Cah. Biol. Mar. (2001) 42 : 109-124

Light harvesting in brown algae

Lise CARON[1], Dominique DOUADY, Alessandra DE MARTINO, Michelle QUINET
Ecole Normale Supérieure, C.N.R.S.-UMR 8543, Dynamique Des Membranes Végétales,
46 rue d'Ulm, 75230 Paris Cedex 05
Fax: 33 2 40 67 50 66 - E-mail: lcaron@nantes.inra.fr
[1]*Present address : INRA, URPOI, 1, rue de la Géraudière, B.P. 71627*
44316 Nantes cedex 03, France.

Abstract: The light harvesting complexes (LHC) of brown algae are embedded in plastid membranes. Besides chlorophyll a, these LHC bind chlorophyll c and fucoxanthin which are efficient for photosynthetic activity. The polypeptides are assembled in vivo into macromolecular complexes with molecular masses ranging from 120 to 700 kDa composed of two or several distinct components of 17-22 kDa. The chlorophyll c-fucoxanthin binding proteins are phylogenetically and structurally related to Chl*a*/*b*-LHC protein. Indeed, the protein contains three membrane-spanning helices and possesses the conserved residues identified in green plants as stabilizing the tertiary structures or binding Chl*a* molecules. However, the localization of Chl*c* and xanthophyll molecules is still unknown. Up to now, it is not clear if in the Chromophyta there are antennae transmitting the absorbed energy specifically to one or the other photosystems. The polypeptides are encoded by a nuclear multigene family but the total number of genes is not yet established in any species. Recently, the expression of Lhc genes has been shown to be regulated by light intensity and under the control of a blue receptor. As perspectives, the reconstitution of complexes in vitro could help to understand the binding of pigments to proteins. Cloning and characterization of the chlorophyll c fucoxanthin binding protein genes allow molecular biology approaches in the studies of the gene expression and also to develop a DNA transformation system for brown algae.

Résumé : Les complexes pigments-protéines qui assurent la collecte de l'énergie chez les algues brunes sont intramembranaires. Ils contiennent comme pigments collecteurs majeurs la chlorophylle a, la chlorophylle c et la fucoxanthine. Ces pigments sont associés, in vivo, à des proteines et constituent des complexes de haute masse moléculaire entre 120 et 700 kDa. Les protéines de ces complexes sont structuralement et phylogénétiquement apparentées aux complexes collecteurs fixant la chlorophylle a et b des plantes supérieures et des algues vertes. En effet, les acides aminés intervenant dans le maintien de la structure des hélices dans la membrane et la fixation des molécules de chlorophylle a sont conservés dans ces protéines. Cependant les sites de fixation des chlorophylles c et des fucoxanthines restent totalement inconnus. Les antennes collectrices proches du photosystème I ou du photosystème II sont indistinguables par leurs propriétés biochimiques ou fonctionnelles. Les protéines de ces complexes sont codées par une famille multigénique, localisée dans le génome nucléaire, et dont le nombre de représentants est encore inconnu. Les gènes sont regulés par l'intensité lumineuse et sont probablement sous le contrôle d'un photorecepteur à la lumière bleue. Plusieurs champs d'investigation semblent prometteurs dans les années à venir concernant d'une part la structure des complexes à fucoxanthine grâce à la reconstitution in vitro, d'autre part l'expression de gènes nucléaires codant pour des protéines chloroplastiques et la transformation génétique des algues brunes.

General introduction

Most of the living organisms obtain the energy to achieve their cellular metabolism directly or indirectly from photosynthesis, a process which enables plants, algae and several bacteria to chemically fix the energy from the solar light. The general principles of photosynthesis are conserved in most of the photosynthetic organisms. Sunlight is absorbed by a light-harvesting or antenna pigment which is a tertrapyrrol or a carotenoid. Absorption of the light brings the pigment in an excited state and this excitation is transferred to other pigments and eventually arrives on a pigment belonging to the reaction centre complex. This process is called light-harvesting. In the reaction centre, the excitation drives charge separation: an electron is ejected from a special chlorophyll (Chl) molecule which supplies a whole set of electron transfers, finally generating chemically fixed free energy in adenosine triphosphate (ATP) and reducing power nicotinamide adenine dinucleotide phosphate (NADPH), then used by the organism in response to the needs of the cells (Fig. 1A).

The most advanced form of photosynthesis is performed by green plants, algae and cyanobacteria. In these organisms the electron needed for photosynthesis electron transfer is abstracted from water and produces oxygen, and a complex machinery is involved in the different events of the photosynthesis. The electron transfer occurs via two serially-functioning photosystems. Each of these photosystem I and II complexes contains the reaction centres and their tightly-bound core antennae.

All the pigments involved in photosynthesis are bound to proteins and constitute pigment-protein complexes folded in a conformation which insures high efficiency of energy transfers. The light-harvesting is essentially performed by large pigment-protein complexes, devoid of reaction centres and named light-harvesting complexes (LHC). They bound the major part of the chloroplast pigments and are able to transfer the energy collected by their pigments to the reaction centres (Fig. 1B).

The LHCs are well diversified in photosynthetic organisms. In red algae, cryptophyceae, cyanobacteria, they essentially contain as collecting pigments Chl*a* and phycobilins and are assembled in big particles (phycobilisomes) protruding outside the photosynthetic membrane, whereas in plants and other algae these complexes bind, besides Chls, carotenoids (lutein, fucoxanthin, violaxanthin...) and are embedded in the membranes (Table 1).

This diversity of pigments contributes to the large ability of the algae to survive under the sea. In marine ecosystem, the light is provided through layers of water which act as filters. So, the light is dramatically attenuated after the first metres under the surface: the water molecules cut off the red

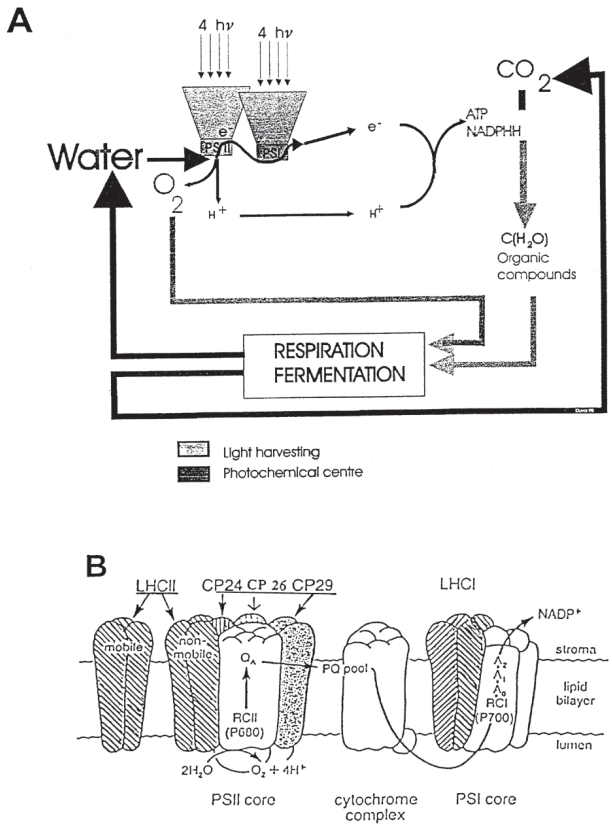


Figure 1. A: Photosynthesis in water, carbon and oxygen cycles.

PSI, PSII =photosystems I and II, hν = photon.

B: Functional organization of pigment-protein complexes involved in photosynthetic electron tranfer in higher plant chloroplasts.

The complexes are embedded in the thylakoid membranes which form closed bag-like structures and delimite two partitions: the external stroma and the inside lumen. The incident light is absorbed by light-harvesting complexes: LHCI, LHCII and minor complexes: CP24, CP26, CP29. Then, the energy is transmitted to reaction centre (RC) P680 in Photosystem II (PSII) and P700 in Photosystem I (PSI). In the RCs a charge separation occurs, which drives a set of electron transfer and induces a proton gradient across the thylakoid membranes leading to the reduction of NADP+ and the formation of ATP.

Figure 1. A. La photosynthèse dans les cycles de l'eau, du carbone et de l'oxygène.

PSI, PSII = photosystèmes I et II, hν = photon.

B : Organisation fonctionelle des complexes pigments-protéines impliqués dans le transfert des électrons dans les chloroplastes des plantes supérieures.

Les complexes sont intégrés dans les membranes des thylakoides qui forment des structures en forme de sac et délimitent deux compartiments : le stroma externe et le lumen interne. La lumière incidente est absorbée par des complexes collecteurs: LHCI, LHCII et des complexes mineurs : CP24, CP26, CP29. L'énergie est ensuite transmise à des centres réactionnels (RC) P680 dans le Photosystème II (PSII) et P700 dans le Photosystème I (PSI). C'est dans les RCs que s'opèrent les séparations de charge qui amorcent une chaîne de transfert d'électrons et induisent un gradient de protons à travers les membranes des thylakoides, conduisant à la réduction du NADP+ et à la formation d'ATP.

Table 1. Light-harvesting complexes of Algae. Chl, chlorophyll; MgDVP, divinylprotochlorophyllide; PE, phycoerythrin; PC, phycocyanin; APC, allophycocyanin; vio, violaxanthin; zea, zeaxanthin; dia, diadinoxanthin; din, dinoxanthin; vau, vaucheriaxanthin; het, heteroxanthin; neo, neoxanthin; fuc, fucoxanthin; *: carotenoid involved in xanthophyll-cycle.

Tableau 1. Les complexes de collecte de l'énergie lumineuse ches les algues. Chl, chlorophylle ; MgDVP, divinylprotochlorophyllide ; PE, phycoérythrine ; PC, phycocyanine ; APC, allophycocyanine ; vio, violaxanthine ; zea, zéaxanthine ; dia, diadinoxanthine ; din, dinoxanthine ; vau, vauchériaxanthine ; het, hétéroxanthine ; neo, néoxanthine ; fuc, fucoxanthine ; *: caroténoide impliqué dans le cycle des xanthophylles.

