indicate that this pigment was derived from a red algal source. Unfortunately, the designation has proven not to be a clean one as another type of phycoerythrin was rapidly discovered from a unicellular red alga, *P. cruentum* and given the subclass designation of B-phycoerythrin (for Bangiophyceae red algae) (Stadnichuk et al., 1997). This nomenclature system has been modified through the years and a number of different designations have developed to cope with R-PE-like phycoerythrins isolated from Cyanobacteria and cryptomonads.

A novel phycoerythrin was recently described by Glazer and colleagues (Glazer et al., 1997) from the freshwater red alga *Audouinella* sp. and a related strain designated as '*Chantransia*' UTEX2623. This PE is unique in that it contains phycocyanobilin, giving it unique spectral characteristics. It has the same molecular weight as B-PE and R-PE (about 240,000 Da), the $(\alpha\beta)_6\gamma$ subunit structure, and the same number of bilins per subunit (α-2, β-3 and γ-3). However, the presence of phycocyanobilin on the β subunit was unique (Glazer et al., 1997). In addition, these authors found that the β^{PE} subunit was not homogeneous, having two slightly different molecular weight β subunits, 20,181 and 20,285 Dalton by mass spectroscopy. The function of these two forms of β subunits was not commented upon and deserves further study. The excitation peaks of the isolated phycoerythrin were 495, 563, and 603 nm, corresponding to PUB, PEB and PCB (phycourobilin, phycoerythrobilin and phycocyanobilin, respectively). Emission of this unique PE was different from all described PEs at 626 nm (Glazer et al., 1997).

Another unique phycoerythrin was isolated recently from *Rhodella reticulata* strain R6, and putatively has no α subunits (Thomas and Passaquet, 1999). The authors isolated the $\beta\beta$-PE and characterized this phycoerythrin is novel in that it contains a large linker polypeptide (L_R^{87}), which they postulated might functionally replace the missing α subunit during the assembly of these proteins. The emission maximum of the $\beta^{PE}_2 L_R^{87}$ purified proteins was found to be 630 nm, unique for a phycoerythrin (Table 1). Its absorbance spectrum showing two maxima at 562 nm and 604 nm was also quite unique.

B. Cyanobacterial Phycobiliproteins

A unique cyanobacterial phycobiliprotein was isolated from *Synechocystis* sp. strain BO8402 from a freshwater lake (Neuschaefer-Rube et al., 2000). This cyanobacterium does not have typical phycobilisomes, but instead forms inclusion bodies containing remnants of phycobilisomes. These inclusion bodies are surrounded by a proteinaceous capsule and contain α^{PC} and β^{PC} subunits, the rod linker polypeptide, $PC{\cdot}L_R^{35}$ and a novel blue colored protein L^{55}. The formation of the L^{55} linker was proposed to be the result of post-translational cross-linking of a β^{PC} subunit and L_R^{PC}, thus offering added stability of these large phycocyanin aggregates unique to *Synechocystis* sp. BO8402.

C. Cryptomonad Phycobiliproteins

Cryptomonad algae are a small group of biflagellated photosynthetic algae (Kugrens et al., 1999; Chapter 1, Douglas et al.). The ability of cryptomonads to harvest light for use in photosynthesis is augmented by antennae systems comprised of both CAC proteins (Chapter 4, Durnford) and phycobiliproteins that assemble into rod substructures located in the thylakoid lumen (Hill and Rowan, 1989; Kugrens et al., 1999). The exact mechanism by which cryptomonads are able to capture photons by phycobiliproteins and transfer that energy to membrane-bound reaction centers remains unknown.

