Chapter 1

An Overview of Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications

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Summary

The chlorophylls are a structurally and functionally distinct group of macrocyclic tetrapyrrole pigments that may occur at the porphyrin, chlorin or bacteriochlorin oxidation levels. Biosynthetically, they are derived from protoporphyrin IX. Structurally, they are characterized by the presence of a fifth ring, isocyclic ring E, which confers the prefix 'phyto' to the porphyrin and chlorin type molecules. Chlorophylls generally have Mg as the central metal and a long-chain esterifying alcohol at C-17[3]. Chemically, they are unstable to both acids and bases, to oxidation and light, and have a pronounced tendency for aggregation and/or interaction with their

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molecular environments. Physically, they are characterized by long-lived excited states and by intense absorptions covering, in mono-disperse solutions, the spectral range from 330 to 800 nm, extending in aggregates or in vivo to 1020 nm.

Due to their high absorption and long-lived excited states, chlorophylls are powerful photosensitizers. The efficient, yet safe transduction of this excited state into chemical energy is the basis of photosynthesis. It requires not only a careful balance between productive energy transduction and excess energy degradation processes, but also tight control of the metabolism of chlorophylls. Interference with these controls is the basis of several herbicides. Chlorophylls can also be applied as natural biocides to non-photosynthetic organisms: the subsequent irradiation with light allows for a spatial and temporal control of phototoxicity, for example in photodynamic therapy of cancer.

Chlorophylls have evolved to fulfill several functions in photosynthesis: incorporated into light-harvesting complexes, they strongly absorb light and transfer the excitation energy with quantum efficiencies near 100% to the photosynthetic reaction center complexes where specialized chlorophylls are active in primary charge separation and energy transduction processes. In both complexes, chlorophylls contribute to the stabilization and regulation of the photosynthetic apparatus; also, there is evidence that they may be directly involved in the degradation of excess energy to heat. Chlorophyll precursors also serve as signals in the regulation of photosynthesis and of plastid-nucleus interactions. The functional relevance of the structural features is discussed.

Content and composition of chlorophylls are important physiological and taxonomic parameters of photosynthetic organisms. Critical physiological parameters can be derived from their absorption and, in particular, fluorescence. Both can be remotely monitored from the cellular level to the global scale from outer space.

I. Introduction

Chlorophylls (Chls) are probably the most abundant and certainly the most obvious biological pigments. Widespread in eubacteria and ubiquitous in plants, they are clearly visible from outer space (Chapter 36, Morel). This relatively small group of cyclic tetrapyrroles performs three major functions in photosynthesis which, by using solar energy, drives the fixation of CO_2 into carbohydrates and provides the energetic basis for the global ecosystem. Firstly, in the light-harvesting complexes or antennas, Chls absorb light efficiently and, secondly, they transfer the excitation energy with high quantum efficiency to the reaction centers where, thirdly, the Chls perform the primary charge separation across the photosynthetic membrane: this separation eventually leads to the generation of reductants and also ATP via a simultaneously generated membrane potential. Only few Chls are found unassociated with the photosynthetic light reactions. A Chl *a* molecule with unknown function is present in the electrogenic cytochrome b_6f complex located between the two photosystems in oxygenic organisms (Pierre et al., 1997; Peterman et al., 1998; Kurisu et al., 2003; Stroebel et al., 2003), and several plant species contain non-photosynthetic, water-soluble Chl proteins which may serve for storage or transport of the pigments (Noguchi et al., 1999; Horigome et al., 2003; K. Schmidt et al., 2003), but are also involved in Chl degradation (K. Watanabe et al., 1999). Likewise, Chl-related structures are relatively rare in nature, which is probably due to the high photodynamic potential of these pigments. They include bonellin, the sex-determining pigment of the marine worm *Bonella viridis* (Dhere, 1932; Pelter et al., 1978; Montforts et al., 1990), a visual pigment of dietary origin in certain deep-sea fish (Campbell and Herring, 1987; Douglas et al., 2000), and bioluminescent pigments in *Euphausid* shrimps (Shimomura, 1980; Topalov and Kishi, 2001). Several chlorophyll metabolites have been identified from animal sources (Ma and Dolphin, 1998) including a

Abbreviations: Acc. – Acariochloris; BChl(s) – bacteriochlorophyll(s); BPhe – bacteriopheophytin; B_X, B_y – higher energy absorption bands of tetrapyrroles in the Vis/UV spectral range, also termed Soret band(s); Chl(s) – chlorophyll(s); Chlide(s) – chlorophyllide(s); FMO – Fenna-Mathews-Olson LHC; GG – geranyl-geraniol; GGPP – geranyl-geranyl pyrophosphate; IC – internal conversion; ISC – intersystem crossing; LHC – light-harvesting complex; NIR – near infra-red spectral range (700–1200 nm); NMR – nuclear magnetic resonance; NUV – near ultraviolet spectral range (300–400 nm); P – phytol; Phe(s) – pheophytin(s); PPP – phytyl pyrophosphate; Proto – protoporphyrin IX; PS – Photosystem; Q_x, Q_y – low energy absorption bands of tetrapyrroles in the Vis/NIR spectral range; RC – reaction center (type I and type II relate to the homologies with PS I and PS II, respectively); *Rsp. – Rhodospirillum*; UV – ultraviolet spectral range (200–400 nm); Vis – visible spectral range (400 – 700 nm); ε – molar extinction coefficient [M^{-1} cm^{-1}]; λ – wavelength [nm]

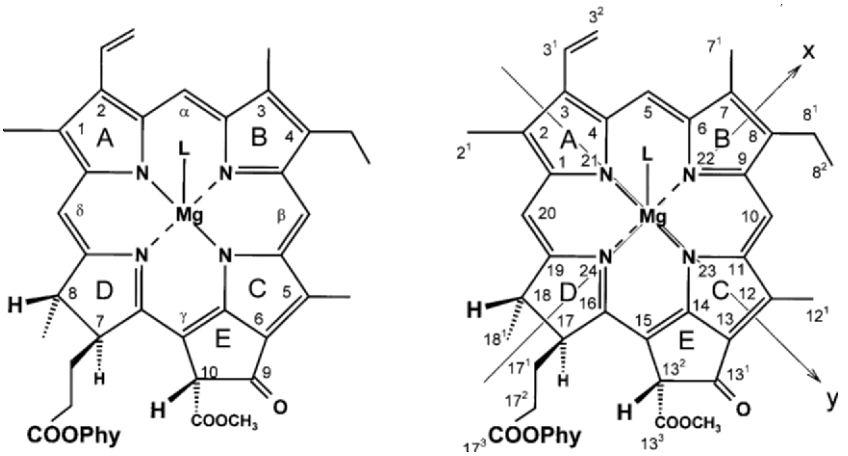


Fig. 1. Chlorophyll *a*: Numbering of rings, C and N-atoms according to Fischer's nomenclature (left) and IUPAC-IUB nomenclature (Moss, 1988) (right). The ligand (L) to the central Mg is shown in the β-position (e.g., above the macrocycle), when it is oriented as shown, with the numbering running clockwise (see footnote). The diagonal arrows in the right structure indicate directions of the two polarization axes, x and y.

variety of pigments with (dark) antioxidant potential from dinoflagellates and marine animals (Karuso et al., 1986; N. Watanabe et al., 1993; Harradine and Maxwell, 1998; Harradine et al., 1996). The tolyporphins are dioxo-bacteriochlorins, lacking the isocyclic ring of Chl, from the cyanobacterium *Tolypothrix tenuis* (*Calothrix* PCC 7601); they may serve a photodynamic defense role (Prinsep et al., 1992, 1998; Minehan and Kishi, 1999). The tunicate *Trididemnum solidum* contains Ni-phytochlorins lacking the 13^2-$COOCH_3$ group and bearing a 3-CH_2OH group that is acylated with a variety of fatty acid alcohols (Bible et al., 1988; Sings et al., 1996): these tunichlorins are clearly derived from chlorophylls. The excited state lifetimes of [Ni]-Chls are so short that photosensitization seems unlikely (Musewald et al., 1999; Fiedor et al., 2000). Chlorophyllous products are also geochemical markers (Chapter 37, Keely). The degradation of chlorophylls in photosynthetic organisms is reviewed in Chapter 17 (Kräutler and Hörtensteiner).

When the photosynthetic apparatus is light saturated, or when the chlorophylls are disconnected from it, the intense absorptions and the long-lived excited states render both Chls and their tetrapyrrole precursors powerful photodynamic agents: when irradiated with visible (vis) or near-infrared (NIR) light in the presence of oxygen, both are capable of generating highly cytotoxic reactive oxygen species. Biosynthesis and degradation of Chls are therefore tightly controlled and co-regulated with the other parts of the photosynthetic apparatus, and several precursors have, vice versa, been directly implicated in affecting regulatory processes (Chapter 10, Grimm and Rüdiger; Chapter 11, Beale; Chapter 12, Jahn et al.; Chapter 13, Yaronskaya and Grimm; Chapter 16, Beck and Grimm). When present in excessive concentrations in photosynthetic organisms, or when incorporated into non-photosynthetic organisms, the photodynamic activities of Chls and their tetrapyrrole precursors can be exploited, for example, as herbicides (Rebeiz et al., 1990), insecticides (Rebeiz et al., 1995) or anti-tumor agents (Chapters 32, 33, Brandis et al.).

Most Chls and bacteriochlorophylls (BChls) are bound to proteins, where they contribute to the

**The nomenclature and numbering system* used throughout this book is that recommended by the Joint Commission on Biochemical Nomenclature (JCBN) of the International Union of Pure and Applied Chemistry and International Union of Biochemistry (IUPAC-IUB) which is described by Moss (1988). The recommended but cumbersome omission-resubstitution nomenclature is not used: substitutions are indicated by placing the modified group in square brackets [ ]; thus, 3-devinyl-3-acetyl-Chl *a* becomes [3-acetyl]-Chl *a*. The esterifying alcohol is generally indicated as a subscript, so that BChl *a* esterified with phytol (P) becomes BChl a_p and BChl *b* esterified with phyta-2,10-dienol becomes BChl $b_{\Delta 2,10}$. The central Mg is always ligated and ligation from above the macrocycle, when it is orientated with the numbering running clockwise, is denoted 'β' and indicated by a solid line (see Fig. 1) while α ligation, from the bottom, is indicated with a dashed line (Moss, 1988; Balaban, 2002).