Algal class	LHC localization	Chls	Carotenoids Phycobilins	most studied genera	Polypeptides MW (kDa)	References
Cyanophyta	Extrinsic	a	PE,PC,APC			(Grossman et al., 1993)
Rhodophyta	Extrinsic	a	PE,PC,APC	*Phorphyridium*	18-24	(Wolfe et al., 1994)
	Intrinsic	a	lut,neo,zea			
Cryptophyta	Extrinsic	a,c2	PE,PC	*Chroomonas*	20,24	(Bhaya & Grossman, 1993)
	Intrinsic	a,c2	alloxanthin	*Cryptomonas*	18-22	(Boekema et al., 1995)
Dinophyta	Extrinsic	a	peridinin	*Amphidinium*	19-24	(Hiller et al., 1993)
	Intrinsic	a,c2	peridinin	*Gonyaulax*	15-17,35	(Jovine et al., 1995)
				Symbodinium		(Iglesias-Prieto et al., 1993)
Chromophyta	Intrinsic	a,c				
Raphidophyceae		a,c1,c2	fuco,dia,din	*Heterosigma*	16-28	(Durnford & Green, 1994)
Prymnesiophyta		a,c1,c2	fuco	*Pavlova*	17-21	(Hiller et al., 1988)
				Isochrysis	18,20,24	(La Roche et al., 1995)
Chrysophyceae		a,c1+c2	fuco,vio*	*Gyraudyopsis*	20	(Passaquet & Lichtlé, 1995)
				Ochromonas	21,26	(Grevby & Sundqvist, 1992)
Fucophyceae		a,c1,c2	fuco,vio*	*Fucus, Dictyota*	17-21	(Caron et al., 1988)
				Laminaria		(Passaquet et al., 1991)
				Macrocystis		(Apt et al., 1995)
Bacillariophyceae		a,c1,c2	fuco,dia*	*Phaeodactylum*	17-20	(Grossman et al., 1990)
				Odontella		(Kroth-Pancic, 1995)
Xanthophyceae		a,c1,c2	dia,vau,het	*Pleurochloris*	17-22	(Büchel & Wilhelm, 1993)
Euglenophyta	Intrinsic	a,b	dia,din,neo	*Euglena*	26-28	(Cunningham & Schiff, 1986)
Chlorophyta		a,b	lut,neo,zea*	*Chlamydomonas*	20-30	(Bassi et al., 1990)
				Dunaliella		
Prasinophyceae		a,b,MgDVP	prasinoxanthin	*Mantionella*	20-25	(Rhiel et al., 1993)
Higher plants		a,b	lut,neo,zea*		22-29	review (Jansson, 1994)

radiations and, in a lesser extend, the UV. Depending on the turbidity of the water, the light environment becomes dim and green with the depth (Fig. 2). Phycobilisome and fucoxanthin-containing algae are well equipped in photosynthetic pigments to absorb the blue-green range of the undersea light. It is likely that these specificities in pigment composition result from an adaptation to light quality in marine ecosystems where radiations in the green region of the spectrum are predominant.

Water-soluble complexes are well understood and especially the structure and the biosynthesis of phycobilisomes. Concerning the membrane complexes, the subcomplex organization of LHC green plant chloroplasts has been intensively studied. Up to ten types of LHC proteins, coded by a multigene family, have been recognized, some of them are specifically bound to photosystem II (LHCII, CP29, CP24, CP26) and other to photosystem I (LHCI) (for review see, Jansson, 1994). For one of these subcomplexes, the LHCIIb of *Pisum sativum*, the conformation of the protein and the spatial distribution of pigment molecules have been determined by means of crystallography (Kühlbrandt et al., 1994). Recently, the obtention of reconstituted complexes mutated at the pigment-binding sites in the proteins has allowed to precise the location of more pigments in the complexes (Pesaresi et al., 1997). We begin to understand the intricacy of the mechanisms which regulate the biosynthesis of the different LHC complexes (Argüello-Astorga & Herrera-Estrella,

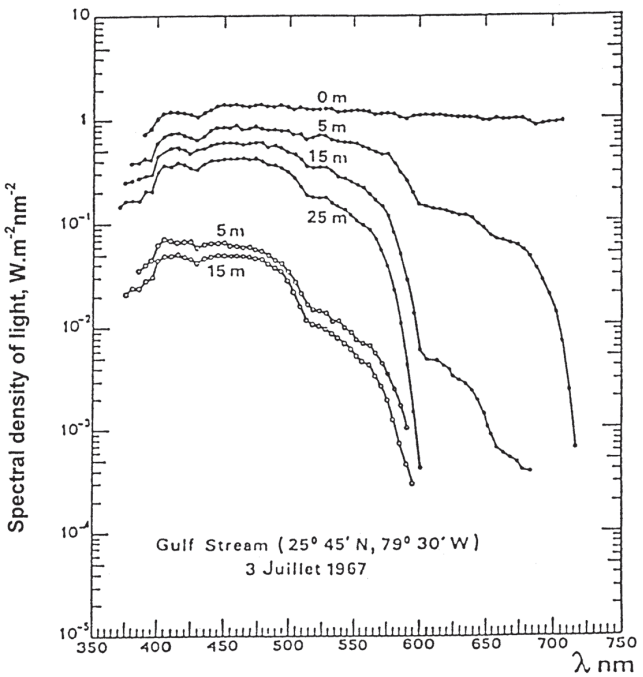


Figure 2. Spectral repartition of the undersea light according to the depth.

The light intensity around 480 nm is reduced by half at 15 metres depth whereas the radiations beyond 600 nm are largely reduced from the first 5 metres. Open circles: descending flux, closed circles: ascending flux (from Ivanoff, 1975).

Figure 2. Répartition spectrale de la lumière en fonction de la profondeur.

L'intensité lumineuse à environ 480 nm est réduite de moitié à 15 mètres, alors que les radiations au delà de 600 nm sont très fortement réduites dès 5 mètres de profondeur. Cercles ouverts : Flux descendant, cercles pleins : flux ascendant (d'après Ivanoff, 1975).

1998; Thompson & White, 1991). Other functions are now also attributed to LHC, in particular a photoprotective role exercised by means of a non-radiative dissipation of light energy. This latter function is correlated with the so-called xanthophyll cycle, i.e. de-epoxidation of violaxanthin in zeaxanthin, and vice-versa. This has been observed in terrestrial green plants as well as in green and brown algae (Demmig-Adams & Adams, 1996).

Our knowledge concerning the other LHCs is less extensive probably because less research efforts have been concentrated on these complexes, but also because of the difficulties to apply the protocoles obtained for the higher plants, in biochemical studies of the membranar complexes of algae, as well as in using molecular biology tools. We will focus the first part of this review on the collection of the light in the most abundant primary producers in marine shore environment: the brown algae.

Fucoxanthin-Chl*c* complexes are embedded in the thylakoid membrane and thus the use of detergent is neccessary to isolate and purify such complexes. The native state of the whole complex and the distribution of pigments are difficult to determine. Different light-harvesting-pigment-protein complexes have been isolated, the use of nonionic detergents has been successful to maintain energy transfer in isolated fractions and thus to obtain complexes in a conformation more closely related to the in vivo state.

Biochemistry of fucoxanthin-binding complexes

In brown algae, by contrast with the green organisms, a pure LHC fraction, entirely devoid of reaction centres, and containing only polypeptides in the 20 kDa range, can be prepared from these organisms by a one-step detergent treatment. It can be considered as the main light-harvesting fraction, as is the so-called LHCIIb in green plants. It has a high content in Chl*c*, fucoxanthin and violaxanthin (LHCF). This LHCF fraction cannot directly be compared to the green plant LHCII because of structural and functional differences. Especially, due to the absence of grana in brown algae, the thylakoids are stacked by three and the LHCF is randomly located all along the thylakoid membranes (Fig. 3) (Berkaloff et al., 1983; Gibbs, 1970). It is currently admitted that fucoxanthin is able to transmit the collected energy equally and efficiently to PSI and PSII centres (Owens, 1986; Smith & Melis, 1987). Up to now, no LHCI and LHCII can be distinghuished by their biochemical and biophysical properties (Schmitt et al., 1993).

Pigments collecting the light

The light is collected in these algae by Chl*a*, Chl*c*1, Chl*c*2 and fucoxanthin as main xanthophyll. Chl*c*1 and 2 molecules are fully unsaturated porphyrin macrocycles without the phytol-C20 chain present in Chl*a* and *b*. The two Chl*c* differ by a single residue on the macrocycle (Fig. 4A), but the absorption properties of both molecules are almost identical. The increase of symmetry in the Chl*c* molecule relative to Chl*a* confers different absorption properties. The most obvious change between Chl*c* and *a* concerns the intensity of the absorption band in the red range, which is greatly reduced for Chl*c* (Fig. 4B) while, in the blue range, the spectrum is shifted toward the longer wavelenghts increasing the absorption around 460nm in vivo.

Fucoxanthin is an allenic xanthophyll (Fig. 4A) which is completly absent in green plants. The fucoxanthin molecule shows a large redshift towards 500-550 nm in their absorption spectra when bound to protein (Fig. 4C). Up to now, this shift is unexplained but it is very useful to test if the complexes have not been disturbed by isolation

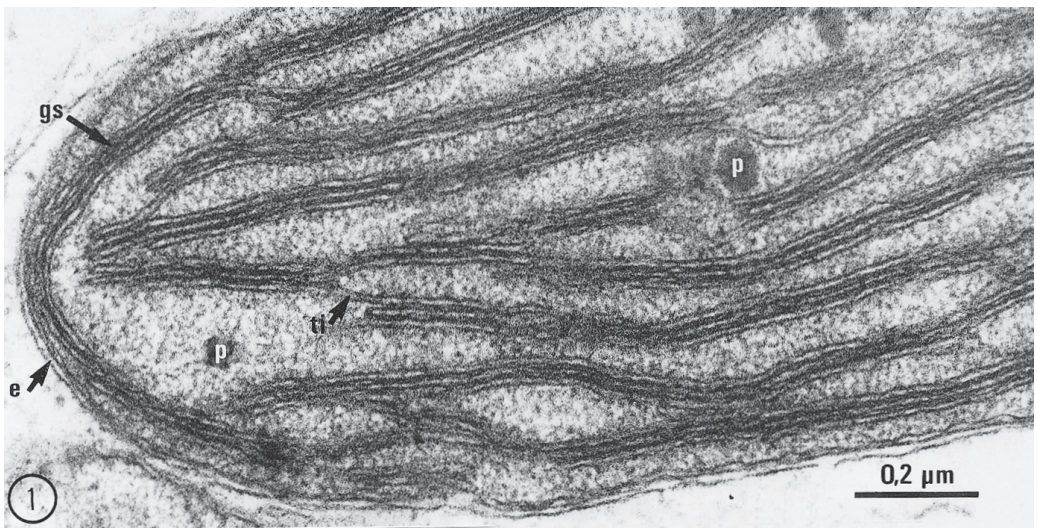


Figure 3. Ultrastructure of brown algal chloroplasts. Transmission electron micrograph of a *Fucus serratus* chloroplast. The thylakoids (ti) are arranged by three; (e) plastid envelope; (p) plastoglobuli; (gs) girdle stack surrounding a large part of the chloroplast (from Berkaloff et al., 1983).

Figure 3. Ultrastructure du chloroplaste des algues brunes. Micrographie de microscopie électronique à transmission d'un chloroplaste de *Fucus serratus*. Les thylakoïdes (ti) sont arrangés par trois; (e) enveloppe plastidiale; (p) globule plastidial; (gs), un groupe de trois thylakoïdes entoure une grande partie du chloroplaste (d'après Berkaloff et al., 1983).

procedures. The excitation fluorescence spectra indicates that fucoxanthin transfers very efficiently the absorbed light to Chl*a*. In the diatom *Phaeodactylum tricornutum*, carotenoid-to-Chl*a* transfers occur in the range 0.5-2.5 ps (Trautman et al., 1990). The mechanisms of energy transfer is unclear, but for efficient excitation transfer from carotenoids to Chl, the distance between the molecules has to be small, at least several nanometers.