Cryptomonads produce either phycoerythrins (Cr-PEs) or phycocyanins (Cr-PCs) that are smaller in size and have different spectral characteristics from red algal, glaucocystophyte and cyanobacterial phycobiliproteins. In any one cryptomonad species, there is only one type of phycobiliprotein present, either a Cr-PC or Cr-PE; APC is never present (Hill and Rowan, 1989). There is one β polypeptide species while there are at least two (sometimes there can be up to four) different α subunits in cryptomonad phycobiliproteins (Schirmer et al., 1985, 1986; Deurring et al., 1991; Ficner et al., 1992; Ficner and Huber, 1993; Brejc et al., 1995; Chang et al., 1996; Ritter et al., 1999; Wilk et al., 1999). Five cryptomonad phycoerythrin subunits have been isolated and characterized from a Cr-PE that have different isoelectric points (ranging in pI from 5.1 to 6.85), and subunit composition (four α and one β subunits). The absorbance spectrum was the same for the different isoforms, and the emission spectra were very similar (Hiller and Martin, 1987). Other isomers of phycoerythrin isolated from *Cryptomonas maculata* had isoelectric points at 7.83, 5.05 and 4.84 (Sidler et al., 1985). These three isomers had

slightly different absorbance and fluorescence emission spectra. Such modifications of the phycobiliproteins to form several isoproteins are typical for cryptomonad phycobiliproteins and might be important for the assembly of the rod structures and/or energy transfer. Each α or α' subunit contains a single bilin linked by a thioether bond, while the β subunit carries three bilins (Wedemayer et al., 1996). The spectral properties of all the characterized isoproteins are very similar, yielding a gap between the biliprotein fluorescence and chlorophyll *a*. In the phycobilisomes, colorless linkers fine-tune the energy transfer steps between the phycobiliprotein chromophores, especially for the transition between the hexamers. Yet unknown proteins could play a similar 'linker-like' role in bridging the energy transfer gap in cryptomonads (energy pathway from the rod structure to the chlorophyll proteins of the photosystems). Alternatively, the high concentration of phycoerythrin within the thylakoid lumen in cryptomonads has also been mentioned in the literature as possibly being responsible for energy transfer to the chlorophyll proteins of the thylakoids (Hiller and Martin, 1987).

D. Glaucocystophyte Phycobiliproteins

Phycobiliproteins are also present in another small Division of unicellular flagellates, referred to as Glaucophyta or Glaucocystophyta. The phycobiliproteins are very similar to Cyanobacterial allophycocyanin and phycocyanins. However, the reason this group has received attention is the origin of the phycobiliprotein-containing plastid of this group, the cyanelle or cyanoplast. In the past, the glaucocystophytes have been classed as green and red algae, but recent phylogenetic analysis of six nuclear markers indicates that the glaucocystophytes are the closest group to green plants and red algae (Moreira et al., 2000). The data of these authors support the previous hypothesis that the glaucocystophytes originated from a photosynthetic endosymbiosis between a cyanobacterium and a eukaryotic host. Using phylogenetic information they extended that hypothesis to contend that red algae, glaucocystophytes and green plants all originated from the same primary endosymbiosis and suggested these three groups comprise the kingdom Plantae. However, they extended the theory to say two separate cyanobacterial symbioses occurred in forming the extant cyanelle (see Chapters 1, Douglas et al. and 2, Larkum and Vesk).

Recent work with *Cyanophora paradoxa* has perfected the isolation of oxygen-evolving PS II complexes and allowed characterization of the associated proteins (Shibata et al., 2001). These authors found that the PsbO protein was closely related to green plant type proteins by N-terminal amino acid sequence analysis. However, a class-II fructose-1,6-bisphosphate aldolase (FBA) was isolated from *C. paradoxa* that was most closely related to cyanobacterial FBA (Nickol et al., 2000). These authors deduced a complicated transport of protein precursors from the cyanelle via leader sequences. The complete sequence of the cyanelle genome from *C. paradoxa* is now available and can be a useful tool for sorting out the complicated evolution of this unique division of flagellated algae (Stirewalt et al., 1995).