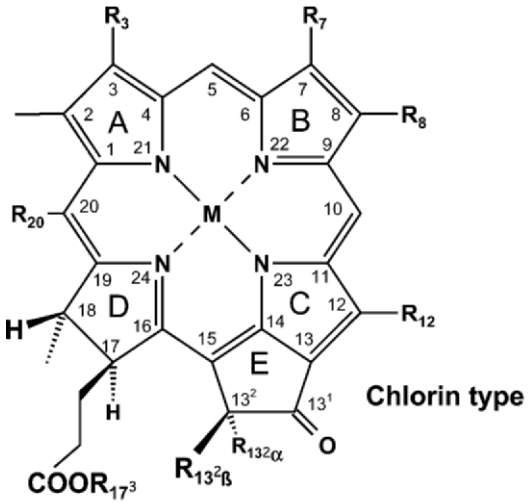
folding and stabilization of the polypeptide chain. Chl-proteins are unusual in the number of cofactor molecules they can bind; for example, 14 Chls plus 4 carotenoids are bound to only 250 amino acids in LHC II (Chapter 26, Paulsen). In the green bacteria, capable of growing at exceedingly low light intensities (Overmann and Schubert, 2002; Blankenship and Matsuura, 2003), this concentration has reached an extreme, since current models even suggest that no protein is directly involved in the short-range organisation of their peripheral antenna, the chlorosome (Chapter 20, de Boer and de Groot). The driving forces for such a dense packing are twofold: firstly, it ensures a high light absorption per unit volume and, secondly, it reduces the amount of protein needed per chromophore thus saving energy input into protein biosynthesis which becomes the more advantageous at the lower light intensities where the cells are growing. A potential danger of dense packing, however, is the so-called 'concentration quenching.' This quenching can be imagined as a statistical process. There is a certain chance that a pigment molecule is defective such that its excited state is very short-lived and relaxes by rapid internal conversion to the ground state. If this pigment is isolated, the effect is negligible but when this defective pigment molecule is tightly coupled to others, it will act as a sink and degrade any excitation of the entire coupled unit to heat, in a time that corresponds to the (average) transfer time from any pigment of the unit to the defective molecule. Therefore, chlorophyll aggregates generally show negligible fluorescence and an extreme shortening of excited state lifetimes (Sauer, 1975, Katz et al., 1976; Scherz et al., 1991). Expanding on this scenario, the photosynthetic apparatus can be viewed as an assembly of pigments in which concentration quenching has evolved from a random to a tightly controlled process where the reaction centers are the productive sinks, leading to 'photochemical quenching' of the excitation: other centers, in particular certain carotenoids, act as safety valves by 'non-photochemical quenching' (Chapter 36, Nedbal and Koblizek).

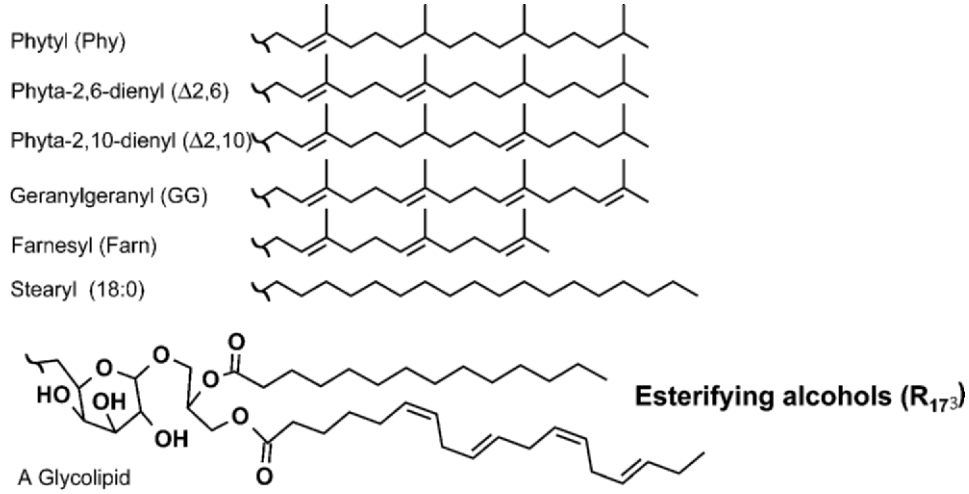
II. Structures

A. Structures and Distribution

The term chlorophyll, the green (Greek *chloros*) of leaves (Greek *phyllos*), was introduced in 1818 (Pelletier and Caventou, 1818) for the pigment(s) extracted from leaves with organic solvents. It was recognized as a mixture of pigments by (partly undisclosed) spectroscopic and solvent partition techniques (Stokes, 1864; Fremy, 1877), but made most obvious perhaps by Tswett's chromatography (Greek *chromos* = color, *graphein* = to write) that separated the blue Chl *a*, the green Chl *b* and several yellow to orange carotenoids (Tswett, 1906). The structural relationship of the tetrapyrrole moiety of Chls *a* and *b* to that of heme, the structure of which was elucidated in 1928 by Hans Fischer's group (Fischer and Stangler, 1927), was suggested early (Verdeil, 1851) although it was based on false evidence. The structural similarity was verified later (Hoppe-Seyler, 1880; Marchlewski, 1909; Willstätter and Stoll, 1913). The latter two books summarized Chl chemistry for the first time. Important aspects of the hydrophobic part of the molecule, phytol, were first identified by Willstätter (1907), including its likely relationship to the isoprenoids. The structure of Chl *a* was established in 1942 (Fischer and Strell, 1947), as the result of more than ten years of work mainly by the groups of Hans Fischer and James Conant (summarized in Fischer and Orth, 1940). The total synthesis of the tetrapyrrole moiety was completed by the groups of Strell in 1962 (Strell and Kalojanoff, 1962) and of Woodward in 1960 using a more rigorous approach (Woodward, 1961). The total synthesis of phytol, the isoprenoid moiety of Chl *a,* was completed a year earlier (Burrell et al., 1959). Chl *a* contains six asymmetric centers: the absolute stereochemistry (Fig. 2A) of the asymmetric C-13[2], C-17 and C-18 of the tetrapyrrole macrocycle was elucidated by Fleming (1967), and those of the asymmetric C-7 and C-11 of phytol by Burrell et al. (1959) and Crabbe et al. (1959). Addition of a single or two different extra ligands to the central Mg creates a sixth asymmetric center, which is of importance in Chl proteins (Balaban et al., 2002; Oba and Tamiaki, 2002).

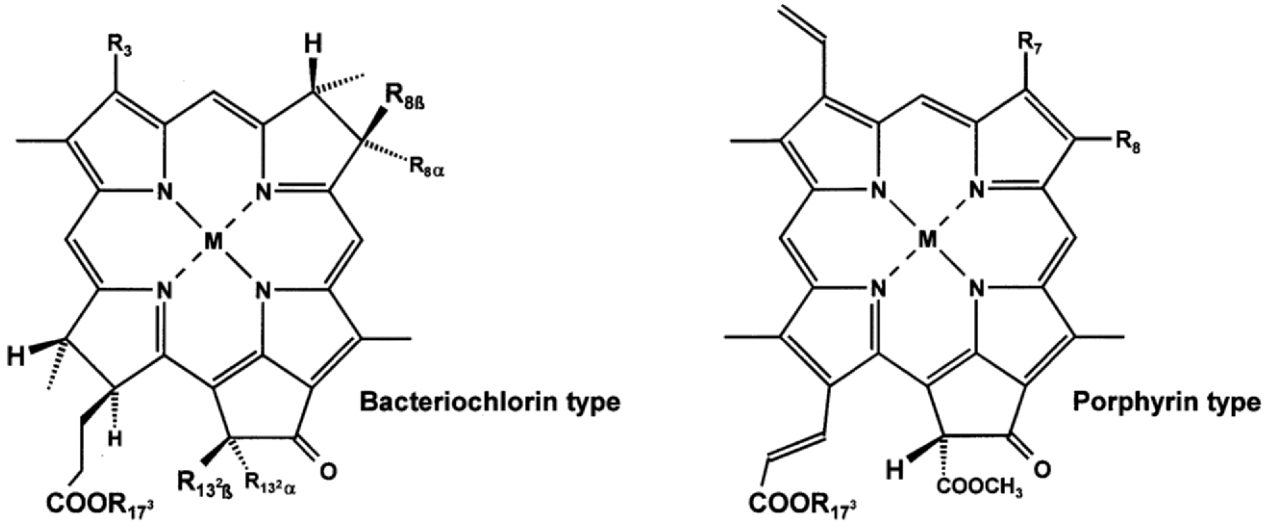
Based on these structures, nearly 100 Chls are known today, with the majority of structures occurring in anoxygenic bacteria, in particular, in the green bacteria. Chls are now defined as cyclic tetrapyrroles carrying a characteristic isocyclic five-membered ring, that are functional in light-harvesting or in charge separation in photosynthesis. The IUPAC-IUB numbering of Chl *a* (Fig. 1) reflects the fact that the isocyclic ring is derived from the C-13 propionic acid side-chain of the common heme and Chl precursor, protoporphyrin IX (Proto) (Moss, 1988). The Chls





Pigment	Abbreviation	R_3	R_7	R_8	R_{12}	$R_{13}^2{}_\alpha$	$R_{13}^2{}_\beta$	R_{17}^3	R_{20}	M	Rem.
Chlorophyll a	Chl a	C_2H_3	CH_3	C_2H_5	CH_3	$COOCH_3$	H	Phy	H	Mg	a)
Chlorophyll a'	Chl a'	C_2H_3	CH_3	C_2H_5	CH_3	H	$COOCH_3$	Phy	H	Mg	
[8-Vinyl]-chlorophyll a	[8-Vinyl]-Chl a	C_2H_3	CH_3	C_2H_3	CH_3	$COOCH_3$	H	Phy	H	Mg	b)
[8-Hydroxyethyl]-chlorophyll a_F	[8-Hydroxyethyl]-Chl a_F	C_2H_3	CH_3	C_2H_4OH	CH_3	$COOCH_3$	H	Farn	H	Mg	
Chlorophyll b	Chl b	C_2H_3	CHO	C_2H_5	CH_3	$COOCH_3$	H	Phy	H	Mg	
[8-Vinyl]-chlorophyll b	[8-Vinyl]-Chl b	C_2H_3	CHO	C_2H_3	CH_3	$COOCH_3$	H	Phy	H	Mg	b)
Chlorophyll d	Chl d	CHO	CH_3	C_2H_5	CH_3	$COOCH_3$	H	Phy	H	Mg	
Chlorophyll d'	Chl d'	CHO	CH_3	C_2H_5	CH_3	H	$COOCH_3$	Phy	H	Mg	
Pheophytin a	Phe a	C_2H_3	CH_3	C_2H_5	CH_3	$COOCH_3$	H	Phy	H	H_2	
Bacteriochlorophyll c	BChl c	CHOH-CH_3	CH_3	$CH_2CH_n(CH_3)_{3-n}$	CH_3/C_2H_5	H	H	Farn/others	CH_3	Mg	c)
Bacteriochlorophyll d	BChl d	CHOH-CH_3	CH_3	$CH_2CH_n(CH_3)_{3-n}$	CH_3/C_2H_5	H	H	Farn/others	H	Mg	c)
Bacteriochlorophyll e	BChl e	CHOH-CH_3	CHO	$CH_2CH_n(CH_3)_{3-n}$	CH_3/C_2H_5	H	H	Farn/others	CH_3	Mg	c)
Bacteriochlorophyll f	BChl f	CHOH-CH_3	CHO	$CH_2CH_n(CH_3)_{3-n}$	CH_3/C_2H_5	H	H	Farn/others	H	Mg	d)