The composition of LHCF from different species are presented in Table 2. The pigment stochiometry of brown algae is rather variable. For example the Chl*a*/*c* and fucoxanthin/Chl*a* ratios are much higher in *Laminaria saccharina* than *in Pelvetia canaliculata*. This could be related to the environmental conditions of the seaweeds. *L. saccharina* is living at low intertidal levels, where the light intensity is low, a positive adaptation could be an increase of the size of antennae. Furthermore these ratios can vary noticeably within one species according to the environmental conditions (Harker et al., 1999). The carotenoids are bound in much higher concentrations per Chl*a* in fucoxanthin-binding complexes than it is reported for lutein-Chl*b* proteins of green plants. Whereas LHCIIb monomer of green plants contains 2 or 3 molecules of xanthophylls for 12-13 Chl and per monomer, Chl and xanthophylls are almost in equal amounts in brown alga LHC (Berkaloff et al., 1990; De Martino et al., 1997; Douady et al., 1994; Katoh et al., 1989; Pascal et al., 1998; Passaquet et al., 1991; Wilhelm, 1990).

Violaxanthin and zeaxanthin are not always present in isolated LHCF, and it is not clear if they are integral components of the complexes or if they are located at the periphery, and are released by the detergent. We will discuss below their role in photoprotection processes.

Polypeptides of fucoxanthin-Chlc complexes

The apoprotein in the functional complex is responsible for a good orientation of the pigments to insure the energy transfer from carotenoids to the last acceptor Chl*a*. Thus it was assumed that the polypeptides were strictly specific of the bound pigments. And indeed, the biochemical characterization of the polypeptides of the isolated LHC's showed great differences.

The apparent molecular weights of the apoproteins of fucoxanthin-Chl*c* containing LHC are smaller (17-25 kDa) compared to the green plant LHC complexes (25-29kDa) (Wilhelm, 1990), (Table 2). Moreover, the immunological studies were conflicting depending on the specifity of the antibodies and the numbers of epitopes. Authors using monoclonal antibody against diatom LHC polypeptides claimed no relationships between Chromophytes (Chl*c*-containing algae) and the green plants (Friedman & Alberte, 1987). Others, with polyclonal antibodies found cross-reactivities between Chl*a*/*c* proteins and Chl*a*/*b* ones suggesting sequence similarities (Caron & Brown, 1987; Fawley et al., 1987; Hiller et al., 1988). The

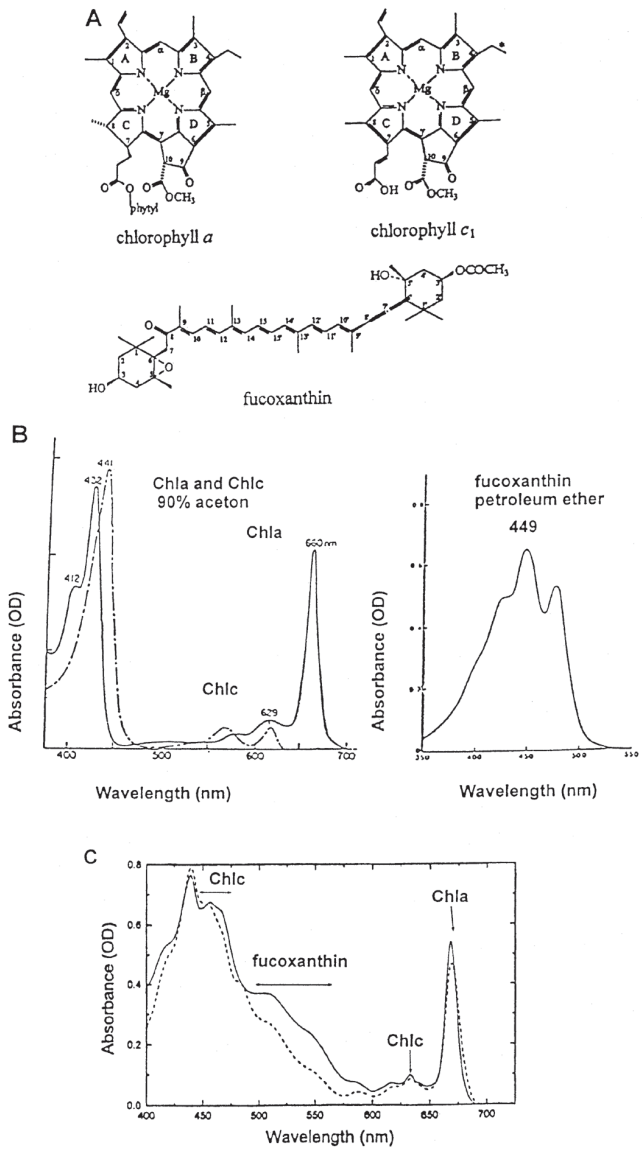


Figure 4. Light-harvesting pigments in brown algae.

A: Molecular structure of Chl*a*, Chl*c*1 and fucoxanthin. Chl*c*2 contains a carbon-carbon double bound at the position marked by an asterisk.

B: Absorption spectra of Chl *a* and *c* in 90% aceton and fucoxanthin in petroleum ether.

C: Absorption spectra of light-harvesting complexes isolated from *Laminaria saccharina* at room temperature.

Figure 4. Pigments collecteurs de lumière chez les algues brunes.

A : Structure chimique des Chl*a*, Chl*c*1 et fucoxanthine. La Chl*c*2 contient une double liaison carbone-carbone à la position marquée par un astérisque.

B : Spectre d'absorption des Chl *a* et *c* dans l'acétone à 90 % et de la fucoxanthine dans l'éther de pétrole.

C : Spectre d'absorption à température ambiante des complexes collecteurs de lumière isolés de *Laminaria saccharina*.

Table 2. Pigment composition of LHC from brown algae obtained by HPLC. The results are expressed in molar percentages relative to Chl*a* (Passaquet et al., 1991).

Tableau 2. Composition en pigments des LHCs des algues brunes déterminée par HPLC. Les résultats sont exprimés en pourcentage molaire par rapport à la molarité en Chl*a* (Passaquet et al., 1991).

	Fucus serratus	*Pelvetia canaliculata*	*Laminaria saccharina*	*Dictyota dichotoma*	*Pylaiella littoralis*
Chlorophyll a	100	100	100±0	100	100
Chlorophyll c	18±1	8	30±4	30±10	30
β-Carotene	4±1	3	2±1	4±01	2
Fucoxanthin	77±3	61	76±7	107±12	85
Violaxanthin	17±2	30	10±1	10±02	6

first complete gene sequences, obtained from the diatom *Phaeodactylum* (Grossman et al., 1990) showed that the fucoxanthin LHC were related to the lutein-LHCs and the authors assumed that all the Chl*a*-binding proteins share common structural features especially in Chl*a*-binding sites.

Then, several LHC sequences from Chromophytes have been published (Apt et al., 1995; Douady et al., 1994; Hiller et al., 1993; Kroth-Pancic, 1995; Laroche et al., 1994; Caron et al., 1996; Passaquet & Lichtlé, 1995) which allow to draw some structural conclusions for the fucoxanthin-Chl*c*-binding proteins in relation to the Chl*a*/*b* binding proteins and to the three-dimensional structure of pea LHCII obtained by crystallography (Kühlbrandt et al., 1994).

Hydropathy plots of all the Chl*a*-binding proteins predict three-membrane helices (TMHs) which are confirmed by the 3D analysis of LHCIIb crystals from *Pisum sativum* (Kühlbrandt et al., 1994). The sequence alignment of fucoxanthin-binding proteins and LHCII of green plants indicates that all the proteins share two highly conserved regions comprising the first and the third TMHs (Fig. 5). These two helices share together considerable sequence similarity. The main observation is that the four amino acids (indicated by dots in Fig. 5) that form the ion pairing between helices 1 and 3 in pea LHCIIb structure are conserved in Chl*a*/*c*-binding proteins, this strongly suggests that the structure and the topology in the membrane of Chl*a*/*c* protein are very similar. The connecting loops between TMHs and the second helix show much lower conservation (Fig. 5).

Pigment-binding sites

The proteins interact with Chls in different ways. One or two ligands from the protein can bind to the central magnesium atom of the Chls. The proteins can also bind the

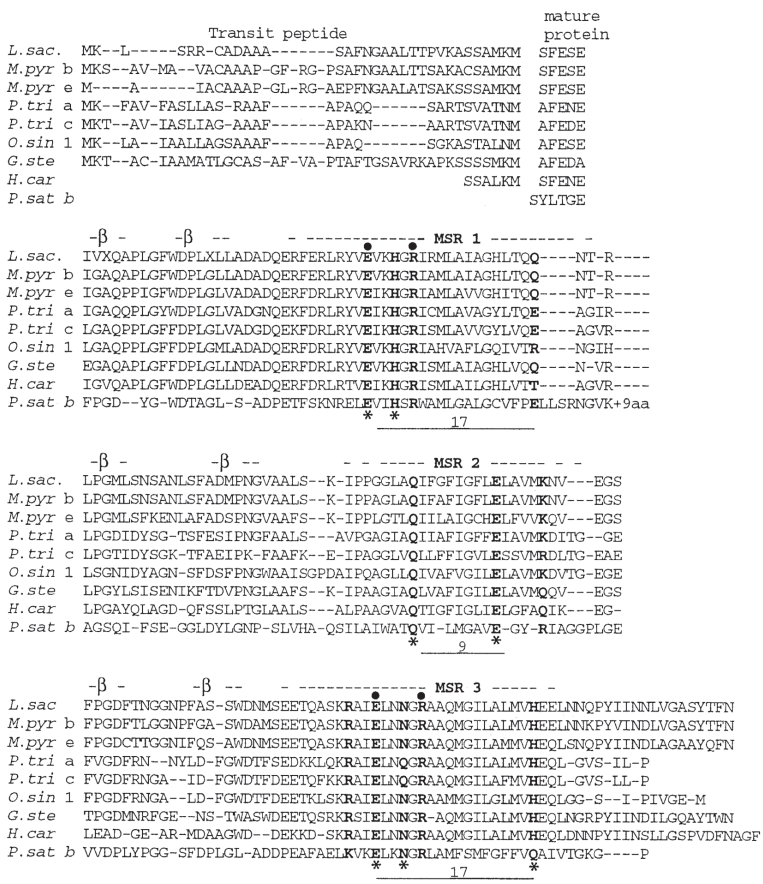


Figure 5 Alignment of the *L. saccharina* LHC amino acid sequence with several Chl*a*/*c* protein sequences from other heterokont algae. The membrane-spanning regions (MSR) correspond to the parts of the protein which span the membrane and thus include the transmembrane helices. The ß-turns upstream the second MSR are more conserved in these sequences than in the LHC proteins of Chl*a*/*b* land plants. *L. sac*.: *Laminaria saccharina,* Fucophyceae (Caron et al., 1996); *M. pyr* (b and e): *Macrocystis pyrifera,* Fucophyceae (MFcpB and MFcpE (Apt et al., 1995); *P. tri.* (a and c): *Phaeodactylum tricornutum,* Bacillariophyceae (Fcp A and Fcp C (Bhaya & Grossman, 1993); *O. sin. Odontella sinensis,* Bacillariophyceae (Kroth-Pancic, 1995); *G. ste* = *Giraudyopsis stellifer* (Passaquet & Lichtlé, 1995); *H. car*.: *Heterosigma carterae,* Raphidophyceae (Durnford & Green, 1994); *P. sat*. b: *Pisum sativum*, Lhcb2 (Kühlbrandt et al., 1994). Dots: residues involved in ion pairing. Stars: putative ligands to Chl molecules. The number of residues between E-E, Q-E and E-H pairs in the 3 helices are indicated above the double arrows.