Glaucocystophyta all contain plastids called cyanelles or cyanoplasts (the phycobiliprotein-containing the remains of an ancient endosymbiont). Like chloroplasts, cyanelles contain a reduced genome (Chapter 1, Douglas et al.). The cyanelle genome has been fully sequenced from *Cyanophora paradoxa* (Stirewalt et al., 1995). The cyanelle genome of 193 genes is surprisingly large compared to the higher plant genome, encoding roughly 50 additional proteins (Stirewalt et al., 1995). However, phylogenetic sequencing of the cyanelle genome shows that strong homology exists between most plastids (Stirewalt et al., 1995). The additional genes, in both glaucocystophytes and rhodophytes, has led these investigators to the theory of a secondary endosymbiosis to introduce additional genes (Stirewalt et al., 1995). This Division is composed of three undisputed genera, *Cyanophora*, *Glaucocystis*, and *Gloeochaete* but may include up to eighty mostly single-species genera (Loffelhardt et al., 1997). *Glaucocystis* is the type genus; however, due to the difficulty in culturing of all but *Cyanophora paradoxa*, the majority of the effort has gone into this algal species. Two other genera have also been linked to this group, *Paulinella* and *Glaucosphaera*, but are now thought to belong elsewhere. *Glaucosphaera* was reclassified as a red alga (Bhattacharya et al., 1995), while *Paulinella* has links to both the glaucocystophytes and testate amoebae.

The cyanelle contains thylakoid membranes that have been shown to contain hemidiscoidal phyco-

bilisomes (MacColl and Guard-Friar, 1987). Allophycocyanin (apcA, apcB, apcD, apcE, apcF but not apcC) and phycocyanin (cpcA and cpcB) are present in these genera and are highly homologous to cyanobacterial phycobiliproteins, showing >80% amino acid sequence homology (Bryant et al., 1985). The phycobiliproteins are encoded on the cyanelle genome. As in other phycobiliprotein containing algae, the phycobiliproteins have been found to be a nitrogen sink that is degraded under nitrogen stressed conditions (Schenk et al., 1983). Unlike other organisms, *C. paradoxa* apparently utilizes the phycobiliproteins as a carbon, nitrogen and sulfur storage system (Muller et al., 1997). This organism, in nitrogen, carbon and sulfur sufficient culture, can accumulate phycobiliproteins in an amount higher than the total of all other cellular proteins combined (Muller et al., 1997).

VI. Phycobiliprotein Crystal Structure

Crystal structures for APC, PC and PE from different Cyanobacteria, red algae and recently cryptomonads have been solved to high resolution. The main conclusion from these structures is that all three major classes of phycobiliprotein display remarkably similar subunit structure (Schirmer et al., 1985, 1986; Deurring et al., 1991; Ficner et al., 1992; Ficner and Huber, 1993; Brejc et al., 1995; Chang et al., 1996; Ritter et al., 1999; Wilk et al., 1999). Most of these crystal structures were done on isolated phycobiliproteins that do not contain their associated linker polypeptides. Recently however, the crystal structure of APC with its associated linker (APC·$L_C^{7.8}$) from *Mastigocladus laminosus* has been determined (Reuter et al., 1999). The location of the γ polypeptide has been modeled by Ritter and colleagues from a phycourobilin-containing PE from a red alga (Ritter et al., 1999).

All crystal structures of phycobiliproteins, except the α subunit of the cryptomonads, demonstrate a similar subunit structure. The formation of a monomer is primarily mediated by hydrophobic interactions between α-helices x and y (amino acid residues 1–35, refer to Figs. 2 and 4) of one subunit and the globular domain of its partner subunit. Three $\alpha\beta$ monomers are arranged around a three-fold symmetry axis to form a trimer, which then assembles face to face with another $\alpha\beta$ trimer for the formation of a hexamer.

A. Allophycocyanin Crystal Structure

APC has been crystallized with and without $L_C^{7.8}$, the small linker polypeptide that helps mediate the APC spectrum. The two subunits of allophycocyanin, α and β, consist of 160 and 161 amino acid residues, respectively. Each subunit has only one covalently attached bilin, phycocyanobilin, bound at αCys82 and βCys82. The structures of the α^{APC} and the β^{APC} subunits are almost identical even though there is low sequence identity between them (Brejc et al., 1995). The primary differences in structure between the APC subunits are a deletion in the β subunit between the b and e helices and a more buried N-terminus of the α subunit due to a two amino acid residue deletion when compared to the β subunit. This region of the b/e loop in the α subunit is completely exposed to solvent and does not interact with the chromophore. However the b/e loop of the β subunit has protein-chromophore interactions with the bilin of a neighboring monomer in the trimer aggregate.