Fig. 2. A: Phytochlorin-type chlorophylls (single bond between C-17/C-18) and esterifying alcohols of chlorophylls. Numbering according to IUPAC-IUB (Moss, 1988). Note that pheophytins lack the central Mg. Footnotes: a) Chl a_F esterified with farnesol has been identified in green sulfur bacteria (see Chapter 4, Kobayashi et al.) b) Pigment is often also termed divinyl-chlorophyll *a* (DV-Chl *a*) to distinguish it from 'normal' (= monovinyl (MV-)) Chl *a*. c) Complex mixture of pigments with varying substituents at C-8 (= R_3), C-12 (= R_4), C-17^3 (= R_7) and epimers at the asymmetric C-3^1. The prevailing sterochemistry at C-3^1 changes from R to S with increasing size of the C-8 substituent R_3. d) Hypothetical



Pigment	Abbreviation	R_3	R_7	$R_{8\alpha}/R_{8ß}$	$R_{13^2\alpha}$	$R_{13^2ß}$	R_{17^3}	M	Rem.
Bacteriochlorophyll a	BChl a	$COCH_3$		H/C_2H_5	$COOCH_3$	H	Phy	Mg	a)
Bacteriochlorophyll a'	BChl a'	$COCH_3$		H/C_2H_5	H	$COOCH_3$	Phy	Mg	a)
Bacteriochlorophyll a_{GG}	BChl a_{GG}	$COCH_3$		H/C_2H_5	$COOCH_3$	H	GG	Mg	a,c)
[Zn]-Bacteriochlorophyll a	Zn-BChl a	$COCH_3$		H/C_2H_5	$COOCH_3$	H	Phy	Zn	a)
Bacteriochlorophyll b	BChl b	$COCH_3$		$=CH-CH_3$	$COOCH_3$	H	Phy	Mg	a)
Bacteriochlorophyll $b_{\Delta 2,10}$	BChl $b_{\Delta 2,10}$	$COCH_3$		$=CH-CH_3$	$COOCH_3$	H	Δ2,10	Mg	a,c)
Bacteriochlorophyll g	BChl g	C_2H_3		$=CH-CH_3$	$COOCH_3$	H	GG	Mg	a)
Bacteriochlorophyll g'	BChl g'	C_2H_3		$=CH-CH_3$	H	$COOCH_3$	GG	Mg	a)
Bacteriopheophytin a	BPhe a	$COCH_3$		H/C_2H_5	$COOCH_3$	H	Phy	H_2	a)
Bacteriopheophytin b	BPhe b	$COCH_3$		$=CH-CH_3$	$COOCH_3$	H	Phy	H_2	a)
Chlorophyll(ide) c_1	Chl(ide) c_1		CH_3	C_2H_5			H	Mg	b,d,e)
Chlorophyll(ide) c_2	Chl(ide) c_2		CH_3	C_2H_3			H	Mg	b,d,e)
Chlorophyll(ide) c_3	Chl(ide) c_3		$COOCH_3$	C_2H_5			H	Mg	b,d,e,f)
Protochlorophyll(ide) a	PChl(ide) a		CH_3	C_2H_5			H	Mg	b,d,g)
[8-Vinyl]-Protochlorophyll(ide) a	[8-Vinyl]-PChl(ide) a		CH_3	C_2H_3			H	Mg	b,d,g)

Fig. 2. B. Bacteriochlorin-type chlorophylls (single bonds between C7/C8 and C-17/C-18, left) and phytoporphyrin-type chlorophylls (right). Numbering according to IUPAC-IUB (Moss, 1988). Note the distinction between chlorophylls (Chl, esterified at C-17^3, R_3 = esterifying alcohol) and chlorophyllides (Chlide, free C-17^3 acid group, R_3 = H), which is often blurred in the literature, in particular on Chls *c*. Footnotes: a) Bacteriochlorin. b) Porphyrin. c) Sometimes esterified with other alcohols. d) Generally free acid, esters found as minor pigments. An extreme case is the glycolipid shown in Fig. 2A . e) Sterochemistry at C-13^2 uncertain. f) Probably mixture with $R_3 = C_2H_3$ and C_2H_5. g) No $17^1/17^2$ double bond.

generally carry Mg as the central metal and, further, a sesqui- (C_{15}) or di-terpenoid (C_{20}) alcohol esterified to the C-17 propionic acid side chain (Fig. 2A), but there are exceptions to both of these characteristics. There are tetrapyrrolic pigments which function in electron or energy transfer, but do not have the central Mg: the central metal is *either* lacking as in the pheophytins, which are involved in electron transfer in type II reaction centers (Satoh, 1993; Dimagno and Norris, 1993), *or* is replaced by Zn in a group of acidophilic purple bacteria (Wakao et al., 1996) (Chapter 4, Kobayashi et al.). Further, large variations in the esterifying alcohols are found in bacteriochlorophylls (BChls) *c, d* and *e* (Fig. 2A; Chapter 15, Friegaard et al.), and most of the *c*-type Chls lack an esterifying alcohol (Fig. 2B; Chapter 3, Zapata et al.).

A major distinction among the various classes of Chls which defines very characteristic spectral features is the degree of unsaturation of the macrocycle (see Fig. 3; Weiss, 1978; Hanson, 1991; Scheer, 2003). The fully unsaturated phytoporphyrin macrocycle, present in the *c*-type chlorophylls of chromophyte algae and some prokaryotes (Fig. 2B), is characterized by an intense absorption in the blue spectral region (Soret or B-bands, $\varepsilon \approx 150{,}000$) and only a moderate absorption in the region around 620 nm (Q_y-bands $\varepsilon \approx 20{,}000$). The phytochlorin system, which is a 17,18-*trans*-dihydrophytoporphyrin, is present in Chls *a, b* and *d* of oxygenic organisms and also in BChls *c, d* and *e* of green anoxygenic bacteria (Fig. 2A). These pigments have (in organic solvents) absorption bands around 440 and 660 nm of equal intensity ($\varepsilon \approx 100{,}000$), with a conspicuous gap in the green spectral region. The bacteriochlorin macrocycle is a 7,8-*trans*,17,18-*trans*-tetrahydrophytoporphyrin system found in BChl *a, b* and *g* of anoxygenic bacteria (Fig. 2B). Here the two major bands ($\varepsilon \approx 100{,}000$) have even moved farther apart into the near ultraviolet (NUV, 350–400 nm) and near infrared (NIR) spectral regions. These pigments also have an appreciably strong absorption in the visible range (Q_x, $\varepsilon \approx 15{,}000$), which has been recognized as a marker band for the state of ligation and H-bonding (T. A. Evans and Katz, 1975; Chapter 34, Yerushalmi et al.). Obviously, the generic nomenclature of the Chls does not relate in a unique way to their degree of unsaturation nor does it necessarily reflect their origin since bacteriochlorophylls include both bacteriochlorins and phytochlorins, while the cyanobacteria also contain the same phytochlorin type Chls as possessed by green plants.

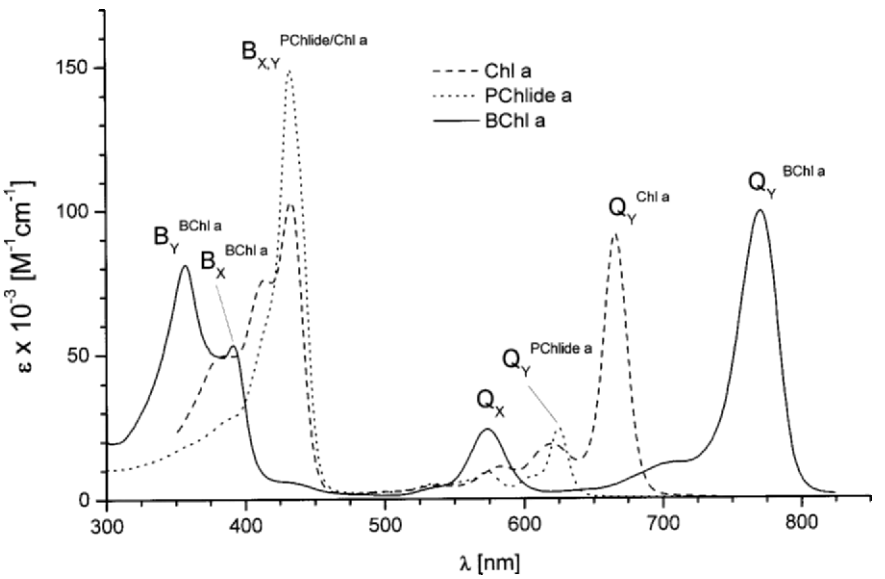


Fig. 3. Absorption spectra of chlorophylls in monodisperse solutions showing the influence of the conjugation system on spectra. Shown are type-spectra of the phytoporphyrin type (PChlide *a* = protochlorophyllide *a*), the phytochlorin type (Chl *a* = chlorophyll *a*) and the bacteriochlorin type (BChl *a* = bacteriochlorophyll *a*) and the assignments of the major absorption bands according to the four-orbital model (Weiss, 1978; L.K. Hanson, 1991). The author is indebted to M. Kobayashi for providing a concise list of extinction coefficients of chlorophylls.