Figure 5 Alignement multiple des séquences en acides aminés des LHC de *L. saccharina* avec les séquences de protéines de liaison Chl*a*/*c* d'autres algues hétérokontes. Les domaines transmembranaires (MSR) correspondent à des parties de la protéine qui comprennent les hélices transmembranaires. Les feuillets ß-turns en amont du second MSR sont mieux conservés dans ces séquences que dans les protéines de liaison Chl*a*/*b* des LHC des plantes supérieures. *L. sac.* : *Laminaria saccharina,* Fucophyceae (Caron et al., 1996) ; *M. pyr* (b and e) : *Macrocystis pyrifera,* Fucophyceae (MFcpB et MFcpE (Apt et al., 1995); *P. tri.* (a et c) : *Phaeodactylum tricornutum,* Bacillariophyceae (Fcp A et Fcp C (Bhaya & Grossman, 1993) ; *O. sin. Odontella sinensis,* Bacillariophyceae (Kroth-Pancic, 1995) ; *G. ste* = *Giraudyopsis stellifer* (Passaquet & Lichtlé, 1995); *H. car.* : *Heterosigma carterae,* Raphidophyceae (Durnford & Green, 1994) ; *P. sat*. b : *Pisum sativum*, Lhcb2 (Kühlbrandt et al., 1994). Points : résidus impliqués dans les paires d'ions. Etoile : ligands putatifs des molécules de Chl. Le nombre de résidus entre les paires E-E, Q-E et E-H des 3 hélices sont indiqués au-dessus des traits fléchés.

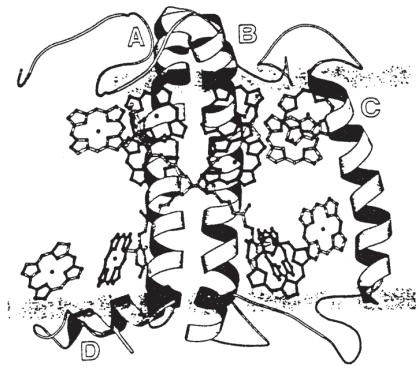
Chls by hydrogen bonds with carbonyl oxygens, in particular the 9-C=O which is implicated in delocalized π-electron on the porphyrin ring.

Eight putative Chl-binding amino acids ligands have been located in the three TMHs and the helix 4 in the LHCII model of Kühlbrandt et al. (1994); some of these ligands have been confirmed in the CP29 Chl*a*/*b* protein by using mutants (Sandonà et al., 1998). Alignments show that seven of the 8 amino acids suspected to bind Chl (indicated by stars in Fig. 5) are well conserved in all Chl*a*/*c* binding proteins and, presumably, they would bind Chl*a* or *c*. More accurately, the 4 Chls bound to the amino acids implicated in ion pairing of helices 1 and 3 are Chl*a* (Fig. 6).

If the binding sites of the Chls seem very common to the Chl*a*/*c* and Chl*a*/*b* proteins, by contrast the location of the xanthophylls are still unknown.

In green plant LHCII complex, biochemical analyses indicated two luteins, 1 neoxanthin per monomer plus violaxanthin in variable substoichiometric amounts (Ruban et al., 1994) and in the crystallized pea LHCIIb, two carotenoid molecules have been assigned in the centre of the monomer (Kühlbrandt et al., 1994). A number of sequence motifs, highly conserved in Chl*a*/*b* proteins and found in the loop regions where they shield the Chls and the xanthophyll head-groups from the water enviromnent, have been assumed to provide xanthophyll-binding sites. These sites are only partially conserved in LHCF; they correspond to the β-β turns followed by the TMH (Fig. 5). In the minor light-harvesting complexes bound to LHCII reconstituted in vitro with mutated proteins and pigments, the presence of an amino acid in the third helix of the protein is neccessary to bind violaxanthin (Giuffra et al., 1996), this aminoacid is also always present in the third helix of the LHCF.

In brown algae, isolated functional monomeric LHC subunit contains 8 fucoxanthin, 4 Chl*a* and 2 Chl*c*1/c2 per monomer. Analysis of this isolated complex by Raman spectroscopy indicated the presence of a few molecules of fucoxanthin (estimated at 2 of 8) in a twisted conformation resulting from a torsion around a carbon-carbon single bound (Pascal et al., 1998). That could indicate interactions between the protein and some fucoxanthin molecules



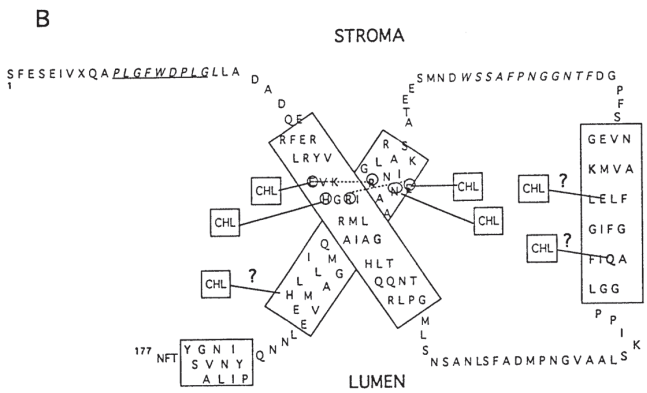


Figure 6. Molecular structure of Chl*a*/*b* and Chl*a*/*c* LHC.

A: Structure of pea LHCII determined by electron microscopy. α-helices are drawn as ribbons. Chl molecules are represented as porphyrin rings. Helices B, C, A and D are the first, second, third and fourth helices (from Kühlbrandt, 1994).

B: hypothetical model for the three-dimensional structure of fucoxanthin-Chl*a*/*c* protein according to the LHCII struture. 4 Chl ligands are well-conserved, 3 others are hypothetical. The binding of at least 8 fucoxanthin molecules on a monomer remains unknown.

Figure 6. Structure moléculaire des complexes LHC Chl*a*/*b* et Chl*a*/*c*.

A : Structure du LHCII de pois déterminée par microscopie électronique. Les hélices α sont représentées en rubans. Les molécules de Chl sont représentées sous forme d'anneaux porphyriques. Les hélices B, C, A et D sont respectivement la première, seconde, troisième et quatrièmes hélices (d'après Kühlbrandt, 1994).

B : Modèle hypothétique de la structure tri-dimensionnelle d'un complexe fucoxanthine-Chl*a*/*c* protéine à partir du modèle de la structure du LHCII. Quatre acides aminés liés à des Chl sont bien conservés, 3 autres sont hypothétiques. Les liaisons d'au moins huit molécules de fucoxanthine sur le monomère demeurent indéterminées.

throughout the whole length of the carbon backbone of the xanthophyll as it has been showed for two lutein molecules involved in the stabilization of the LHCII structure. Nevertheless, resonnance Raman spectra do not indicate such twisting for lutein molecules in Chl*a*/*b* complexes. Thus, the sequence alignments and biophysical data give some arguments to locate at least two molecules of fucoxanthin in the central part of the complex, but where are the other fucoxanthins? In an oligomeric form of LHCF, an highly efficient and fast energy transfer (near 100% in 3 ps) was observed from fucoxanthin to Chl*a* molecules (Mimuro & Katoh, 1991); thus each of the 8 molecules of fucoxanthin by monomer are neccessary close to a Chl*a* or a Chl*c* molecule or they can funnel the excitation on one of them which transmits the excitation to one Chl, in this case only one fucoxanthin has to be very close to one Chl.

Furthermore, as in green plant the oligomeric LHC complex of brown algae contains violaxanthin (Wilhelm, 1990; De Martino et al., 1997; Passaquet et al., 1991). Where is violaxanthin located into fucoxanthin-containing complexes? This pigment is associated with LHC fractions during the first isolation step. When more drastic procedures are applied, it is no more part of functional complexes, but is found in fractions which present no more energy transfer between fucoxanthin and Chl*a*. Thus it is not clear if violaxanthin is intrinsic to a complex and is artefactly released by isolation procedures or if it is sticked at the periphery of the complexes (De Martino et al., 1997).

Phylogenetic implications

The sequence similarity relating the first and the third TMH supported the suggestion that the occurrence of these two helices were the result of the duplication of an ancient gene during the evolution (Hoffman et al., 1987). Recently in two species of cyanobacteria and also in chloroplast genome of red algae, a gene has been discovered, encoding a small proteins with one membrane-spanning region which is related to the first and the third helices of LHC (Dolganov et al., 1995). These one-helix proteins have conserved amino acids involved in Chl binding and the ion pairing and thus could form dimers (Green & Kühlbrandt, 1995). These proteins provide a common link between algal antennae and suggest that a *Lhc*-type gene was present in the first photosynthetic eukaryote.

To be more specific about the evolution relationships between Chl*a*/*b* and Chl*a*/*c* LHC phylogenetic trees based on the amino acid sequences, have been built using parsimony or distance methods (Caron et al., 1996; Durnford et al., 1996). They showed, as expected, that the genes encoding the LHCF from heterokont algae (Chl*c* - containing algae) and dinophyta derive from an ancestral gene which also gave rise to the *Lhc* genes of Chl*a*/*b* plants.

Heterogeneity of the fucoxanthin-Chl*c* -proteins.

Structural heterogeneity has been already observed in bulk fractions of brown algae. Polypeptide analysis, by SDS-polyacrylamide gel electrophoresis (PAGE) showed that the antenna system of one species contain up to ten related polypeptides with apparent molecular weight of 17-23 kDa (Caron et al., 1988; Durnford & Green, 1994; Passaquet et al., 1991).

These proteins have similar size and many comigrate in polyacrylamide gels: in *Laminaria saccharina*, two peptidic populations differing by their hydrophobicity have been detected by reverse phase FPLC, although only one peptidic band was detected on SDS-PAGE (Douady et al., 1994). In other cases, LHC subfractions with very similar peptidic components may present more simple pigment composition with Chl*a*, Chl*c* and violaxanthin only (Barrett & Anderson, 1980) or with Chl*a*, Chl*c* and fucoxanthin only (De Martino et al., 1997).

Recently, the presence of several genes, with high sequence homology, have been shown in different species of fucoxanthin-containing algae (Apt et al., 1995; Bhaya & Grossman, 1993; Caron et al., 1996; Durnford et al., 1996; Kroth-Pancic, 1995; de Martino et al., 2000). Clearly, LHCF constitute a family of abundant proteins but the exact number of different LHCF in the thylakoid membranes is unknown.

After the steps of separation and purification to obtain the isolated complexes even with the low-denaturing flat-bed isoelectric focusing (IEF) (De Martino et al., 1997), the use of detergent induces significant modification of the complexes such as loss of pigment and state of oligomerization. Finally, it is difficult to make sure of the in vivo structure of the complexes. But it has been shown, using cryofracture and electronic microscopy that the LHCF complexes in brown algal chloroplast membranes are assembled in relatively big particles and by consequence are composed of several apoproteins (Berkaloff et al., 1983). The use of very mild detergents leads to isolate LHCF oligomers of 120 up to 700 kDa (Passaquet et al., 1991; Katoh & Ehara, 1990; Katoh et al., 1989).

These results suggest that fucoxanthin-light harvesting antennae are intricate systems composed of very similar pigment-protein components.