Monomeric APC is spectrally different from trimeric APC by a 40 nm red shift (614 to 640 nm, respectively) (Liu et al., 1999). Brejc and co-workers solved the structure of APC from *Spirulina platensis* where two trimers are associated in a 'back to back' manner (Brejc et al., 1995). The association between two APC trimers is loose and is mediated through the β subunits. In crystal structures of APC isolated from the red alga *Porphyra yezoensis* (*P. yezoensis*), the contact between two trimers is mediated primarily through the α subunits and is much tighter. The assembly of the APC hexamer from *P. yezoensis* is similar to that for C-PC from *Fremyella diplosiphon* (Cyanobacteria) and R-PE from *Polysiphonia urceolata* (red alga), where the α subunits provide the contacting surface between trimers (Brejc et al., 1995). All APC crystal structures obtained without the presence of the small linker polypeptide show loose association between the trimers associating to form hexamers.

Recently Reuter and associates crystallized APC-linker complex, APC·$L_C^{7.8}$, from the phycobilisomes of the cyanobacterium, *Mastigocladus laminosus* (Reuter et al., 1999), and demonstrated critical interactions between this small linker polypeptide and APC. The $L_C^{7.8}$ resides at the surface face of the APC trimers and is in contact with two of the three β-subunits. A large portion (45.3%) of this linker is buried in the APC core complex, where it interacts

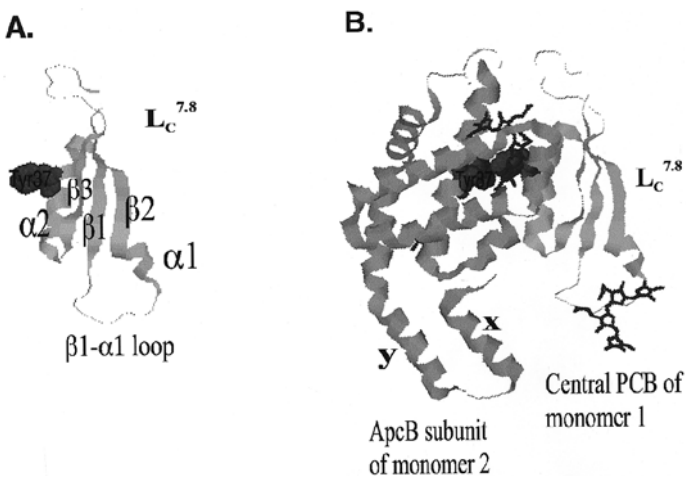

Fig. 4. Structure of the small APC linker polypeptide ($L_C^{7.8}$) and its interactions with surrounding central phycocyanobilin chromophores covalently attached to allophycocyanin monomers 1 and 2. Panel A) Structure of the $L_C^{7.8}$ polypeptide, showing α-helices $\alpha 1$ and $\alpha 2$ and three β-sheets, $\beta 1$, $\beta 2$ and $\beta 3$. The Tyr37 residue, located on $\alpha 2$, is depicted as space-filled atoms. The linker polypeptide is shown as a ribbon structure. Panel B) the same three-dimensional structure of the $L_C^{7.8}$ linker polypeptide is shown along with its interactions with central phycocyanobilins (PCB) attached to Cys82 residues on allophycocyanin monomers 1 and 2. The PCBs of monomers one and 2 are shown as stick figures. The allophycocyanin monomers are shown as ribbon structures. Residue Tyr37 located in $\alpha 2$ of the linker polypeptide is shown as space-filed atoms, as is residue Tyr87 of the ApcB subunit of monomer 2. The interaction of Tyr37 with Tyr87 results in a conformational change between monomer 2 and its chromophore, specifically where ring B of PCB is forced in the opposite direction of Tyr87. This interaction is not found in the remaining two monomers of the APC trimer. Structures were generated using Rasmol and the PDB coordinates for APC·$L_C^{7.8}$ from the phycobilisomes of *Mastigocladus laminosus* (Reuter et al., 1999). See Color Plate 4.