B. 'Green' Chlorophylls of Oxygenic Organisms: Chlorophylls a, b, and d

The most abundant Chl, Chl *a* (Figs. 1, 2A), is present in the reaction centers and the core antennas of almost all oxygenic organisms (Tables 1 and 2). In green algae and plants it is the major pigment of the peripheral antenna complexes and, in a slightly modified form carrying a second vinyl group at C-8 (see Fig. 2A), also occurs in the type II cyanobacteria, sometimes referred to as prochlorophytes (Goericke and Repeta, 1992; Chapter 4, Kobayashi et al.). Its absorption maxima are intense, but restricted to two narrow bands at the edges of the visible spectrum (≈430 and ≈680 nm); it is, therefore, almost always accompanied by other pigments to broaden the absorption range for photosynthetically active radiation. These supplementary pigments are in many cases other Chls. The type II cyanobacteria and the green photosynthetic eukaryotes (green algae, plants) contain Chl *b* (Fig. 2A). With absorption maxima at $\lambda_{max} \approx 460$ and $\lambda_{max} \approx 650$ nm, Chl *b* reduces the green absorption gap to the spectral region between 500 and 600 nm. Chl *d*, a relatively rare pigment of the phytochlorin-type (see Fig. 2A), has distinctly red-shifted absorption maxima as compared to Chl *a*. Originally reported in red algae (Allen, 1966), Chl *d* has now been identified as the major pigment in several oxygenic prokaryotes, including *Acaryochloris* (*Acc.*) *marina* (Miyashita et al., 1997), which also contain Chl *c* (Chapter 4, Kobayashi et al.; Chapter 18, Larkum). Chl *d* largely replaces Chl *a*, including in the primary donor of PS I (Hu et al., 1998), while the pheophytin acceptor and possibly (part of) the primary donor remain Phe *a* and Chl *a*, respectively (Itoh et al., 2001; Mimuro et al., 2004; Chen et al., 2005; Tomo et al., 2005).

Table 1. Occurrence and functions of major chlorophylls (majaor pigments bold, minor pigments in brackets). 'A' denotes antenna or light-harvesting pigments, 'R' reaction center pigments. See Figs. 2 A,B for structures, and Chapter 4 for occurrence and functions of minor chlorophylls.

Pigment / Organism[a]	Chloroflexaceae	Chlorobiaceae	Heliobacteria	Purple bacteria	Cyanophyta I[b], Glaucophyta	Cyanophyta II[b]	Rhodophyta	Heterokonts	Bacillariophyceae (Diatoms)	Cryptophyta	Dinophyta (Dinoflagellates)[c]	Euglenophyta	Chlorarachniophyta	Prasinophyceae	Chlorophyceae	Green plant plastids
Chlorophyll *a*					AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR
[8-Vinyl]-Chlorophyll *a*						AR										
Chlorophyll *b*						A					(A)[c]	A	A	A	A	A
[8-Vinyl]-Chlorophyll *b*						A										
Chlorophylls *c*						(A)		A	A		(A)[c]			A		
Chlorophyll *d*						AR[d]	(A)									
Bacteriochlorophyll *a*	AR	AR		AR												
Bacteriochlorophyll *b*				AR												
Bacteriochlorophylls *c,d*	A	A														
Bacteriochlorophylls *e*		A														
Bacteriochlorophyll *g*			AR													

a) See Green et al. (2003) for taxonomy of algae; b) Cyanophyta I have the classical pigmentation (Chl a plus phycobilins). Cyanophyta II contain Chl *b* in addition to Chl *a*, some abundant marine strains contain large amounts of [8-vinyl]-Chl *a* and *b* (Goericke and Repeta, 1992). c) Pigmentation depends on endosymbiotic photosynthetic organisms, which can be useful as phylogenetic markers. d) Identified only in few species, but here the major pigment. *Acc. marina* (cyanophyta) contains mainly Chl *d*, traces of Chl *a* and Chl *c* and biliproteins (Marquardt et al., 1997)

C. 'Green' Chlorophylls of Anoxygenic Bacteria: Bacteriochlorophylls c, d and e

Both green filamentous bacteria, such as *Chloroflexus aurantiacus,* and green sulfur bacteria like *Chlorobium tepidum,* contain large amounts of a group of closely structurally related, but diverse Chls of the phytochlorin type, namely, BChls *c, d, e*: the anticipated BChl *f*, homologous to BChl e but lacking the C-20 methyl group, is yet to be found in nature (Fig. 2A). In solution, the absorption spectra of BChl *c* and *d* are very similar to that of Chl *a*, and those of BChls *e* and *f* carrying a 7-CHO group are very similar to that of Chl *b*. In situ, the absorption maxima of BChls *c, d* and *e* are strongly red-shifted to 700–750 nm. This red-shift is due to a unique form of aggregation which is related to the presence of the 3^1-OH group and the absence of the sterically demanding 13^2-$COOCH_3$ substituent. It can be mimicked well in vitro by self-organizing aggregates of structurally related porphyrins, chlorins and bacteriochlorins (Balaban et al., 2004; Kunieda et al., 2004; Tamiaki et al., 2004b; Chapter 20, de Boer and de Groot). A similar type of aggregation is probably the key

Table 2. Location, functions and basic spectroscopic properties of photosynthetic pigments. The author is indebted to M. Kobayashi for providing a concise list of extinction coefficients of chlorophylls.

Pigment	Type	Location [a)]	Function	Absorption in solution[b)] λ_{max} (ε) [solvent]	Emission in solution [b,c)] λ_{max} [solvent]	Absorption in situ λ_{max}	Ref [d)]
Chl *a*	Phytochlorin	PA, CA, RC	LH, ET	430, 662(78.8) [A] 430,662(90)[D]	668 [A] 666 [D]	~440, 670-720	1,2[f)]
Phe *a*	Phytochlorin	RC	ET	408,505,534,667(55.2)[D]	673[D]	~680	2,3
Chl *b*	Phytochlorin	PA	LH	457,646(46.6)[A] 454,644(56.3)[D]	652 [A] 646 [D]	~460, 630-680	1,2 [f)]
Chl *c* [g]	Phytoporphyrin	PA	LH	446,578,629(23.9)[AP] 446,579,628[D]	~633 [A][a]	~400, 500-620	1
Chl *d*	Phytochlorin	PA, CA, RC	LH, ET	447, 688 (98.3)[D]	695[D]	~440, ~690	4
BChl *a*	Bacteriochlorin	PA, CA, RC	LH, ET	357,391,573,772(91)[D] 365,608,772(60)[M]	800	<400, ~600, 800–960	2 [f)], 4,5
Bphe *a*	Bacteriochlorin	RC	ET	357,525,749(66.2)[D]	762[D]	770	2,6
BChl *b*	Bacteriochlorin	PA, CA, RC	LH, ET	368,408,578,794(106)[D] 368,407,582,795 [A]	810	<400, ~600, 800–1020	4[f)]
Bphe *b*	Bacteriochlorin	RC	ET	398,776(1:0.42)[D]	785[D]	780-795	7,8
BChl *c*	Phytochlorin	PA	LH	432,622,660 (92.7) [D] 435,620,670 (70,9) [M]	667[D]	~460, 730-760	2 [f)],4[e)]
BChl *d*	Phytochlorin	PA	LH	425,612,650 (87.9) [D] 427,612,659 (64) [M]		~440,720-750	4[e,f)]
BChl *e*	Phytochlorin	PA	LH	338,456,594,649(48.9) [A] 476, 660(41)[M]		~460,~715	9[f)]
BChl *g*	Bacteriochlorin	CA, RC	LH, ET	365,405,566,762(76)[A] 364,767(0.8:1) [D]		<400, 780-850	7,10

a) PA = peripheral antenna, CA = core antenna, RC = reaction center; b) λ_{max} in nm, ε in $cm^{-1}mM^{-1}$, solvent abbreviations: A = acetone, AP = acetone/1% pyridine, D = diethylether, DO = dioxan, M = methanol; c) The fluorescence yield of the pigments varies with their environment; d) 1 = Jeffrey et al., 1997; 2 = Goedheer, 1966; 3 = Germano et al., 2001; 4 = Oelze, 1985; 5 = Permentier et al., 2001; 6 = J. H. C. Smith and Benitez, 1955; 7 = M. Kobayashi, personnel communication; 8 = H. Scheer, unpublished; 9 = Borrego et al., 1999; 10 = Kobayashi et al., 1992; e) extinction coefficients calculated from specific extinction coefficients using the 8-ethyl-11-methyl-17^4-farnesyl structures; f) see Chapter 7 (Porra) for extinction coefficients in other solvents; g) family of pigments, absorptions vary with structure, in Chl $c_{1,2}$ and [8-Vinyl]-Pchlide *a* the band at ≈625 is more intense than that at ≈580 nm, in Chl c_3 and Chl c_{CS-170} it is less intense.

to the tight packing of these pigments in the unique peripheral antenna of green bacteria, the extra-membranous chlorosomes (Blankenship and Matsuura, 2003), where pigment-protein interactions seem to play little or no role (Niedermeier et al., 1992) in the long-range organization (Chapter 6, Kobayashi et al.; Chapter 20, de Boer and de Groot).

In spite of their names, BChls *c, d* and *e* are not bacteriochlorins, but phytochlorins. Their unifying structural features are the lack of the 13^2-$COOCH_3$ substituent on the isocyclic ring, and the presence of a CHOH-CH_3 substituent at C-3. The biosynthesis of these three BChls is only now beginning to be unravelled (Senge and K. M. Smith, 1995; Chapter 15, Friegaard et al.). The following five additional features of these BChls generate a remarkable structural diversity, the significance of which is only partly understood at present (Oelze and Golecki, 1995; Senge and K. M. Smith, 1995; Kunieda et al., 2004; Tamiaki et al., 2004a,b). Firstly, the *c*-and *e*-type BChls are characterized by a 20-CH_3 substituent, which is lacking in BChls *d* and *f*. In both cases, steric hindrance of the C-20 methyl group induces, in vitro and in vivo, a red-shift (~10 nm) when compared to the respective C-20 unsubstituted pigment. Secondly, BChl *e* type homologues (as well as the putative BChl *f*) carry a 7-CHO substituent like Chl *b*, leading to similar spectral characteristics as for Chl *b*. Thirdly, they show large variations in the esterifying alcohols (Larsen et al., 1995; Caple et al., 1978; Glaeser et al., 2002; Chapter 15, Frigaard et al.; Chapter 37, Keely). Fourthly, the pigments can be methylated once (at $C\text{-}12^1$ or C-20) or up to three times (at $C\text{-}8^2$) (Airs et al., 2001; Chapter 15, Friegaard et al.; Chapter 37, Keely). Fifthly, both epimers of the newly generated asymmetric $C\text{-}3^1$ are found in nature (Fig. 2A) (K. M. Smith and Simpson, 1986; Tamiaki et al., 1994). Not all the theoretically possible combinations of these features have been identified from natural samples, but BChls *c, d* and *e* still provide more than 50% of the currently known chlorophyll structures, even though the green bacteria make a relatively limited contribution to total global photosynthesis. Understanding the role of these variations in the assembly and function of the chlorosome core, which appears to be largely devoid of proteins, is currently a major challenge in chlorophyll research (Chapter 15, Friegaard et al.; Chapter 20, de Boer and de Groot). Unlike most other Chl aggregates, those present in chlorosomes are, in a way not understood, highly fluorescent and avoid concentration quenching (see above).