In Chl*a*/*b*-containing plants, two series of LHC proteins have been associated with one or the other photosystem (for review, see Jansson, 1994). According to phylogenetic trees (Caron et al., 1996; Durnford et al., 1996), it has been suggested that Chl*a*/*c*-binding proteins diverged from Chl*a*/*b* lineage prior to the functional separation of the light-harvesting complex associated with PSI and PSII (LHCI and LHCII respectively). In fact two distinct photosystems I and II are also present in Chl*c*-containing algae, but it not known if two types of LHCF preferentially associated to one photosystem can be distinguished. Indeed, light-harvesting complexes have been isolated from PSII and PSI-enriched fractions, they showed polypeptides with the same molecular weight as the major light-harvesting complex isolated by the first step of the purification procedures. Both are enriched in fucoxanthin, violoxanthin and Chl*c*, the differences observed in pigment composition between these two LHCF can be due to purification procedures (Berkaloff et al., 1990; Douady et al., 1993). The only difference which has been shown is that LHC isolated from PSI fraction contain more hydrophobic peptidic components than LHC from PSII fraction (Douady et al., 1994). In the few species where several amino acid LHCF sequences from the same species have been published, the distinction between LHCFI and LHCFII cannot be demonstrated (Apt et al., 1995; Bhaya & Grossman, 1993; De Martino et al., 2000).

Role of light-harvesting complexes in the regulation of the energy distribution between both photosytems.

In Chl*a*/*b* plants, such mechanisms implicate the phosphorylation of LHCII, i.e. Lhcb1 and Lhcb2; this is triggered by the redox state of a component (plastoquinone or one inside the cytochrome b6/f complex, Fig. 7) in the photosynthetic electron transport chain between both photosystems. Phosphorylation leads to a lateral movement of LhCb1 and Lhcb2 from PSII in the grana regions towards the PSI in the stroma unstacked regions where it can transfer the absorbed energy to PSI. This is assumed to be due to the short-term regulation of energy balance between PSI and PSII in response to modifications of the environmental light conditions (Allen, 1992).

Although it has been shown that a minor part of LHCF pool can be phosphorylated in the light (Caron et al., 1988; Douady et al., 1994), the green plant model cannot be directly applied to the Chromophytes. This is supported by the fact that the mechanims involved in the regulation of the energy balance between the two photosystems have not been evidenced in fucoxanthin-Chl*c* containing algae (Owens, 1986; Smith & Melis, 1987; Ting & Owens, 1994).

The photosynthetic apparatus of fucoxanthin-containing algae shows peculiar features: the thylakoids in chloroplasts have appressed regions corresponding to the grana as they have in green plants or green algae. They are arranged in parallel bands of three (Berkaloff et al., 1983; Gibbs, 1970) and the distribution of LHCF in the thylakoids is homogeneous as well as PSI complexes (Lichtlé et al., 1992).

Interestingly, in the Chl*a*/*b*/*c*-containing alga

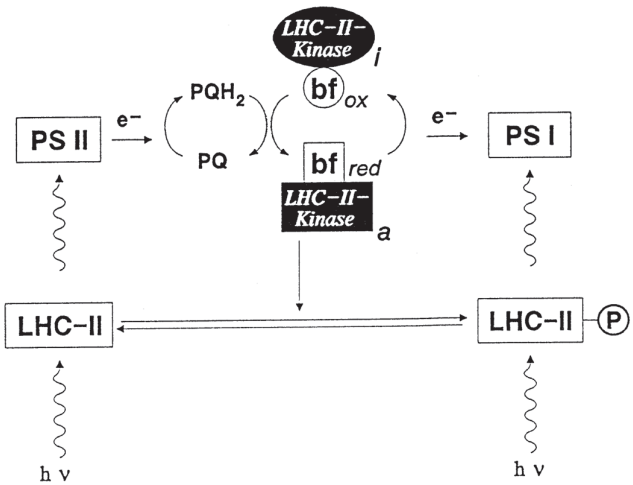


Figure 7. Phosphorylation of LHCII in higher plants. The LHCII-kinase is activated by the cytochrome b6-f-complex (bf) when it is reduced by plastoquinone (PQ) during the electron transfer occuring between Photosystem II (PSII) and I (PSI). The suffixes i and a stand for "inactive" and "active" form of the kinase (Hauska et al., 1996)

Figure 7. Phosphorylation du LHCII chez les plantes supérieures. La LHCII-kinase est activée par le complexe cytochrome b6-f (bf) quand il est réduit par la plastoquinone (PQ) durant les transferts d'électrons entre les Photosystèmes II (PSII) et I (PSI). Les suffixes i et a correspondent aux formes "inactive" et "active" de la kinase (Hauska et al., 1996).

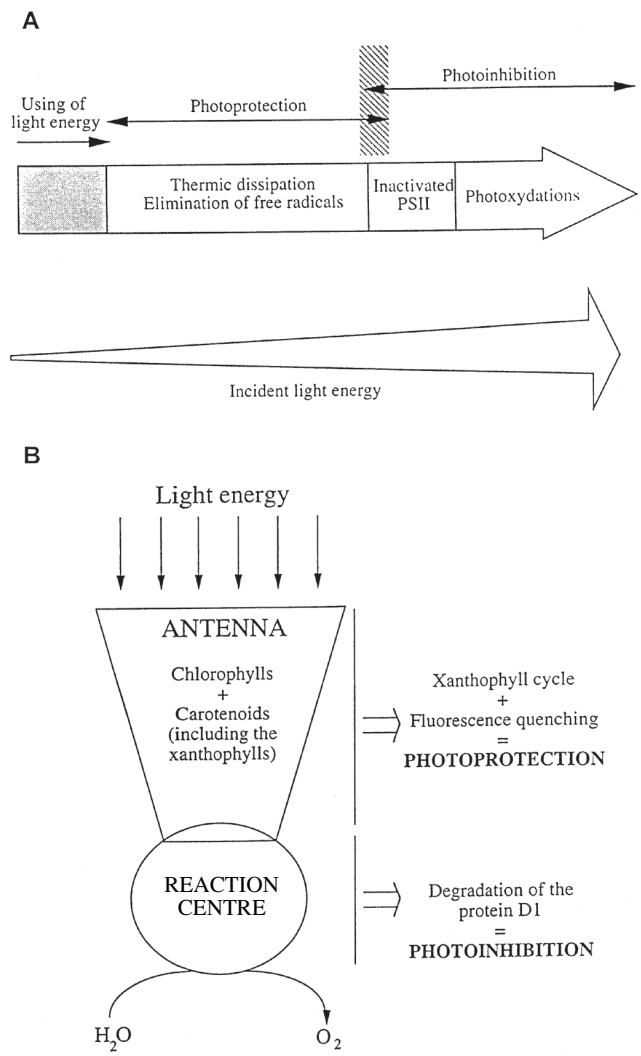


Figure 8. Responses of the photosynthetic apparatus to the absorption of increasing light intensities.

A: Schematic representation of the photoprotection and photoinhibition processes in photosynthetic organisms. As the absorbed light increases, the organisms develop photoprotective mechanisms, i.e. elimination of free radicals ($^1O_2$*) and thermic dissipation of the excess of absorbed energy in the antennae. When these mechanisms are not enough protective, PSII damages predominate and photoinhibition occurs (adapted from Demmig-Adams & Adams III, 1992).

B: Localization of the photoprotection and photoinhibition processes in a Photosystem II unit. The xanthophyll-cycle occurs simultaneously with the quenching of the fluorescence from the antenna Chl. Thus it is assumed that zeaxanthin is a quencher of energy excitation. When an excess of light arrives on the PSII, protein D1 component of the reaction centre is proteolysed, leading to the decline of the photosynthetic activity.

Figure 8. Réponses de l'appareil photosynthétique à l'augmentation de l'intensité lumineuse absorbée.

A : Représentation schématique des processus de photoprotection et de photoinhibition dans les organismes photosynthétiques. Quand la lumière absorbée augmente, les organismes développent des mécanismes de protection, comme l'élimination des radicaux libres ($^1O_2$*) et la dissipation thermique de l'excès d'énergie absorbée dans les antennes. Quand ces mécanismes sont insuffisants, les dommages sur le PSII prédominent et il y a photoinhibition (adapté de Demmig-Adams & Adams III, 1992).

B : Localisation des processus de photoprotection et de photoinhibition dans le Photosystème II. Le cycle des xanthophylles s'opère simultanément avec la disparition de fluorescence de l'antenne à Chl. On suppose ainsi, que la zéaxanthine agit comme un dissipateur de l'énergie d'excitation. Quand un excès de lumière arrive au PSII, la protéine D1 du centre réactionnel est protéolysée, conduisant à une baisse de l'activité photosynthétique.

Mantionella squamata which is thought to represent a primitive green alga, there is no specific LHCI or LHCII antennae and the LHC appears to transfer the absorbed energy to both photosystems (Schmitt et al., 1993). The thylakoid membranes of this prasinophycean alga are also not arranged in grana and the LHC is homogeneously distributed in the chloroplast membranes. The homogeneous ditribution of antennae is accompanied by the lack of transition states. In this alga, both photosystems may be excited by the same (biochemically undistinguishable) antenna complex and it could be the same situation in fucoxanthin-Chl*c*-containing algae (De Martino et al., 2000).

Role of light-harvesting complexes in photoprotection.

The light because it provides the energy required for photosynthesis, is one of the most important factors

influencing the primary production in ocean. Turbulence motions in seawater, meteorological changes, tidal regimes induce large fluctuations of the light available to the seaweeds. The algae, as all the plants, have the ability to adapt to their natural habitats and to improve short-term-regulation mechanisms for the working of the both photosystems, especially against the harmful subsaturating lights, which notably damages PSII centres (Fig. 8A). The carotenoids play a key role in these photoprotective mechanisms of the photosynthesis apparatus, essentially via the quenching of dangerous products induced by photosynthetic activity and via the dissipation of the excess of absorbed light by Chl (Fig. 8B).

Quenching of the triplet state of Chl and singlet oxygen.

Absorption of light by the pigments does not lead only to the transfer of electrons between the reaction centres and the primary acceptors. This photochemical reaction competes with other processes due to the excitement of the pigments: fluorescence, heat, formation of triplet excited states of the Chl molecules (^{3}Chl).

Even when energy transfer and charge separation are efficient, part of the excitement of Chl*a*, *b* and probably Chl*c* by light aborption leads to the formation of ^{3}Chl. These states are high enough in energy to transfer its excitement to oxygen in its ground state ($^3O_2$) and form singlet oxygen ($^1O_2$*) (Fig. 9). $^1O_2$* is a very oxidizing agent, thus it can cause severe damages to the cells. The photosynthetic organisms are equipped against the dangers of singlet oxygen. Indeed, carotenoids with nine or more double bonds (all carotenoids in plants and algae) can react with singlet oxygen yielding ground state oxygen and excited carotenoid (^{3}Car). And, they can also prevent singlet oxygen formation directly by exchanging triplet electron with ^{3}Chl (Fig. 9 (Siefermann-Harms, 1987; Siefermann-Harms & Angerhofer, 1998)).

In vitro, carotenoids can quench $^1O_2$* directly, although it is not well-established if these molecules are effective in pigment-protein complexes. In a carotenoporphyrin complex, there is no quenching of $^1O_2$* (Moore et al., 1994). But, in isolated LHCIIb trimers, which contain several xanthophylls: 2 luteins, 1 neoxanthin, 1/2 violaxanthin per monomer, ^{3}Chl-triplet state are totally quenched by xanthophylls and more than 2 xanthophylls seem to be implicated in this quenching (Peterman et al., 1995).