directly with the central chromophores of two distinct β-subunits. The presence of at least monomeric APC complexes is required for correct folding of this linker polypeptide (Betz et al., 1993). $L_C^{7.8}$ has a similar folding pattern as topoisomerase II and the prosegment of procarboxypeptidase A (which has two additional helices) (Reuter et al., 1999). The linker polypeptide is elongated and consists of three-stranded β-sheets ($\beta 1$, Leu-3 to Leu-9; $\beta 2$, Tyr-26 to Pro32; $\beta 3$, Lys-49 to Leu-55) and two α-helices ($\alpha 1$, Leu-22 to Thr-25; $\alpha 2$ Tyr-33 to Met-46). The structure of $L_C^{7.8}$ is shown in Fig. 4A. The $\alpha 1$ helix is very short, consisting of approximately one turn and is connected to the $\alpha 2$ helix by a random coil segment. This association of APC with $L_C^{7.8}$ influences its spectroscopic properties. Structural changes are induced in APC through interactions with the linker polypeptide, which are specifically mediated by polar and hydrophobic interactions between the N-terminal residue of the long linker helix ($\alpha 2$ helix) and monomer 2 of the trimer. This interaction results in a change of conformation between monomer 2 and its chromophore, specifically induced by Phe37 of the $L_C^{7.8}$ linker polypeptide inserting itself between Tyr87 (of β^{APC}) and ring B of the chromophore, thereby displacing them in opposite directions. The stacking interaction between Tyr87 and pyrrole ring B, observed for the other two monomers in the APC core complex, is thereby broken.

A second linker-chromophore interaction occurs between charged and polar residues in the loop region between β-sheet, $\beta 1$ and α-helix $\alpha 1$ of the $L_C^{7.8}$ linker polypeptide and the normally solvent-exposed side of the chromophore found on monomer 1. This loop domain of the linker polypeptide ($\beta 1$-$\alpha 1$ loop) covers the exposed chromophore on monomer 1 but does not change the conformation of the bilin, only the surrounding environment of the chromophore on monomer 1. These interactions of APC monomers with $L_C^{7.8}$ (shown in Fig. 4B) and others contract the hourglass-like shape of the APC trimer to a more flattened shape. This conformational change in the APC trimer is caused by a slight rotation of the three monomers bringing three β-chromophores closer to each other through $L_C^{7.8}$ contacts, thus modifying the APC spectrum.

B. C-Phycocyanin (C-PC) Crystal Structure

C-Phycocyanin has an α subunit comprised of 162 amino acids with one bilin-binding site at αCys82 (Deurring et al., 1990). The β subunit has 172 amino acids and two phycocyanobilins (PCB) covalently attached at βCys82 and βCys155. Attachment of the peripheral bilin (β155) occurs between α-helices g and h in the β subunit. The locations of the both central bilins in CpcA and CpcB are shown in Fig. 2, along with the peripheral phycocyanobilin found in CpcB. Upon alignment of the linear α and β sequences of C-PC and R-PC, this structural domain for attachment of the β155 phycocyanobilin was found to be missing in the α subunits where ten amino acids between α-helices g and h have been deleted (Schirmer et al., 1995, 1987; Deurring et al., 1991). Similar to APC, the central bilins for C-PC (βCys82 and αCys82) extend into a cleft formed by α-helices e and f. The overall structures of the individual subunits of C-PC, in addition to being similar to each other, are strikingly similar to APC. Structurally, the α subunit displays more differences, as was the case in the APC α subunit. APC and C-PC both have covalently attached phycocyanobilins at βCys82 and αCys82. However, their spectral properties differ due most likely to the variation of protein-chromophore interactions, including linker interactions. In C-PC, all three phycocyanobilins have similar interactions with the protein where they arch around aspartate residues (α87, β87 and β39) and most of the proprionic side chains of the bilins form salt bridges with arginine and lysine residues (Schirmer et al., 1987). These aspartate residues, located close to the central bilin, are held rigid by α-helices x and y (refer to Figs. 2 and 4), thus stabilizing the light absorption properties of the phycobiliprotein assemblage (Kikuchi et al., 2000). The crystal structure of C-PC contributed to defining the energy transfer mechanism between the chromophores, since the distance between the chromophores and their relative orientations gave more information regarding the role of central β82 chromophores in energy transfer.