D. Chlorophylls of the Phytoporphyrin Type: The Chlorophylls c

Various Chl *c*-type pigments are found in heterokonts and the type II cyanobacteria (Stauber, 1988; Jeffrey, Mantoura, and Wright, 1997; Chapter 3, Zapata et al.; Chapter 8, Garrido and Zapata). The *c*-type Chls are phytoporphyrins, and most contain an acrylic acid side chain at C-17 (Fig. 2B). They absorb only moderately (ε ~20,000 $M^{-1}cm^{-1}$) in the region of the 'green gap,' the spectral region where Chls *a* and *b* absorb only weakly (Fig. 3, Table 2). Accordingly, more chromophores are needed to provide the same absorption. They have, however, a relatively intense absorption near 400 nm, which may be advantageous in clear waters (Chapter 18, Larkum). A structural feature of most *c*-type Chls is the unesterified acrylic- or propionic-acid side-chain at C-17; thus, these pigments should be more correctly called chlorophyllides (Chlides), with the ending 'ide' denoting an unesterified acid side chain at C-17. These Chl(ide)s *c* are therefore much more polar than the other Chls. With the development of analytical techniques suitable for pigments with a free C-17 acid side chain (Chapter 8, Garrido and Zapata), the diversity of known *c*-type Chls has dramatically increased in recent times.

The *c*-type Chls may have evolved first among the Chls (Chapter 18, Larkum): [8-vinyl]-protochlorophyllide *a*, also known as 3,8-divinyl-pheoporphyrin *a*-Mg-monomethylester, may be the most ancient Chl still active in photosynthesis: it is also a precursor to most Chls, but has been identified as a genuine antenna pigment in symbiotic prokaryotes (Helfrich et al., 1999). Recently, an increasing number of hitherto only partially characterized Chls *c* have been discovered, which are esterified at $C\text{-}17^3$ by an alcohol including the unique galactolipid shown in Fig. 2A (Chapter 3, Zapata et al.). In view of the immense variety of photosynthetic organisms, especially of marine microalgae, more surprises can be anticipated. The functional significance of these variations still requires clarification. Remarkably, the 'classical' *c*-type Chls, lacking an esterifying alcohol at $C\text{-}17^3$, are bound to proteins of the LHCII type which, in green plants, bind Chls *a* and *b* esterified with phytol.

E: Bacteriochlorin-type Chlorophylls: Bacteriochlorophylls a, b and g

While all pigments of anoxygenic photosynthetic

bacteria are known as BChls, only three of them, BChls *a, b* and *g,* are true bacteriochlorins, that is, 7,8-*trans*,17,18-*trans*-tetrahydrophytoporphyrins or their Δ8,8′-derivatives (see Fig. 2B). The spectroscopic consequence of this hydrogenation of rings B and D is a considerably increased gap among the absorption bands (Fig. 3). The Q_Y-absorption band is red-shifted to 750–800 nm in mono-disperse solution, and even more so (800–1020 nm) in situ (Drews and Giesbrecht, 1966), while the Soret-band is blue-shifted (<400 nm) and split. The organisms containing these Chls thereby extend the useable spectrum in both directions beyond those of all other Chls. BChl *a* is the most widely distributed BChl. It is present in RC and the core-antennas of most anoxygenic bacteria, as well as in the peripheral antennas of the purple bacteria (Tables 1, 2). In some purple bacteria, it is replaced by the structurally related BChl *b*, carrying an 8-ethylidene instead of the 8-ethyl group present in BChl *a* (Scheer et al., 1974; Steiner et al., 1981). BChl *g* is found in the strictly anaerobic Gram-positive heliobacteria, both as a light-harvesting and as an electron transfer pigment in the functionally integrated type I-RC complex (Michalski et al., 1987; Neerken and Amesz, 2001; Chapter 4, Kobayashi et al.).

F. Structural Variants of Chlorophylls

The number of recognized Chls is constantly increasing. Structural variants are found in most photosynthetic organisms, often only in small amounts, and their functions are not always known. Some have been established as biosynthetic precursors or products of (bio)degradation and include pigments esterified with terpenoid alcohols other than phytol including phytadi-, tri- or tetra-ene alcohols (Schoch et al., 1977; Shioi, 1991), and also phytochlorin-pigments bearing a C-8 vinyl rather than an ethyl substituent (Rebeiz et al., 1994). However, many of these unusual Chls are functional in photosynthesis. Pigments with more highly unsaturated terpenoid alcohols can replace phytol in BChl *a* (Katz et al., 1972; Walter et al., 1979) or BChl *b* (Steiner et al., 1981) in certain purple bacteria and, often, these modified pigments are the major Chls present and, therefore, must be functional; the role of the modified alcohols, however, remains unclear (Section III.3). In at least one organism, *Rhodospirillum* (*Rsp.*) *rubrum*, pigments with different alcohols are segregated among different functions: in this organism the BChl *a* present in the RC is esterified with phytol while that in the antennae with geranylgeraniol (Walter et al., 1979). In the elucidated structures of photosynthetic complexes, the esterifying alcohols often interact with other hydrophobic structures including not only the respective alcohols of neighboring Chls, but also lipids and carotenoids. In this context, the presence of non-covalently bound lipid molecules inside the native proteins and in close proximity to Chls is noteworthy. Such lipids have been found in the PS I core (Jordan et al., 2001; Ben-Shem et al., 2003), in PS II (Biesiadka et al., 2004; Ferreira et al., 2004) and even in the water-soluble peridinin-Chl protein (PCP) from *Amphidinium carterae* (Hofmann et al., 1996).

Another variation concerns the central metal which, in almost all cases, is Mg. Several closely related strains of purple bacteria (*Acidiphilium* sp.), however, contain up to 90% [Zn]-BChl (Kobayashi et al., 1999; Stewart et al., 2001; Chapter 4, Kobayashi et al.), which is not only functional but also largely replaces BChl *a* in all pigment proteins, including the primary donor, P870, of the RC (Matsuura and Stewart, 2004). While Mg- and Zn-containing Chls have largely similar properties, a notable chemical difference is the increased stability of the [Zn]-Chls towards acid. Since *Acidiphilium* has been isolated from acidic mine ponds, it is likely that pH constituted the evolutionary pressure responsible for the change of the central metal. As the Zn-concentrations of the ponds were not unusually high, it is probable, therefore, that the Zn was actively incorporated, *either* into the bacteria (where it can then compete by mass action with Mg), *or* by a specialized Zn-chelating enzyme. It is unknown, however, whether the Zn is introduced by a specialized Proto Zn-chelatase, or replaces Mg at a later biosynthetic stage. In vitro, the Mg-containing Chls can be replaced in all accessible sites of antennas and LHC by their Zn-containing analogues without impairing their functions (Kobayashi et al., 1992, 1999; Davis et al., 1996; Noy et al., 1998; Lapouge et al., 2000), and most enzymes accept Chls with either Mg or Zn as central metal as substrates (Schoch et al., 1995). It should be noted that a chlorophyll precursor containing Zn, namely Zn-Proto, has been found in another acidophilic organism, the rhodophyte *Galdieria sulphuraria (Cyanidium caldarium)*, where it was considered as a precursor for biliproteins (Csatorday et al., 1981); however, since biliproteins are formed frome heme, another function is possible. No [Zn]-Chls have been found in this organism.

Transmetallated Chls, with Hg, Cd, Ni, Cu or Pb replacing Mg, have been isolated from lichens, microalgae and, more recently, from water plants grown on media enriched in the respective metals (Küpper et al., 1996, 1998; Chapter 5, Küpper et al.). In these organisms, photosynthesis is strongly impaired (Caspi et al., 1999). It is interesting in this context that prospecting for Cu has been attempted via determination of Chl fluorescence (Lanaras et al., 1993). If judged by experiments with isolated light-harvesting complexes, the afore-mentioned metals (which bind much more strongly than Mg to the tetrapyrrole) can replace the latter spontaneously at concentrations likely to exist within the cells. The rapid internal conversion of metals with unfilled d-shells can well explain the impaired photosynthetic capacity of plants that are stressed with heavy metals (Küpper et al., 1998). [Ni]-BChl *a* shows internal conversion (IC) on a time-scale <100 fs (Musewald et al., 1999; Noy et al., 2000). If it is incorporated in bacterial LH1 complexes, it can compete efficiently with energy transfer at time-scales down to 50 fs. It therefore acts as a 'black hole': any exciton that passes by a [Ni]-complex is degraded to heat. In vitro, this can be used to study unit-sizes and exciton delocalisation (Fiedor et al., 2000; see below).

Unusual or minor Chls with specialized functions have been identified in all RCs. The metal-free pheophytin (Phe) *a* is found in the PS II-RC. Because its redox-potential is ≈200 mV more positive than that of Chl *a* (T. Watanabe and Kobayashi, 1991), it serves as an early electron acceptor from P680 and transfers this electron on to the quinone, Q_A (M. W. C. Evans and Nugent, 1993). In the type II RC of purple (Reed and Peters, 1972) and filamentous green (Vasmel et al., 1983) bacteria, two BChl *a* (or *b*) molecules are structurally and functionally replaced by BPhe *a* (or *b*). One of them, termed H_A, has been shown to be very rapidly (≈1 ps) reduced by the primary acceptor, BChl-B_A (Schenck et al., 1981; S. Schmidt et al., 1995) and to donate its electron subsequently within 200 ps to Q_A (Dimagno and Norris, 1993). The minor Chls of type I-RCs are the 13^2(S)-epimers of the respective major Chls, the so-called 'prime'-Chls. Photosystem I (PS I) of most oxygenic organisms contains Chl *a*′ (Kobayashi et al., 1988), the 13^2(S)-epimer of Chl *a*, while the Chl *d*-containing *Acc. marina* contains Chl *d*′ (Akiyama et al., 2001) and heliobacteria contain BChl *g*′ (Kobayashi et al., 1991). The location of Chl *a*′ as one of the pigments of the special *pair has* been verified by the X-ray structure of a cyanobacterial PS I (Jordan et al., 2001). By contrast, RCs from green sulfur bacteria, which are also likely to be of the type I, do not contain a prime-chlorophyll but rather Chl $a_{\Delta 2,6}$, namely, the 'normal' Chl *a* macrocycle esterified with Δ2,6-phytadienol (Kobayashi et al., 2000). In the RCs of *Heliobacterium chlorum,* Chl *a* bearing a hydroxyethyl substituent at C-8 and esterified with farnesol (8^1-hydroxy-Chl a_F) has been identified: it is believed to function as primary acceptor A_0 (Van de Meent et al., 1991). Finally, the Chl *a*-$\Delta 13^1,13^2$-enol shows some of the spectral features of P700 in PS I-RCs (Wasielewski et al., 1981). The current X-ray structure is still inconclusive in this respect. One Chl *a* of P700 is strongly distorted, but seems incompatible with an enol (Jordan et al., 2001). RC of *Rsp. rubrum* contain BChl a_P (i.e., esterified with phytol) and BPhe a_{GG} esterified with geranygeraniol: the antenna contains only BChl a_{GG} (Walter et al., 1979).