Dissipation of energy excess absorbed by Chl.

The responses of brown algae to saturating light are closely similar to those of many green algae and higher plants. From the first minutes of illumination, the room temperature fluorescence of Chl declines; the main part of this decrease has been referred to a non-photochemical fluorescence

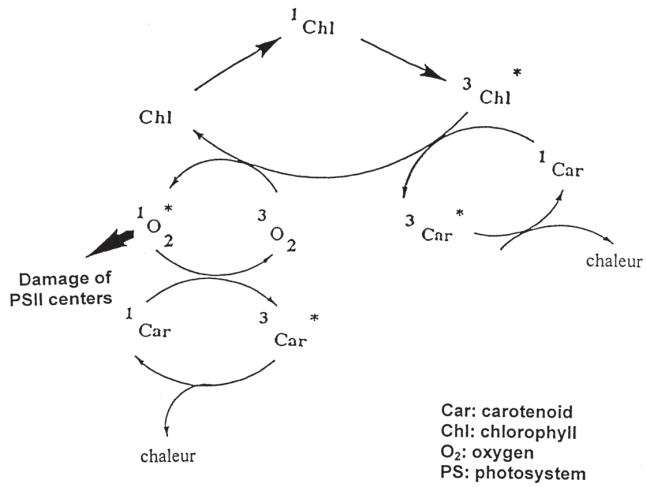


Figure 9. Photobiological function of carotenoids in the quenching of the Chl triplets (^{3} Chl). A Chl triplet is high enough in energy to transfer its excitation energy to oxygen ($^3O_2$*). In this process a singlet-excited $^1O_2$** is formed which is a very oxidizing component. The prevention of singlet oxygen formation is achieved by carotenoids (^{1}Car) either by quenching ^{3} Chl or by quenching $^1O_2$*.

Figure 9. Fonction photobiologique des caroténoides dans le piégeage des triplés de Chl (^{3} Chl). Un triplé de Chl à une énergie suffisante pour transférer son énergie d'excitation à l'oxygène. Dans ce processus un singulet $^1O_2$** est formé, qui est un oxydant très fort. La prévention de la formation de ce singulet est réalisée par les caroténoides (^{1}Car) soit par le piégeage de ^{3} Chl, soit par la consommation de $^1O_2$*.

quenching (NPQ) caused by the thermal dissipation of excess absorbed photons, that most probably occurs in LHC, and more accurately in LHCII in green plants. This fluorescence quenching occurs in parallel with the interconversion of carotenoids via de-epoxidation of xanthophylls. Several observations have strengthened the idea that de-epoxidated xanthophylls play a role in NPQ (Demmig-Adams, 1990) and given rise to two main hypotheses: i) direct interaction of Chl and carotenoid involves energy transfer and leads to de-excitation of Chl and the quenching of the Chl fluorescence (Demmig-Adams & Adams, 1996; Wagner et al., 1996), ii) carotenoids induce a change in the conformation of the LHC which decreases the absorption capacity of the complexes (Horton et al., 1996).

Quenching of the Chl fluorescence mediated by zeaxanthin.

Certain carotenoids undergo reversible interconversions referred as "xanthophyll cycle" (X-cycle, Fig. 10A). The majority of the carotenoids involved in photosynthesis of higher plants and algae are C40 compounds with carbon-carbon bonds implicated in a π-electron conjugation. The

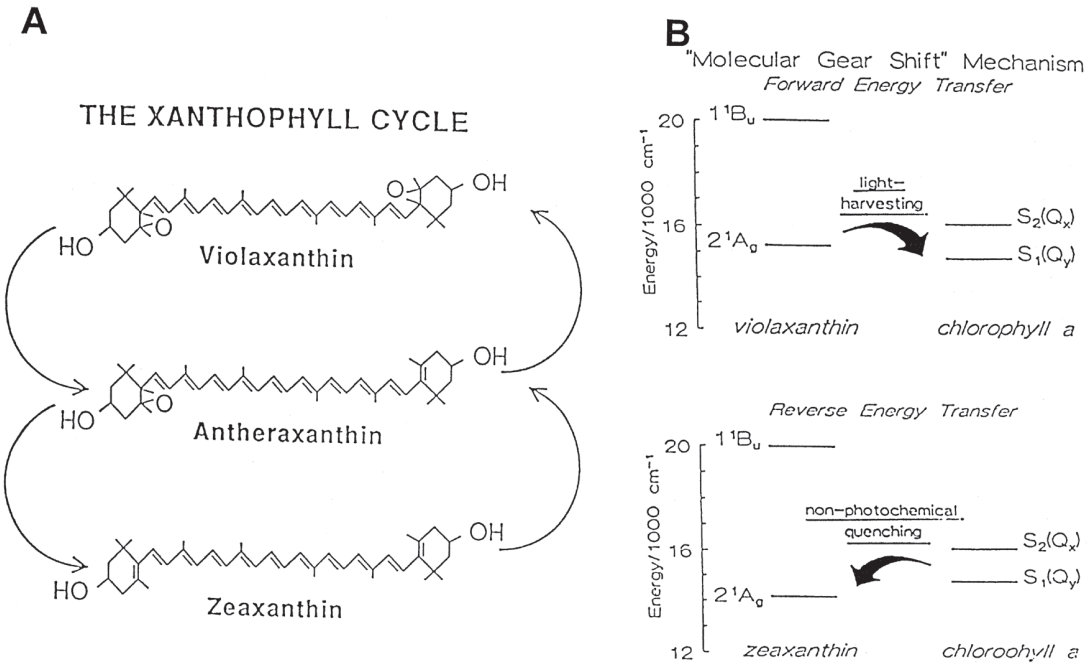


Figure 10. The xanthophyll-cycle.

A: The cycle operates as a transmembrane system where de-epoxidation (violaxanthin ---> antheraxanthin ---> zeaxanthin) occurs on the lumen side and epoxidation on the stroma side. Epoxidase activity is maximal at pH 7.0 and violaxanthin de-epoxidation is optimal at pH 5.0. The enzyme violaxanthin de-epoxidase (VDE) has been isolated and was reported to co-purify with LHCII (Gruszecki & Krupa, 1993).

B: Energy transfers between Chls and carotenoids.

When a photon is absorbed by violaxanthin, an electron reaches the 2^1Ag energy level which is higher than S1 level of Chl*a* , energy transfer can occur from violaxanthin to Chl*a*, the carotenoid plays a role in light-harvesting. In excess of light, violaxanthin is de-epoxidated in zeaxanthin, the 2^1Ag energy level of zeaxanthin is lower than S1 level. Energy transfer occurs from Chl*a* to zeaxanthin, the carotenoid plays a role in photoprotection.

Figure 10. Le cycle des xanthophylles.

A : Ce cycle fonctionne comme un sytème transmembranaire ou la dé-époxidation (violaxanthin ---> anthéraxanthin ---> zéaxanthin) a lieu dans le lumen et l'époxidation dans le stroma. L'activité époxidase est maximale à pH 7,0 et la de-époxidation de la violaxanthine est optimale à pH 5,0. L'enzyme violaxanthin dé-époxidase (VDE) a été isolée et elle co-purifie avec le LHCII (Gruszecki & Krupa, 1993).

B : Transferts d'énergie entre les Chls et les caroténoides.

Quand un proton est abosrbé par la violaxanthine, un électron atteint le niveau d'énergie 2^1Ag qui est plus élevé que le niveau S1 de la Chl*a* , le tranfert d'énergie peut avoir lieu entre la violaxanthine et la Chl*a*, et les caroténoides jouent un rôle dans la collecte de l'énergie lumineuse. En excès de lumière, la violaxanthine est dé-époxidée en zéaxanthine, le niveau d'énergie 2^1Ag de la zéaxanthine est plus bas que le niveau S1. Le tranfert d'énergie a lieu entre la Chl*a* et la zéaxanthine et les caroténoides jouent un rôle de photoprotection.

extent of the π-electron delocalization greatly determines the spectral properties of the molecules and the energies of their excited states. The interconversion of violaxanthin to zeaxanthin induces a change in the extent of the conjugated double-bond system (from 9 in violaxanthin to 11 in zeaxanthin). This implicates modifications in the energies and the lifetimes of the excited singlet states which are important in the role of the carotenoids in photosynthetic systems. Following absorption of a photon by a carotenoid, the electronic transition occurs from the ground state 1^1Ag to the 1^1Bu state, then by internal conversion the electron goes down and reaches 2^1Ag state. An increase in the conjugation system results in a decrease of 1^1Ag and 2^1Ag energies. The precise determination of the energy levels is crucial to demonstrate the energetically possible events (Frank & Cogdell, 1993). Indeed, the lowest excited singlet state (S1) of Chl*a* has been determined and is lower than that determined for the 2^1Ag state of violaxanthin, this allows this carotenoid to function as a light-harvesting pigment using energy transfer from its 2^1Ag state to Chl. In the case of zeaxanthin, the S_1 state of Chl*a* is higher than that of the carotenoid, thus an energy tranfer is possible from S_1 state of Chl*a* to the 2^1Ag state of zeaxanthin, leading to a quenching of Chl fluorescence (Fig. 10,B). The role of antheraxanthin is not clear as its 2^1Ag state is isoenergetic with that of Chl*a*.

Another model for the zeaxanthin-mediated fluorescence quenching is built on a correlation between the fluorescence quenching and the aggregation state of the isolated green plant LHCII. In this model, when plants are exposed to the light, a proton gradient is maintained across the photosynthetic membranes and generates low pH domains in LHCII. The protonation of certain aminoacids leads to the LHCII aggregation, zeaxanthin acts as an amplifier of this aggregation which induces the fluorescence quenching, rather than being directly implicated in de-excitation of Chl*a* (Horton et al., 1996; Ruban et al., 1997). Some authors suggest that the proton gradient and zeaxanthin cooperate, generating a membrane conformation change which induces the Chl quenching (Bilger & Bjorkman, 1994; Bilger et al., 1995; Pfündel & Bilger, 1994). A recent study shows that the zeaxanthin is a quencher of Chl fluorescence in presence of a proton gradient (ΔpH), but the data do not fit well with the aggregation model. The authors suggest that the ΔpH enhances the rate of different energy dissipation pathways: one directly at the PSII centres via charge recombination and the other at the antenna via the thermal dissipation of Chl excitation (singlet-singlet transfer) (Wagner et al., 1996).

In green plants, where several discrete Chl*a*/*b* LHC are associated to each PSI and PSII (Fig. 1B), the reports concerning the binding sites of X-cycle carotenoids are still conflicting. Probably, because a part of violaxanthin and zeaxanthin are weakly bound to the complexes.

Minor complexes (CP24, CP26, CP29) are specifically enriched in X-cycle carotenoids providing the support for a role of minor complexes in the dissipation of light excess (Bassi et al., 1993). As regards LHCIIb, there are more reports but the opinions are more confuse. The LHCIIb trimer binds one molecule of violaxanthin and when it dissociates into monomeric form, the violaxanthin is loss. Some reports indicate that LHCIIb has the ability to synthesize zeaxanthin from violaxanthin (Gruszecki & Krupa, 1993; Phillips et al., 1995; Ruban et al., 1994), other not (Bassi et al., 1993).