Early crystal structures of C-PC demonstrated the similarity between the phycobiliprotein subunits' globular domains (α-helices a through h) and the globin family (Schirmer et al., 1985). The early events in the folding pathway for apomyoglobin have been proposed as a model for the folding of the phycobiliprotein subunits, where α-helices a, g and h would be necessary for the initiation of folding, forming a highly ordered, compact structure and the remaining helices would have a very small amount, if any of secondary structure (Anderson and Toole, 1998). The globular domain is then locked into place by lyase-mediated attachment of the correct bilin moiety, similar to what occurs in the globins when heme is attached to the apoprotein (Pastore and Lesk, 1990).

C. Phycoerythrin Crystal Structure

The major differences between C-PC and R-PE (and C-PE) again result from deletions or insertions of amino acids in order to accommodate specific protein-chromophore interactions. Phycocyanin has three phycocyanobilins attached to cysteinyl residues α82, β82 and β155, whereas R-PE and C-PE have four phycoerythrobilins (PEB) covalently attached to cysteinyl residues, α82, α140, β82 and β155. The fifth bilin is attached to the β subunit through ring A to βCys50 and through ring D, βCys61. The B-PE subunits of *Porphyridium sordidum* (*P. sordidum*), α and β, have 177 and 164 residues, respectively. The α-helices of R-PE and C-PE show high similarity to each other and to C-PC, where the main differences in structure between C-PC and R-PE/C-PE are in regions of insertion and deletion. The deletion of two amino acids in the α subunit of R-PE/C-PE (α67 and α68) changes the b/e loop region. This loop is close to the central bilin attachment site on the α subunit, αCys82, and results in additional protein-chromophore interactions similar to those found in the APC crystal structure (Brejc et al., 1995; Chang et al., 1996; Ficner et al., 1992). The insertion of four amino acids in the α subunit extends the loop region between the g and h α-helices and includes the αCys140 residue for attachment of the second phycoerythrobilin (PEB). Residues 138 to 144 of the α subunit differ in their confirmation from that found in C-PC crystal structure to form a pocket thereby accommodating this additional bilin. The β subunit also has an insertion of five amino acids in the loop region that connects helices g and h, thus accommodating the doubly attached β50/61 phycoerythrobilin (Ficner and Huber, 1993). When the carbon backbones of the α and β subunits of C-PE/R-PE are superimposed upon the α-carbon backbone of C-PC α and β subunits, there is very little deviation. The protein-chromophore interactions differ slightly from R-PE/C-PE to C-PC. However the interactions

between the subunits are well retained. For example, the interacting pairs for α and β monomer formation, αAsp13 and βArg93 are found both in R-PE/C-PE and C-PC. The specific interactions that occur in trimer formation also appear to be well conserved between R-PE/C-PE and C-PC.

D. Cryptophyte Phycoerythrin Crystal Structure

The crystal structure of the heterodimeric $\alpha_1\alpha_2\beta\beta$ Cr-PE_{545} from the cryptophyte *Rhodomonas* CS24 has been determined at 1.63 Ångstrom resolution (Wilk et al., 1999). Structurally, cryptomonad phycobiliprotein β-subunits (shown in Fig 5A) are very similar to cyanobacterial and red algae phycobiliprotein subunits, all having 9 α-helices connected by irregular loops and a tertiary structure that resembles hemoglobin. There is approximately 70% sequence identity between the β subunits of cryptomonads and those of red algae (Hill and Rowan, 1989). In one case, Cr-PE_{566} β subunits demonstrate 84.2% identity, where out of 177 positions 149 residues show identity to *Porphyridium cruentum* B-phycoerythrin β subunits. The *P. cruentum* α and β subunits are comprised of 70 to 80 and 177 amino acid residues, respectively (Sidler et al., 1985).

