q_B site) (Hearst and Sauer, 1984) led to the inhibition of photosynthetic activity. Such a result is not surprising in the light of the new knowledge ascertained from the *Rps. viridis* crystal structure, that this histidine is involved in binding the P960 bacteriochlorophyll-*b* special pair and not Q_B.

The final objective of the genetic engineer is to induce artificially a mutation which will either help to gain a better understanding of how a protein works or will alter its activity to obtain a desirable organism with 'improved' or 'modified' characteristics. That is, the replacement must change the activity of the protein without causing total inhibition. An example which exists in photosynthesis research is the work of Gutteridge *et al.* (1984), who modified the activity of the water-soluble enzyme RuBPCase of *R. rubrum*. As mentioned above, this work was restricted to the L_2 form of the enzyme and did not achieve the goal of increasing the carboxylase/oxygenase activity

ratio. The expression and assembly of the active cyanobacterial L_8S_8 enzyme in *E. coli* (Gatenby, van der Vies and Bradley, 1985) does, however, represent a major step forward in establishing an experimental system which uses the techniques of molecular genetics to explore the enzymology of a RuBPCase similar to that found in higher plants. Such studies would be greatly enhanced if the structure of the enzyme was known to high spatial resolution and, indeed, crystal forms of L_8S_8 and of L_2 are being actively analysed by X-ray diffraction (Brändén *et al.*, 1986; Chapman *et al.*, 1986).

Genetic engineering of the photosynthetic apparatus of higher plants, unlike that of prokaryotic organisms, is technically very difficult. Although procedures exist to modify DNA within the nuclear chromosomes using Ti-plasmid vectors (Schell *et al.*, 1985) no well-established transformation system has been developed for the chloroplast genome. Therefore, at the moment it is not possible, for example, purposely to engineer higher plants to have resistance to triazine herbicides, despite the fact that the relevant information is available for the site-specific modifications. Procedures for manipulating chloroplast DNA may soon become available, however: for example, recently a claim was made that a direct transformation of the chloroplast genome was achieved and expressed as maternally inherited antibiotic resistance (de Block, Schell and van Montagu, 1985). Another interesting approach was reported by Van den Broeck *et al.* (1985), who were able to introduce a foreign protein into the chloroplast by fusion to the leader sequence of the RuBPCase small subunit. In this system the fused gene was introduced using an *Agrobacterium* Ti-plasmid and the hybrid polypeptide was synthesized on cytoplasmic ribosomes and imported into the chloroplast with concomitant processing to remove the *rbc* S-derived leader sequence.

Even when the barrier of manipulating chloroplast DNA is overcome, there are other serious problems to be solved, such as regenerating plants from the transformed protoplast—a technique which is, so far, limited to certain species such as tobacco. Monocotyledons, compared with dicotyledons, are even more problematical in that not only are they difficult to regenerate from protoplasts but also the modification of their nuclear DNA, as well as their chloroplast DNA, is difficult.

Without any doubt, the application of molecular genetics to photosynthesis research is having an impact on our understanding of the basic processes. This knowledge is growing side by side with rapid developments in techniques of *in vitro* manipulation of higher-plant chromosomes. Thus, it seems realistic to look forward to when plant molecular biology will be an established part of biotechnology, and when the agricultural and horticultural industries will be creating their wealth from the growth of crops which have been genetically engineered to produce new varieties with higher photosynthetic efficiencies and with better adaptation to their environment. Moreover, genetic engineering of plants will have an impact on the herbicide and pesticide industries, as well as offering an opportunity to grow new types of crops designed for the production of novel compounds.

Acknowledgements

We wish to thank the Agricultural and Food Research Council for financial support and Drs Tristan Dyer and David Chapman for their advice during the preparation of this chapter.

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