The xanthophyll-cycle and its location in brown algae

X-cycles are widely used in plant kingdom; in *Phaeophyceae*, the two-step cycle involving violaxanthin, antheraxanthin and zeaxanthin is currently admitted (Benet et al., 1994) but some authors failed to demonstrate its presence in some species (Vershinin & Kamnev, 1996). Under excess light and from the first minutes of illumination, violaxanthin is de-epoxidated into zeaxanthin via antheraxanthin. If the light level noticeably decreases or is turn off, the process is reversed and the photosynthesis activity is not altered (Fig. 8). But the recovery is slow and generally incomplete when the PSII centres have been damaged by a too long or too high illumination, this being revealed by as a decrease of the rate of the photosynthesis (Harker et al., 1999; Uhrmacher et al., 1995) (for review, see Franklin & Forster, 1997).

The link between the X-cycle and the light-harvesting complexes in brown algae as in Chl*a*/*b* plants, is confirmed by biochemical studies which localize xanthophyll cycle carotenoids in LHC fractions associated to PSI and PSII (Berkaloff et al., 1990; Douady et al., 1993), and after a light stress, LHC fractions are enriched in zeaxanthin (De Martino et al., 1997; Lichtlé et al., 1995).

More precise binding sites in LHCF are not known. After dissociation of the oligomeric forms, the monomers of the major LHCF bind any X-cycle xanthophylls. By contrast, the X-cycle carotenoids are associated with fractions where interactions between pigments and proteins are greatly disturbed. This suggests that the X-cycle xanthophylls are not tightly associated with proteins in the monomeric complexes (De Martino et al., 1997).

In other fucoxanthin-containing algae as diatoms, the photoprotection is not ensured by violaxanthin/zeaxanthin interconversion but by an other one-step xanthophyll cycle (diatoxanthin/ diadinoxanthin interconversion) which appears to act as similar Chl*a* quenchers (Arsalane et al., 1994; Olaizola et al., 1994).

Perspectives

During the last ten years, major progresses have been made in the understanding of the fucoxanthin-Chl*c* antennae concerning their biochemistry and the genes encoding their constituting proteins.

The most important conclusion drawn from the comparison of the protein sequences is that intrinsic Chl*a* - antennae share common ways to bind Chl molecules. By contrast, the binding of carotenoids is more diversified. To determine the binding sites of pigments, experiments of in vitro reconstitution is now possible using proteins over-expressed in bacteria and directed mutagenesis. The prelimary experiments that have been performed in our laboratory indicate a functionnal re-association of Chl*a* and Chl*c* in reconstituted complexes, while fucoxanthin appears more difficult to reassociate. The “real” binding of pigments could be controlled by Raman spectroscopy.

Up to now, only one work, published by Apt et al. (1995), is devoted to the expression of the LHCF genes in a brown alga. The transcription of several genes is controlled by the intensity of the light and is probably under the control of a blue light receptor. Genetic tools are now available to initiate macroalga transformation and to study the regulation of gene expression.

References

Allen J. F. 1992. Protein phosphorylation in regulation of photosynthesis. *Biochimica et Biophysica Acta*, **1098**: 275-335.

Apt K. E., Clendennen S. K., Powers D. A. & Grossman A. R. 1995. The gene family encoding the fucoxanthin chlorophyll proteins from the brown alga *Macrocystis pyrifera*. *Molecular and General Genetics,* **246**: 455-464.

Argüello-Astorga G. & Herrera-Estrella L. 1998. Evolution of light-regulated plant promoters. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**: 525-555.

Arsalane W., Rousseau B. & Duval J. C. 1994. Influence of the pool size of the xanthophyll cycle on the effects of light stress in a diatom: Competition between photoprotection and photoinhibition. *Photochemistry and Photobiology*, **60**: 237-243.

Barrett J. & Anderson J. M. 1980. The P-700-chlorophyll alpha-protein complex and two major light-harvesting complexes of *Acrocarpia paniculata* and other brown seaweeds. *Biochimica et Biophysica Acta*, **590**: 309-23.

Bassi R., Pineau B., Dainese P. & Marquardt J. 1993. Carotenoid-binding proteins of photosystem II. *European Journal of Biochemistry*, **212**: 297-303.

Bassi R., Rigoni F. & Giacometti G. M. 1990. Chlorophyll binding proteins with antenna function in higher plants and green algae. *Photochemistry and Photobiology*, **52:** 1187-1206.

Benet H., Bruss U., Duval J. C. & Kloareg B. 1994. Photosynthesis and photoinhibition in protoplast of the marine brown alga *Laminaria saccharina*. *Journal of Experimental Botany*, **45**: 211-220.

Berkaloff, C., Caron, L. & Rousseau, B. 1990. Subunit organization of PSI particles from brown algae and diatoms: polypeptide and pigments analysis. *Photosynthesis Research*, **23**: 181-193.

Berkaloff C., Duval J. C., Hauswirth N. & Rousseau B. 1983. Freeze fracture study of thylakoids of *Fucus serratus*. *Journal of Phycology*, **19**: 96-100.

Bhaya D. & Grossman A. R. 1993. Characterization of gene clusters encoding the fucoxanthin chlorophyll proteins of the diatom *Phaeodactylum tricornutum*. *Nucleic Acids Research*, **21**: 4458-66.

Bilger W. & Bjorkman O. 1994. Relationships Among Violaxanthin Deepoxidation, Thylakoid Membrane Conformation, and Nonphotochemical Chlorophyll Fluorescence Quenching in Leaves of Cotton (*Gossypium hirsutum* L). *Planta*, **193**: 238-246.

Bilger W., Fisahn J., Brummet W., Kossmann J. & Willmitzer L. 1995. Violaxanthin cycle pigment contents in potato and tobacco plants with genetically reduced photosynthetic capacity. *Plant Physiology*, **108**: 1479-1486.

Boekema E. J., Hankamer B., Bald D., Kruip J. & Nield J. 1995. Supramolecular structure of the photosystem II complex from green plants and cyanobacteria. *Proceedings of the National Academy of Sciences USA*, **92**: 175-179.

Büchel C. & Wilhelm C. 1993. Isolation and characterization of a Photosystem-I-associated antenna (LHC-I) and a Photosystem-I core complex from the chlorophyll-c-containing alga *Pleurochloris-Meiringensis* (Xanthophyceae). *Journal of Photochemistry and Photobiology*, **20**: 87-93.

Caron L. & Brown J. 1987. Chlorophyll carotenoid protein complexes from the diatom *Phaeodactylum tricornutum*: spectrophotometric, pigment and polypeptide analyses. *Plant Cell Physiology*, **20**: 775-785.

Caron L., Douady D., Quinet-Szely M., De Goër S. & Berkaloff C. 1996. Gene structure of a chlorophyll a/c-binding protein from a brown alga: Presence of an intron and phylogenetic implications. *Journal of Molecular Evolution*, **43**: 270-280.

Caron L., Remy R. & Berkaloff C. 1988. Polypeptide composition of light-harvesting complexes from some algae and diatoms. *FEBS letters,* **229**: 11-15.

Cunningham F. X. & Schiff J. A. 1986. Chlorophyll-protein complexes from from *Euglena gracilis* and mutants deficient in chlorophyll b. *Plant Physiology,* **80**: 231-238.

De Martino A., Douady D., Quinet-Szély M., Rousseau B., Crépineau F., Apt K. & Caron L. 2000. The light-harvesting antenna of brown algae: almost identical proteins encoded by a multigene family. *European Journal of Biochemistry* **267**: 5540-5549.

De Martino A., Douady D., Rousseau B., Duval J. C. & Caron L. 1997. Characterization of two light-harvesting subunits isolated from the brown alga *Pelvetia canaliculata*: Heterogeneity of xanthophyll distribution. *Photochemistry and Photobiology,* **66**: 190-197.

Demmig-Adams B. 1990. Carotenoids and photoprotection in plants. A role for the xanthophyll zeaxanthin. *Biochimica et Biophysica Acta*, **1020**: 1-24.

Demmig-Adams B. & Adams W. W. 1992. Photoprotection and other responses to high light stress. *Annual Review of Plant Physiology and Plant Molecular Biology*, **43**: 599-626.

Demmig-Adams B. & Adams W. W. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science*, **1**: 21-26.

Dolganov N. A. M., Bhaya D. & Grossman A. R. 1995. Cyanobacterial protein with similarity to the chlorophyll a/b binding proteins of higher plants: Evolution and regulation. *Proceedings of the National Academy of Sciences USA*, **92**: 636-640.

Douady D., Rousseau B. & Berkaloff C. 1993. Isolation and characterization of PSII core complexes from a brown alga: *Laminaria saccharina*. *FEBS Letters*, **324**: 22-26.

Douady D., Rousseau B. & Caron L. 1994. Fucoxanthin chlorophyll a/c light-harvesting complexes of *Laminaria saccharina* : partial amino acid sequences and arrangement in thylakoid membranes. *Biochemistry*, **33**: 795-796.

Durnford D. G., Aebersold, & Green R. 1996. The fucoxanthin-chlorophyll proteins from a chromophyte alga are part of a large multigene family: Structural and evolutionary relationships to other light harvesting antennae. *Molecular and General Genetics*, **253**: 377-386.

Durnford D. G. & Green B. R. 1994. Characterization of the light harvesting proteins of the chromophytic alga, *Olisthodiscus luteus* (*Heterosigma carterae*). *Biochimica et Biophysica Acta*, **1184**: 118-123.

Fawley M. W., Morton J. S., Stewart K. D. & Mattox K. R. 1987. Evidence for a common evolutionary origin of light-

harvesting fucoxanthin a/c complexes of *Pavlova gyrans* (Prymnesiophyceae) and *Phaeodactylum tricornutum* (Bacillariophyceae). *Journal of Phycology*, **23**: 377-381.

Frank H. A. & Cogdell R. J. 1993. The photochemistry and function of carotenoids in photosynthesis. In *Carotenoids in photosynthesis*. (A. Young, and G. Britton, eds), pp 253-326. Chapman & Hall, London.

Franklin L. A. & Forster R. M. 1997. The changing irradiance environment: Consequences for marine macrophyte physiology, productivity and ecology. *European Journal of Phycology,* **32**: 207-232.

Friedman A. L. & Alberte R. S. 1987. Phylogenetic distribution of the major diatom light-harvesting pigment-protein determined by immunological methods. *Journal of Phycology,* **23**: 427-433.

Gibbs S. 1970. The comparative ultrastructure of the algal chloroplast. *Annals of the New York Academy of Sciences,* **175**: 433-444.

Giuffra E., Cugini D., Croce R. & Bassi R. 1996. Reconstitution and pigment-binding properties of recombinant CP29. *European Journal of Biochemistry,* **238**: 112-120.

Green B. R. & Kühlbrandt W. 1995. Sequence conservation of light-harvesting and stress-response proteins in relation to the three-dimensional molecular structure of LHCII. *Photosynthesis Research,* **44**: 139-148.

Grevby C. & Sundqvist C. 1992. Characterization of light-harvesting complex in *Ochromonas danica* (Chrysophyceae). *Journal of Plant Physiology,* **140**: 414-420.