The α subunits of cryptomonads are shorter and unrelated to other sequenced proteins available in the database (Wilk et al., 1999). The molecular weights of α and β subunits isolated from cryptomonads are approximately 10 to 12 kDa and 15 to 21 kDa, respectively. These are isolated in a 1:1 ratio (MacColl and Guard-Friar, 1983b). The subunit structures of the cryptophycean phycoerythrin and phycocyanin are described in the literature as $\alpha_1\alpha_2\beta\beta$, where one bilin is carried on α and three on the β subunit at completely conserved attachment positions: αCys18 (or 19), βCys50/61, βCys82, and βCys158 (MacColl et al., 1999b). There are often multiple forms of cryptomonad phycobiliproteins with isoelectric points that range from slightly acidic to slightly basic, and Hiller and Martin suggested that these multiple forms may function in energy transfer in a method analogous to phycobilisomes (Hiller and Martin, 1987).

VII. Bilin Chromophores

The chromophores of phycobiliproteins are linear tetrapyrroles that are covalently linked to the protein backbones of the phycobiliproteins at very specific cysteine residues. The bilin type, number and location within the protein serve as the primary factors determining the visible absorption and fluorescence spectrum and energy transfer pathway for any given phycobiliprotein. The red algae have three major bilins, while the cryptomonad algae have several additional novel bilins that provide their unique spectral properties (Table 1). In cryptomonads, the β subunits have >80% identity with red and

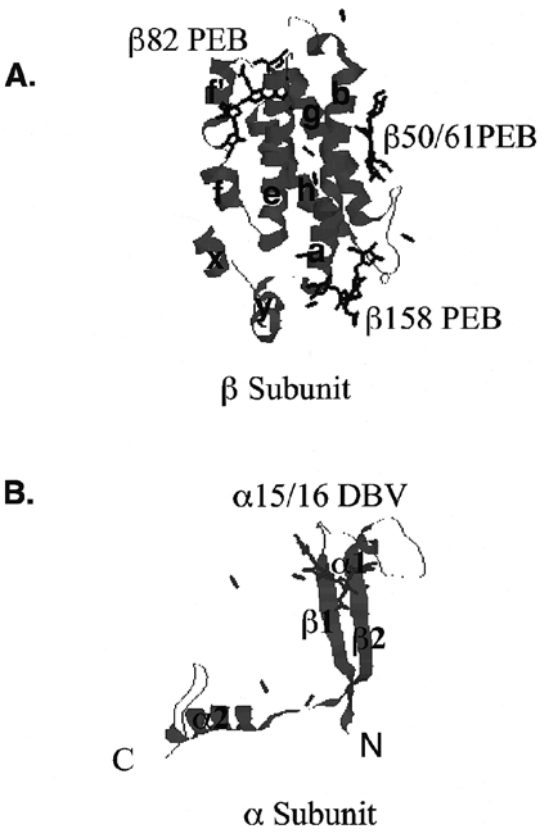

Fig. 5. Cryptomonad phycobiliprotein structure. Panel A) The Cr-phycoerythrin 545 β subunit is shown in ribbon with the nine α-helices labeled x-y-a-b-c-f-f'-g and h. Three phycoerythrobilin chromophores (β82, β158, β50/61) are shown as stick structures and colored black. Panel B) The Cr-PE_{545} α subunit is shown in ribbon and α-helices one and two are labeled α1 and α2, respectively. There are two antiparallel β-sheets labeled β1 and β2, respectively. A dihydrobiliverdin (DBV) chromophore is covalent and labeled β15/16 DBV. Structures were generated using Rasmol and the PDB coordinates for Cryptomonad PE_{545} (Wilk et al., 1999).