Antenna complexes can also contain modified pigments. Probably the most striking example is the presence of [8-vinyl]-Chls *a* and *b* as the most abundant pigments in marine *Prochlorococcus* species living down to considerable (≈150 m) depths (Goericke and Repeta, 1992), and contributing a large fraction of global photosynthesis. The functional significance of this substituent is currently unclear. In the red region, the [8]-vinyl-Chls absorb only slightly different from the corresponding Chls *a* and *b* bearing the usual 8-ethyl group. However, the Soret bands are shifted by approximately 8 nm to the red, which may be of relevance in the clear oceanic waters where blue light is prevailing.

III. Why Chlorophylls?

Nature has almost exclusively and universally selected Chls for the primary reactions of photosynthesis. In reaction centers, Chls are indispensable as primary electron donors and acceptors, transporting the electron within a few picoseconds across half the thylakoid membrane. They also serve to transport triplet excitation energy to the protective carotenoids (Angerhofer et al., 1998). In most photosynthetic organisms, Chls also provide the majority of light-harvesting pigments which are only supplemented by linear tetrapyrroles and carotenoids. What are the advantages of the Chls over these and other pigments?

The answer is probably a unique combination

of factors: the macrocycle, the central metal, the peripheral substituents and the hydrophobic esterifying alcohol all together provide some unique physical and chemical properties. Among them, the macrocycle and the central Mg, seem to be most important, if judged by their conservation and by their known influence on the chemical and physical parameters of the pigments. Variations in the substituents provide the necessary plasticity for chemical interactions and modulation of certain properties including light absorption and redox potential, while the functional relevance of the hydrophobic esterifying alcohol is less clear.

A. The Macrocycle

The large, aromatic tetrapyrrole macrocycle is the basis for both the absorption and the redox chemistry of Chls. Starting from the last common precursor with hemes, namely protoporphyrin (Proto), the macrocycle has been considerably modified for improved function, by substitution and reduction.

Proto has an intense absorption in the blue spectral range but only a moderate absorption in the visible. The absorption in the visible (VIS) is doubled in porphyrins containing the isocyclic ring which is a characteristic of all Chls, and is slightly red-shifted, which may have been an important factor in the early evolution of Chls. These effects arise from a reduced symmetry of the macrocyclic π-system (Weiss, 1978; Hanson, 1991). They become much more pronounced upon reduction of the 17,18-double bond in ring D, and, even more so, upon further reduction of the 7,8-double bond in the BChls. Thus, the absorbance of an individual chromophore in the Vis and NIR range, respectively, is increased two-three-fold, and the accessible spectral range nearly doubled, without any specific protein interaction required. This contrasts with the second group of photosynthetic tetrapyrrole chromophores, the bilins (Scheer, 2003). Here, the absorbance of the chromophores is similarly high, but due to a conformational change that requires more extensive pigment-protein interactions, which, in turn, require a considerably larger investment of energy in protein biosynthesis (Table 3).

The reduction and oxidation potentials of Chls are closely related to their optical spectra, in particular, their differences should correspond roughly to the energy of the Q_y-band. Accordingly, this gap decreases from porphyrins to chlorins and bacteriochlorins (Fuhrhop, 1975; Felton, 1978). However, the changes of the individual potentials vary considerably with substituents (Watanabe and Kobayashi, 1991), ligation to the central metal (Felton, 1978; Chapter 34, Yerushalmi et al.), H-bonding (Chapter 19, Allen and Williams) and distortions of the macrocycle (Fajer et al., 1990), which are difficult to separate from the influence of ring-reduction per se. In the tetraphenylporphyrin-series, reduction to the chlorin affects mainly the oxidation potential, while further reduction to the bacteriochlorin affects mainly the reduction potential (Felton, 1978). In a series of metallo-octaethylporphyrins, the first oxidation potential is always decreased by ~300 mV if the macrocycle is reduced to the chlorin level (Fuhrhop, 1975), and the same decrease is seen from Mg-octaethylchlorin to Mg-octaethylbacteriochlorin (Felton, 1978). In a series of (B)Chls and (B)Phes, the 1st reduction potential is hardly changed when going from the chlorin to the bacteriochlorin while maintaining the substituents, but the 1st oxidation potential is more affected and lowered by ~0.25 V (Geskes et al., 1995a). When this is compared to the respective changes of ~0.14 and ~0.05 V upon exchanging the 3-acetyl- to a 3-vinyl-

Table 3. Investment into chromophores in different antennas (IP = isoprenoid).

Antenna	Esterifying alcohol investment	Protein investment (kDa)	
	IP units/chromophore	per chromophore	per tetrapyrrole)
Chlorosome (green bacteria)	3	<1	≈ 1
LHCII (green plants)	4	1.7	2.25
LHCII (heterokonts, dinophytes)	4 (Chl *a*) 0 (Chls *c*)	1.7	2.25
LH1 (purple bacteria)	4	4.3	6.5
LH2 (purple bacteria)	4	2.6	4.3
PS I (type I RC with integrated antenna)	4	3,5	4
Allophycocyanin	–	16	16
C-Phycocyanin	–	11	11
C-Phycoerythrin	–	7.5	7.5

substitutent (Geskes et al., 1995a) or the change in oxditation potential of ~100 mV per H-bond (Chapter 19, Allen and Williams), it becomes clear that the redox potentials are only moderately influenced by the reduction of the macrocycle from the porphyrin to the bacteriochlorin.

The aromatic macrocycle is also one reason for the pronounced stacking of Chls. Certain aggregates in organic solvents, but especially in amphiphilic environments, are formed mainly by π-π interactions of the macrocycle (Scheer et al., 1985; Scherz et al., 1991). In contrast to other types of aggregates, they do not require the presence of the central Mg for their formation. These π-π interactions also contribute to pigment-protein interactions in photosynthetic complexes.

Distortions of the macrocycle introduce a red-shift (Gudowska-Nowak et al., 1990). In isolated pigments, this is possible by steric hindrance of peripheral substituents (Woodward et al., 1960): for example, the absorption of BChl *c* is 10 nm red-shifted relative to that of BChl *d*, since the 20-H of the latter is replaced by a CH_3-group in BChl *c* (Senge and K. M. Smith, 1994) (Table 2). Much larger effects have been introduced in porphyrins and chlorins (Medforth et al., 1992; Gentemann et al., 1994; Senge et al., 1998; Senge, 2000; Chapter 2, Senge et al.). Distortions can also be introduced into isolated porphyrins by central metals that do not fit snugly into the central hole of the macrocycle (Buchler, 1975; Sparks et al., 1993). In situ, non-planarity of the macrocycle can also be introduced by interactions with the proteins. This is seen in all highly-resolved structures of chlorophyll-proteins, and is particularly pronounced in one of the BChl-B850 of the purple bacterial LH2 where it probably contributes to the lowering of the site-energy (Robert et al., 2003)

B. The Peripheral Substituents

Considerable variations in the properties of chromophores are possible by changing the substituents. Conjugated carbonyl groups are introduced at several sites. The C-13^1 oxo group, for instance, is present in all Chls. BChls *a* and *b* and Chl *d* also possess C=O groups at C-3 while Chls *b* and c_3 and the BChls *e* (and *f*) have them at C-7. The redox potential is shifted in all cases by about 200 mV. The Soret band system ($B_{x,y}$) is always red-shifted, but the spectral effect on the Q_y band depends on the site to which the C=O group is bound: if they are situated along the y-axis (C-3, C-13^1, see Fig. 1 for definition of the axes), they result in a red-shift of the major visible Q_y-absorption band. On the other hand, if a C=O group is located along the x-axis (C-7 on ring B), it results in a blue-shift of this band. A fine-tuning of these absorption shifts is possible by changing the orbital overlap of the C=O groups with the π-electrons of the macrocycle: this has been studied especially for the 3-acetyl group of BChl *a*, where shifts up to 30 nm are discussed (McLuskey et al., 1999; Robert et al., 2003). The influence of C=O groups on both the spectral and the redox properties can furthermore be modulated by H-bonding. Allen et al. have, for example, shifted the redox potential of the primary donor, P870, of bacterial reaction centers by about 100 mV per H-bond (Chapter 19, Allen and Williams). H-bonding has also been shown for the 3^1-carbonyl group of one of the Chl *d* in the primary donor, P740, of PS I in *Acc. marina* (Sivakumar et al., 2003) or to the 13´-C=O group of BChl in the LH2 antenna (Kwa et al., 2004; Chapter 27, Garcia-Marin et al.).

Another important effect that can be introduced by peripheral substituents is steric hindrance (see above), by which the macrocycle becomes distorted (Barkigia and Fajer, 1993). The red-shifts of BChl *c* vs. BChl *d,* and of BChl *e* vs. BChl *f,* can be rationalized by steric hindrance of the 20-CH_3 group (Section III. A). Considerable distortions of the macrocycle are obvious for several Chls in high-resolution X-ray structures, where they are induced by site-specific interactions with their apo-proteins (Yeates et al., 1988; Deisenhofer and Michel, 1993; Schiffer and Norris, 1993; Tronrud and Matthews, 1993; McDermott et al., 1995; Hofmann et al., 1996; Jordan et al., 2001; Spiedel et al., 2002; Liu et al., 2004; Standfuss et al., 2005): the site-energies of the respective Chls are expected to be modified by such distortions, but their functional significance remains to be explored experimentally.