Grossman A.G., Manodori A. & Snyder D. 1990. Light-harvesting proteins of diatoms: Their relationship to the chlorophyll a/b binding proteins of higher plants and their mode of transport into plastids. *Molecular and General Genetics*, **224**: 91-100.

Grossman A. R., Schaefer M. R., Chiang G. G. & Collier J. L. 1993. The phycobilisome, a light-harvesting complex responsive to environmental conditions. *Microbiological Reviews,* **57**: 725-749.

Gruszecki W. I. & Krupa Z. 1993. LHCII, the major light-harvesting pigment-protein complex is a zeaxanthin epoxidase. *Biochimica et Biophysica Acta*, **1144**: 97-101.

Harker M., Berkaloff C., Lemoine Y., Britton G., Young A., Duval J. C., Rmiki N. & Rousseau B. 1999. Effects of high light and desiccation on the operation of the xanthophyll cycle in two marine brown algae. *European Journal of Phycology,* **34**: 35-42.

Hauska G., Schütz M. & Büttner M. 1996. The cytochrome b6f complex-composition, structure and function. In: *Oxygenic photosynthesis: the light reactions*, vol. 4. *Advances in photosynthesis* (ed. D. R. a. Y. Ort, C.F.), pp. 377-398. Kluwer academic publishers, Dordrecht.

Hiller R., Larkum A. W. D. & Wrench P. 1988. Chlorophyll-proteins of the prymnesiophyte *Pavlova lutherii* comb. nov.: Identification of the major light-harvesting complex. *Biochimica et Biophysica Acta*, **932**: 223-231.

Hiller R. G., Wrench P. M., Gooley A. P., Shoebridge G. & Breton J. 1993. The major intrinsic light-harvesting protein of *Amphidinium*: characterization and relation to other light-harvesting proteins. *Photochemistry and Photobiology*, **57**: 125-131.

Hoffman N. E., Pichersky E., Malik V. P., Castresana, C., Ko K., Darr S. C. & Cashmore A. R. 1987. A cDNA clone encoding a photosystem I protein with homology to photosystem II chlorophyll a/b-binding polypeptides. *Proceedings of the National Academy of Sciences USA*, **84:** 844-848.

Horton P., Ruban A. V. & Walters R. G. 1996. Regulation of light harvesting in green plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **47:** 655-684.

Ivanoff A. 1975. *Introduction à l'oceanographie. Propriétés physiques et chimiques des eaux de mer.* Vuibert, Paris.

Iglesias-Prieto R., Govind N. S. & Trench R. K. 1993. Isolation and characterization of 3 membrane-bound chlorophyll protein complexes from 4 dinoflagellate species. *Philosophical Transactions of the Royal Society of London Serie B*, **340**: 381-392.

Jansson S. 1994. The light-harvesting chlorophylla/b-binding proteins. *Biochimica et Biophysica Acta*, **1184**: 1-19.

Jovine R. V. M., Johnsen G. & Prezelin B. B. 1995. Isolation of membrane bound light-harvesting-complexes from the dinoflagellates *Heterocapsa pygmaea* and *Prorocentrum minimum*. *Photosynthis Research,* **44**: 127-138.

Katoh T. & Ehara T. 1990. Supramolecular assembly of fucoxanthin-chlorophyll-protein complexes isolated from a brown alga, *Petalonia fascia*. *Plant Cell Physiol*ogy, **31**: 439-447.

Katoh T., Mimuro M. & Takaichi S. 1989. Light-harvesting particles isolated from a brown alga, *Dictyota dichotoma*. A supramolecular assembly of fucoxanthin-chlorophyll-protein complexes. *Biochimica et Biophysica Acta*, **976**: 233-240.

Kroth-Pancic P. A. 1995. Nucleotide sequence of two cDNAs encoding fucoxanthin chlorophyll a/c proteins in the diatom *Odontella sinensis*. *Plant Molecular Biology,* **27**: 825-828.

Kühlbrandt W. 1994. Structure and function of the plant light-harvesting complex, LHC-II. *Current Opinion in Structural Biology*, **4**: 519-528.

Kühlbrandt W., Wang D. N. & Fujiyoshi Y. 1994. Atomic model of plant light-harvesting complex by electron crystallography. *Nature*, **367**: 614-621.

Laroche J., Henry D., Wyman K., Sukenik A. & Falkowski P. 1994. Cloning and nucleotide sequence of a cDNA encoding a major fucoxanthin-chlorophyll a/c-containing protein from the chrysophyte *Isochrysis galbana*: Implications for evolution of the cab gene family. *Plant Molecular Biology,* **25**: 355-368.

La Roche J., Partenski P. & Falkowski P. 1995. The major light-harvesting chl binding protein of *Prochloroccus marinus* is similar to CP43', a chl binding protein induced by iron-depletion in cyanobacteria. *Journal of Plant Physiology,* **38**: 678-684.

Lichtlé C., Arsalane W., Duval J. C. & Passaquet C. 1995. Characterization of the light-harvesting complex of *Giraudyopsis stellifer* (Chrysophyceae) and effects of light stress. *Journal of Phycology*, **31**: 380-387.

Lichtlé C., Spilar A. & Duval J. C. 1992. Immunogold localization of light-harvesting and photosystem I complexes in the thylakoids of *Fucus serratus* (Phaeophyceae). *Protoplasma*, **166**: 99-106.

Mimuro M. & Katoh T. 1991. Carotenoids in photosynthesis: absorptionn transfer and dissipation of light energy. *Pure and Applied Chemistry*, **63**: 123-130.

Moore T., Gust D. & Moore A. 1994. Carotenoids: nature's unique pigments for light and energy processing. *Pure and Applied Chemistry*, **66**: 1033-1040.

Olaizola M., Laroche J., Kolber Z. & Falkowski P. 1994. Non-photochemical fluorescence quenching and the diadinoxanthin cycle in a marine diatom. *Photosynthesis Research,* **41:** 357-370.

Owens T. G. 1986. Light-harvesting function in the diatom *Phaeodactylum tricornutum*. II. Distribution of excitation energy between the photosystems. *Plant Physiology,* **80**: 739-746.

Pascal A. A., Caron L., Rousseau B., Lapouge K., Duval J. C. & Robert B. 1998. Resonance Raman spectroscopy of a light-harvesting protein from the brown alga *Laminaria saccharina*. *Biochemistry*, **37**: 2450-2457.

Passaquet C. & Lichtlé C. 1995. Molecular study of a light-harvesting apoprotein of *Giraudyopsis stellifer* (Chrysophyceae). *Plant Molecular Biology*, **29**: 135-148.

Passaquet C., Thomas J. C., Caron L., Hauswirth N., Puel F. & Berkaloff C. 1991. Light-harvesting complexes of brown algae: biochemical characterization and immunological relationships. *FEBS Letters,* **280**: 21-26.

Pesaresi P., Sandonà D., Giuffra E. & Bassi R. 1997. A single point mutation (E166Q) prevents dicyclohexylcarbodiimide binding to the photosystem II subunit CP29. *FEBS Letters,* **402**: 151-156.

Peterman E., Dukker F., Vangrondelle R. & Vanamerongen H. 1995. Chlorophyll a and carotenoid triplet states in light-harvesting complex II of higher plants. *Biophysical Journal,* **69**: 2670-2678.

Pfündel E. & Bilger W. 1994. Regulation and possible function of the violaxanthin cycle. *Photosynthesis Research,* **42**: 89-109.

Phillips L., Cowan A., Rose P. & Logie M. 1995. Operation of the xanthophyll cycle in non-stressed and stressed cells of *Dunaliella salina* Teod in response to diurnal changes in incident irradiation: A correlation with intracellular beta-carotene content. *Journal of Plant Physiology,* **146**: 547-553.

Rhiel E., Lange W. & Morsche E. 1993. The unusual light-harvesting complex of *Mantoniella squamata* - supramolecular composition and assembly. *Biochimica et Biophysica Acta*, **1143**: 163-172.

Ruban A., Phillip D., Young A. & Horton P. 1997. Carotenoid-dependent oligomerization of the major chlorophyll a/b light harvesting complex of photosystem II of plants. *Biochemistry*, **36**: 7855-7859.

Ruban A. V., Young A. J., Pascal A. A. & Horton P. 1994. The effects of illumination on the xanthophyll composition of the photosystem II light-harvesting complexes of spinach thylakoid membranes. *Plant Physiology,* **104**: 227-234.

Sandonà D., Croce R., Pagano A., Crimi M. & Bassi R. 1998. Higher plants light harvesting proteins. Structure and function as revealed by mutation analysis of either protein or chromophore moieties. *Biochimica et Biophysica Acta*, **1365**: 207-214.

Schmitt A., Herold A., Welte C., Wild A. & Wilhelm C. 1993. The light-harvesting system of the unicellular alga *Mantioniella squamata* (Prasinophyceae): Evidence for the lack of a photosystemI-specific antenna complex. *Photochemistry and Photobiology,* **57**: 132-138.

Siefermann-Harms D. 1987. The light-harvesting and protective functions of carotenoids in photosynthetic membranes. *Physiologia Plantarum,* **69**: 561-568.

Siefermann-Harms D. & Angerhofer A. 1998. Evidence for an O-2-barrier in the light-harvesting chlorophyll-a/b-protein complex LHC II. *Photosynthesis Research*, **55**: 83-94.

Smith B. M. & Melis A. 1987. Photosystem stoichiometry and excitation distribution in chloroplasts from surface and minus 20 meters blades of *Macrocystis pyrifera,* the giant kelp. *Plant Physiology*, **84**: 1325-1330.

Thompson W. F. & White M. J. 1991. Physiological and molecular studies of light-regulated nuclear genes in nuclear genes in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **42**: 423-466.

Ting C. & Owens T. 1994. The effects of excess irradiance on photosynthesis in the marine diatom *Phaeodactylum tricornutum*. *Plant Physiology*, **106:** 763-770.

Trautman J. K., Shreve A. P., Owens T. G. & Albrecht A. C. 1990. Femtosecond dynamics of carotenoid to chlorophyll energy transfer in thylakoid membrane preparations. *Chemical and Physical Letters,* **98**: 369-376.

Uhrmacher S., Hanelt D. & Nultsch W. 1995. Zeaxanthin content and the degree of photoinhibition are linearly correlated in the brown alga *Dictyota dichotoma*. *Marine Biology*, **123**: 159-165.

Vershinin A. O. & Kamnev A. N. 1996. Xanthophyll cycle in marine macroalgae. *Botanica Marina*, **39**: 421-425.

Wagner B., Goss R., Richter M., Wild A. & Holzwarth A. R. 1996. Picosecond time-resolved study on the nature of high-energy-state quenching in isolated pea thylakoids - Different localization of zeaxanthin dependent and independent quenching mechanisms. *Journal of Photochemistry and Photobiology,* **36:** 339-350.

Wilhelm C. 1990. The biochemistry and physiology of light-harvesting processes in chlorophyll b- and chlorophyll c-containing algae. *Plant Physiology and Biochemistry*, **28:** 293-306.

Wolfe G. R., Cunningham Jr F. X., Grabowski B. & Gantt E. 1994. Isolation and characterization of photosystems I and II from the red alga *Porphyridium cruentum*. *Biochimica et Biophysica Acta*, **1188:** 357-366.