The functional significance of the most conspicuous and ever-present peripheral carbonyl substituent of the Chls, namely, the C=O group at C-13^1 which is located on the isocyclic ring derived from the oxidized 13-propionic acid side chain of Mg-Proto, is largely unknown. Cyclization renders this C=O group at C-13^1 coplanar with the macrocycle, which may be significant in terms of a pronounced red-shift. It may also increase the stiffness of the macrocycle, and thereby reduce losses in photosynthesis due to internal conversion of excited states. H-bonding to the 13^1-CO group has been recognized as an im-

portant factor for interaction with the protein, e.g. in the LH2 complex of purple bacteria (Chapter 27, Garcia-Martin et al.). Even less is known of the significance of the β-ketoester system, of which the 13^1-CO group is part. This chemically reactive system is present in all Chls except BChls *c, d, e* and *f*, which lack the 13^2-$COOCH_3$ group. The 13^2-$COOCH_3$ group interferes with aggregation (Scheer and Katz, 1975; Oba and Tamiaki, 1999), which is probably a reason for its absence in BChls *c, d* and *e*, which form very large regular aggregates (Blankenship and Matsuura, 2003; Balaban et al., 2004; Chapter 20, de Boer and de Groot). The β-ketoester system is a potential site for chelation, but such chelates have only been observed in vitro (Scheer and Katz, 1978). The β-ketoester group also facilitates enolization, but again there is little hard evidence that enols play a photosynthetic role in vivo (Jordan et al., 2001). It facilitates the formation of the 13^2-epimers and these 'prime' pigments like Chl *a*′ form part of the primary donor of type I RCs (Chapter 4, Kobayashi et al.). Aggregation is influenced by this change in stereochemistry (Oba et al., 1996), as well as the planarity of the macrocycle (Furukawa et al., 2000), which may both be relevant for the formation and symmetry of the *special pair* present as primary donor in these reaction centers (Katz et al., 1978a; Feiler et al., 1995; Plato and Moebius, 1995; Rautter et al., 1995; Bratt et al., 1996; Artz et al., 1997; Bratt et al., 1999; Kaess et al., 2001).

The significance of ring E is further emphasized by site-selective exchanges in a variety of chlorophyll proteins (Scheer and Hartwich, 1995; Davis et al., 1996; Chapter 26, Paulsen) and the substrate specificity of metabolising enzymes (Helfrich et al., 1994; Klement et al., 1999; Chapter 13, Yaronskaya and Grimm; Chapter 14, Rüdiger; Chapter 17, Kräutler and Hörtensteiner). While, in general, considerable variations of substituents are possible on rings A and B without impairing binding or physiological functions, variations on ring D and, in particular, on the isocyclic ring E are critical. Most of these studies have been carried out in vitro; thus, it remains a challenge to complement them with in vivo studies (Vavilin et al., 2003).

C. The Central Metal

The central metal has a decisive influence on the excited state kinetics of tetrapyrroles and, therefore, on their function in photosynthesis. It appears that nature has selected Mg as the central metal which maximizes excited state lifetime, while maintaining low intersystem crossing to the highly toxic triplet (see Chapter 6, Kobayashi et al.). Transition metals with open d shells, as well as all heavy metals, would be deleterious in this respect. Of the remaining metals, K, Na, Li and Ca form very labile complexes, while Be seems too small; the trivalent metals are possibly avoided because they introduce a charge. Thus, only H^+, Mg^{++} and Zn^{++} remain and they are, indeed, the only central metals found in nature as functional chlorophyllous pigments. The preference of Mg over Zn may then relate to its lower mass and concomitantly reduced intersystem crossing (ISC) properties; in other aspects, the [Zn]-Chls seem to be very similar to the respective Mg-complexes. They both have similar redox potentials; indeed, they are the lowest of all sufficiently stable metal complexes, which seems to be another reason for their selection (T. Watanabe and Kobayashi, 1991; Geskes et al., 1995b). [Zn]-Chls can replace the respective Mg-complexes in all complexes where such exchanges have been studied (Scheer and Hartwich, 1995; Davis et al., 1996; Lapouge et al., 2000; Chapter 5, Küpper et al.; Chapter 25, Nango; Chapter 26, Paulsen), and in synthetic chlorophyll-binding proteins (Struck et al., 1998; Rau et al., 2001; Chapter 24, Noy et al.; Chapter 25, Nango). They can also replace the Mg-complexes in all biosynthetic enzymes involved in Chl formation beyond Mg-chelatase, which is the only enzyme specific for Mg (Rüdiger, 2002; Chapter 13, Yaronskaja and Grimm; Chapter 14, Rüdiger).

It has long been recognized that the Mg in Chls binds extra ligands so strongly that it is practically never four coordinate. Removal of the extra ligand in unpolar solvents results in aggregation with another Chl donating a ligand; for example, the highly conserved 13^1-CO group (Katz et al., 1978b). Interactions with the central metal and the 3^1-OH group in BChls *c*, *d* and *e* are likewise important in chlorosomes (Umetsu et al., 2003; Chapter 20, de Boer and de Groot). The central Mg is also important for the interactions of Chls with their apo-proteins. A variety of ligands has been identified in the crystal structures of Chl proteins: in more than 50% of known binding sites the ligand is histidine, but glutamine, asparagine, backbone C=O groups, and even water, are known to be ligated (Yeates et al., 1988; Deisenhofer and Michel, 1993; Schiffer and Norris, 1993; Tronrud and Matthews, 1993; Kühlbrandt et al., 1994; McDermott et al., 1995; Hofmann et al., 1996; Savage

et al., 1996; Jordan et al., 2001; Spiedel et al., 2002; Ben-Shem et al., 2003; Liu et al., 2004; Standfuss et al., 2005). These interactions may be of considerable importance in the organization of Chl proteins, especially in a hydrophobic environment. A Chl ligated to histidine may, for example, be considered a highly modified amino acid. Ligand interactions are known to modulate the electron density of the macrocycle and, thereby, its properties (Alia et al., 2001; Chapter 34, Yerushalmi et al.). They are used as a tool to replace (B)Chls by (B)Phes, and vice versa (Coleman and Youvan, 1990; Shen and Vermaas, 1994; Palaniappan et al., 1995; Czarnecki et al., 1997): when there is no space provided to carry in a ligand, the replacement of, for example, a liganding histidine by a hydrophobic amino acid results in the introduction of a (B)Phe, and the Phe specificity of the PS II-RC is maintained when Phe *a* is replaced by Phe *b* (Vavilin et al., 2003).

Only recently in the study of Chl ligation has the stereochemical aspect been recognized. Due to the dissymmetric arrangement of the substituents, the two 'faces' of the molecule are diasterotopic (see legend Fig. 1 for nomenclature). The two ligation states differ in their energies, with α-ligation (from *below,* α-type) being favored by ~4 kJ/mole over β-ligation (from *above*, β-type) (Oba and Tamiaki, 2002), and they are unevenly distributed in chlorophyll proteins (Balaban et al., 2002; Balaban, 2003). A functional significance is suggested by the preservation of geometries during evolution, but requires further exploration.

D. The Esterifying Alcohol

Of all the structural features of Chls, least is known about the functional significance of the phytol. The influence of the long-chain esterifying alcohol on ground and excited state properties of the tetrapyrrole is negligible. In mono-disperse solution it has, at most, a minor influence on absorption. It is noteworthy, however, that the alcohol moiety has a considerable affect on the aggregation of Chls, especially in micellar systems (Scheer et al., 1985; Agostiano et al., 2000, 2002a). This suggests a function of the long-chain alcohols in interactions with the environment including the apo-protein, other Chls, carotenoids and lipids. The alcohols appear to be important packing factors, to ensure the proper spacing and orientation among the pigments and other cofactors which govern energy and electron transfer. In particular, electron (and the related triplet energy) transfer can be affected by even minor variations of the pigment arrangement, due to changes in orbital overlap. A spacing function for the long-chain alcohol in positioning of the Chls is supported by all available X-ray structures: the esterifying alcohols are generally remarkably well resolved and engaged in interactions, especially among each other and with the carotenoids (Yeates et al., 1988; Deisenhofer and Michel, 1993; Schiffer and Norris, 1993; Tronrud and Matthews, 1993; Kühlbrandt et al., 1994; McDermott et al., 1995; Hofmann et al., 1996; Savage et al., 1996; Jordan et al., 2001; Spiedel et al., 2002; Ben-Shem et al., 2003; Biesiadka et al., 2004; Ferreira et al., 2004; Liu et al., 2004; Standfuss et al., 2005). Certain conformations of the respective alcohols are even conserved in an evolutionary sense over long periods; for example, from bacterial RC to plant PS II-RC (W. Zinth, personal communication).

While terpenoid alcohols function in many proteins as membrane anchors, there is relatively little direct interaction of the Chl esterifying alcohol with the membrane lipids. Thus, the alcohol may be a handle to Chls as is the phosphate group to carbohydrates which, due to their stereochemical complexity, also require precise positioning, for example, in enzymatic reactions. It should be noted in this context that, in *Rsp. rubrum,* the alcohol in the reaction center is different from that of the antenna (Walter et al., 1979), that hydrolysis of the alcohol is an early step in Chl degradation (Chapter 17, Kräutler and Hörtensteiner), and that the long-chain alcohol is retained even in water-soluble Chl proteins (Noguchi et al., 1999; Horigome et al., 2003; K. Schmidt et al., 2003; Reinbothe et al., 2004) including the light-harvesting peridinin-Chl protein (Hofmann et al., 1996) and the FMO complex (Tronrud and Matthews, 1993). It is surprising, therefore, that the alcohol is missing in most Chls *c* which are also membrane located, emphasizing the need for an X-ray structure of a Chl *c*-containing protein.

IV. Functions

A. Light Absorption and Energy Transfer

The first two functions are relevant for light harvesting. Antennas serve to increase the absorption cross-section of reaction centers by orders of magnitude, which is greatly assisted by the intense absorptions ($\varepsilon \approx 10^5 M^{-1}cm^{-1}$) of most Chls. Their individual ab-

sorptions are narrow, but the bands can be shifted by hundreds of cm^{-1} by interactions with protein, and a further spread of the useful absorption is realized by combination with other pigments including other Chls. In combinations, the Chls have strong absorptions over nearly the entire range of the photosynthetically useful spectrum (330 –1050 nm) with, however, the exception of the 'green gap' around 500 nm. Since this spectral range is particularly important in aquatic environments, where blue-green light is often prevalent, algae and photosynthetic bacteria have exploited this 'gap' not only by using *c*-type Chls but also with special carotenoids and biliproteins (Scheer, 2003). There is a cost, however, since excited carotenoids and bile pigments have only very short lifetimes which require considerable 'investments' in the form of binding proteins and biosynthetic enzymes. Plants, however, appear to have evolved a different strategy by investing more heavily in structure in the competition for light, both on a macroscopic (growth towards the light) and on a microscopic scale (leaf structure and internal reflection). For a recent comprehensive treatment of light-harvesting in photosynthetic organisms see Green and Parson (2003).

In the antennas, energy is frequently transported over tens of nm from the site of primary excitation to the reaction center where energy transduction takes place, and this transport is only efficient when conducted by *radiationless* transfer. Among Chls, Förster and exciton mechanisms are considered the dominant modes of transfer (Parson and Nagarajan, 2003), and both require long-lived excited states that are especially important in the vicinity of the reaction center where 'storage rings,' composed of Chls, are believed to hold the excited energy over relatively long (ns) periods.

That energy transfer can also be efficiently conducted by electron exchange (Dexter mechanism) (Parson and Nagarajan, 2003) has been proposed, in particular, for singlet energy transfer between carotenoids and Chls. An extremely fast transfer is necessary in these cases to compete with the very rapid internal conversion of most carotenoids (Section IV.C). The close contact between the donor (carotenoid) and acceptor (Chl) is clearly seen in all sufficiently-resolved structures of light-harvesting Chl-carotenoid-proteins (Freer et al., 1996; Koepke et al., 1996; Hofmann et al., 1996; Cline, 2003; Liu et al., 2004; Standfuss et al., 2005). Since electron exchange, like electron transfer, requires orbital overlap, it is expected to be critically dependent on very small geometrical changes (Section III.D). Electron exchange is also the major mechanism of triplet energy transfer which is especially important in protection against excessive light (Section IV.C).

B. Electron Transfer

Chlorophylls are indispensable for the primary charge separation in photosynthesis (Deisenhofer and Norris, 1993; Chapter 31,Wachtveitl and Zinth). The primary donor is a dimer (*special pair*) of (B)Chl which upon excitation donates within <3 ps an electron to the first acceptor, which also is a (B)Chl in all known RC. The secondary acceptor is also a (B)Chl in type I RCs (present in PSI of oxygenic phototrophs, green sulfur bacteria, heliobacteria), whereas it is replaced by a (B)Phe in type II RCs (present in PS II of oxygenic phototrophs, purple bacteria, green filamentous bacteria). In purple bacteria, the second transfer step is even faster (~1 ps) than the first step (Chapter 31, Wachtveitl and Zinth). The secondary acceptor is, in both types of RC, located halfway across the membrane. Electron transfer across the membrane is completed ~200 ps later when it is passed on to a quinone. All these first steps proceed on only one of the two (nearly) symmetrical branches of the RC. In reaction centers of the purple bacterium, *Rba. sphaeroides,* the other branch serves to protect the RC by transferring, and thereby quenching, triplet excitations to a carotenoid (Angerhofer et al., 1998; Arellano et al., 2004) (see below). For a comprehensive treatment of photosynthetic reaction centers see Deisenhofer and Norris (1993).

C. Protection of the Photosynthetic Apparatus Against Light-induced Damage

The long lifetimes of excited states of Chl are helpful for both energy transfer and efficient photochemistry in the reaction centers: both have to compete with radiationless de-excitation. In the proper structural context, this allows for efficient photosynthesis with minimal energy losses. On the other hand, the relatively long-lived excited states of Chls are potentially hazardous due to the concomitantly increased chance of triplet formation which, in turn, can generate toxic reactive oxygen species (ROS) (Chapters 32, 33, Brandis et al.). Carotenoids are the pigments most intimately involved in photoprotection and protect the photosynthetic apparatus by a variety of direct and indirect mechanisms against damage by excess

light (Frank et al., 1999). Carotenoid-less mutants are highly light-sensitive in all photosynthetic organisms and are unable to survive in the natural environment. Comparatively little is known about the protective role, if any, of Chls: Chl cation radicals, which can be generated in antennas under saturating light conditions, are good quenchers of Chl excited states (Law and Cogdell, 1998). Most chlorophyll aggregates are also excellent quenchers (Katz et al., 1978b; Scherz et al., 1991), with the notable exception of those of BChls *c*, *d* and *e* in the chlorosomes (Blankenship and Matsuura, 2003; Chapter 20, deBoer and deGroot). It has recently been proposed that a light-induced structural change may convert the latter also to a quenching state (Kakitani et al., 2003).

D. Structure Stabilization

As products of a co-evolution, all chromophores are integral components of the light-harvesting and energy transducing systems. As such, they are also integral to the stability of the systems. Chls show a remarkable potential for self-organization in both hydrophobic (Katz et al., 1978b; van Rossum et al., 2001) and hydrophilic media (Gottstein and Scheer, 1983; Katz et al., 1991; Scherz et al., 1991; Agostiano

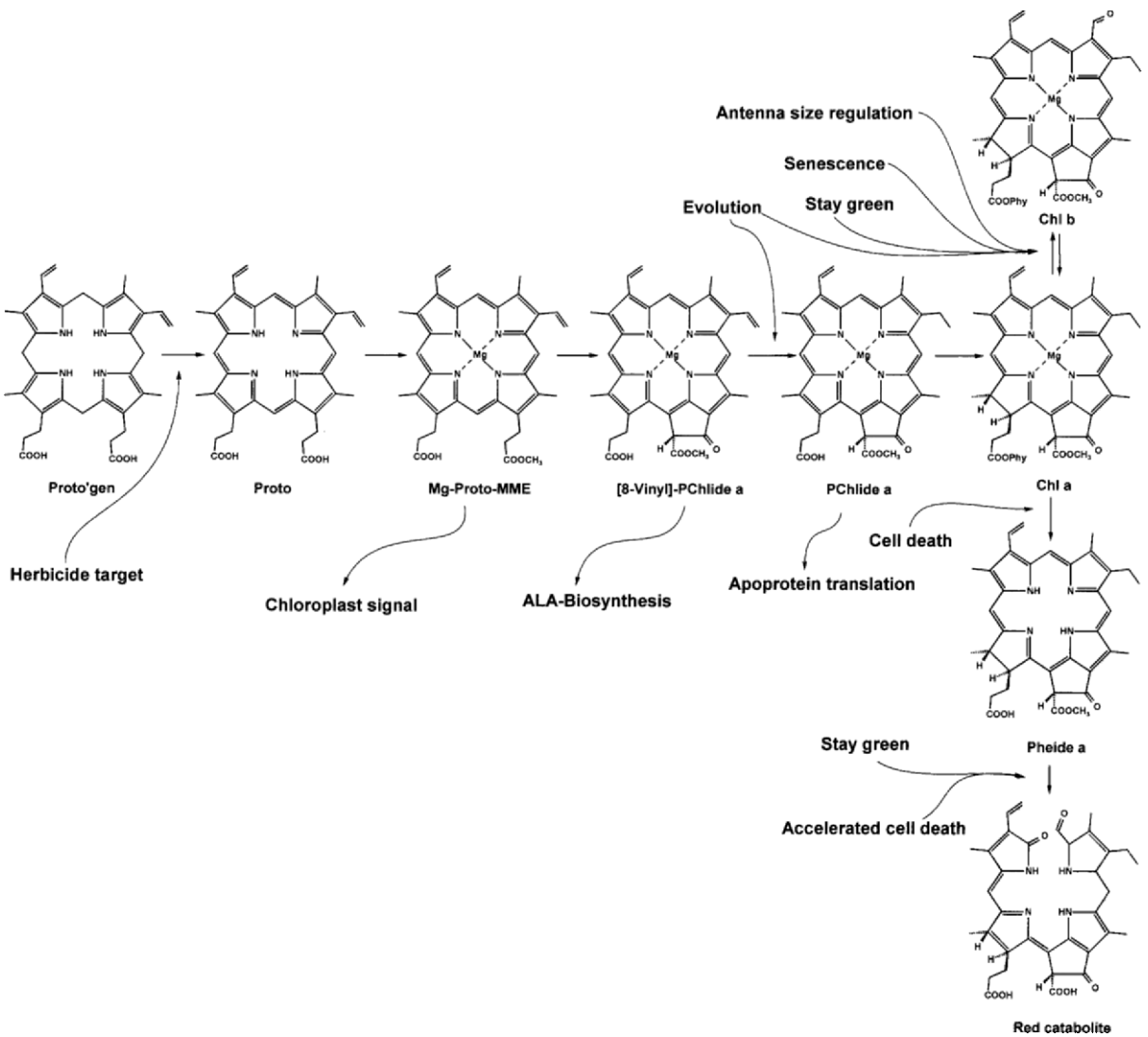


Fig. 4. Involvement of chlorophylls in physiological responses and evolution of photosynthetic organisms. This is a modified version of the scheme presented by A. Tanaka at the International Congress for Photosynthesis, Montréal, 2004).

et al., 2002). Before the identification of Chl proteins, speculation on the organization of light-harvesting complexes had focused on the self-aggregation of Chls as a driving force. This notion has been recently revived in two ways. Firstly, the presence of only very small amounts of protein in the chlorosomes of green bacteria (Chapter 6, Kobayashi et al.) has revived the idea of Chl-only aggregates in these antennas (Hirota et al., 1992; van Rossum et al., 2001; but see Niedermeier et al., 1992). Secondly, X-ray structures of light harvesting systems have shown that Chls are often so close to each other that Chl-Chl interactions can be expected (Tronrud and Matthews, 1993; Freer et al., 1996; Koepke et al., 1996; Jordan et al., 2001; Cline, 2003; Liu et al., 2004; Standfuss et al., 2005), with the pigments in geometric arrangements that are very similar to those found in Chl aggregates in mixed organic-aqueous media (Scherz et al., 1991). Strong interactions among the Chls have been confirmed especially in bacterial antennas (Sundström and Grondelle van, 1995; van Rossum et al., 1998; Leupold et al., 1999; Fiedor et al., 2000; Chapter 21, Köhler and Aartsma; Chapter 29, Leupold et al.). Assembly problems are frequently observed when amino acids binding the central Mg are modified, and, further, apo-protein complexes fail to assemble in the absence of Chls (Eichacker et al., 1996): this strongly suggests that Chls contribute to the formation and stability of light-harvesting pigment-protein complexes. As an example for a quantitative study, the thermodynamics of the influence of the central metal in the association of bacterial LH1 has been evaluated (Lapouge et al., 2000).

E. Other Functions

Chlorophyll metabolism is a central part of metabolism of all photosynthetic organisms and subject to many regulations. Vice versa, chlorophyll precursors are also involved in feedback control of their biosynthesis, in regulating the translation and import of apoproteins of pigment-protein complexes and in the interaction of the plastid with the nucleus in eukaryotic organisms (see Chapter 16, Beck and Grimm, and Fig. 4). Modifications of chlorophylls (Scheer and Hartwich, 1995; Chapter 2, Senge et al.; Chapter 4, Kobayashi et al.; Chapter 24, Noy et al.; Chapter 25, Nango; Chapter 26, Paulsen; Chapter 34, Yerushalmi et al.), Chl interactions with proteins (Chapter 19, Allen and Williams; Chapter 27, Garcia-Martin et al.) and Chl biosynthesis (see Section 'Metabolism' in this volume) have all been key factors in the evolution of photosynthetic organisms (Chapter 18, Larkum). They are primary targets to monitor and control plant health and productivity (Chapter 34, Yerushalmi et al.; Chapter 35, Nedbal and Koblizek; Chapter 36, Morel). Due to the stability of the tetrapyrrole, they also become increasingly important in the interpretation of the fossil record of photosynthesis (Chapter 37, Keely).

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