cyanobacterial β subunits. In Cyanobacteria and red algae (and presumably glaucocysteophytes), the βCys82 position holds the terminal energy accepting bilin also called the central bilin (Ong and Glazer, 1987, 1991; Toole et al., 1998). However, this position was not held constant in cryptomonad phycobiliproteins (Wedemayer et al., 1996). These authors found that at αCys18 position in Cr-PC_{645} (a mesobiliverdin), Cr-PE_{566} (a bilin 618) and Cr-PE_{545} (a 15,16-dihydrobiliverdin) were the terminal acceptor chromophores. In Cr-PE_{555} the terminal bilin was hypothesized to be a dihydrobiliverdin (DBV) at βDiCys50/61 (Wedemayer et al., 1996). They concluded that cryptomonad phycobiliproteins do not control the position of their terminal acceptors as rigidly red algal and cyanobacterial phycobiliproteins. This could be due to the production of phycobilisome supramolecular complexes formed by the red algal and cyanobacterial classes of phycobiliproteins that are not present in cryptomonad systems.

A. Bilin Types

The bilins for Cyanobacteria, glaucocysteophytes and red algae are usually of three types, phycoerythrobilin, phycocyanobilin and phycourobilin (Table 2). All of these three common phycobilins as well as phycobiliviolin (also known as cryptoviolin after its initial isolation in cryptomonads) are isomers of one another (Fig. 6B). The bilins are linked to specific cysteine residues in the phycobiliprotein backbone through thioether bonding. There can be either one or two of these covalent bonds per bilin. The structure of the protein and the bonding affect the spectral characteristics of the bilin and the overall protein. The cryptomonad algae have additional distinct bilins that give them unique spectral characteristics. One bilin found in the cryptomonads first then in Cyanobacteria is cryptoviolin, now referred to phycobiliviolin (Fig. 6B). It is an isomer of PUB, PEB and PCB, the common cyanobacterial and red algal bilins. The recent work of Wedemayer and colleagues has made the more unique cryptomonad bilins more numerous and understandable (Wedemayer et al., 1991, 1992, 1996). They identified two bilin isomeric groups that seem unique to the cryptomonads. The bilin 584 is closely related to the phytochrome bilin (phytochromobilin) (Fig. 6C). Then a larger group of bilin isomers, bilin 618, mesobiliverdin and dihydrobiliverdin (Fig. 6A) makes up the remainder of the currently identified unique cryptomonad bilins. These bilins give the cryptomonad phycobiliproteins complex spectra and different emission profiles compared with the phycoerythrins and phycocyanins from Cyanobacteria, glaucocystophytes and red algae.

The role of the protein/bilin interaction is to stabilize the three dimensional bilin structures in relationship to itself and surrounding bilins. The increased rigidity of the bilin ring structure allows different conformations resulting in different spectral properties. This association with the proteins minimizes non-radiative relaxation of the excited states of these molecules to aid in efficient transfer of energy from one bilin to another.

B. Bilin Attachment

In vitro studies investigating the spontaneous attachment of bilins to the apoprotein suggest that, in vivo, this reaction is mediated by specific enzymes called lyases (Arciero et al., 1988). When apophycocyanin is incubated without lyase addition, at neutral pH with phycocyanobilin, phycoerythrobilin and biliverdin, all formed covalent attachments with the apoprotein at the central bilin sites, α82 and β82. No spontaneous attachment of bilin occurred at the β155 site, even in the presence of excess bilin. These bilin-apoprotein complexes were spectroscopically distinct from holophycocyanin, and NMR analysis showed reduced forms of these products. When the open chain, cyclic tetrapyrroles were incubated with the apoprotein (in the presence of 5 M urea thus abolishing its three-dimensional structure), there was no attachment between the bilins and apoprotein. This result indicated the necessity of some tertiary structure for the reaction to proceed. The absence of attachment at the β155 site, the variation of condensation products formed at α82 and β82, and the lack of attachment of denatured apoproteins, suggest that specific enzymes might be responsible for the covalent attachment of bilins to the apoproteins.

Two cyanobacterial genes, *cpc*E and *cpc*F, located downstream of the C-PC operon in *Synechococcus* sp. PCC7002 were found to be one of the minor cotranscripts for these structural genes (Zhou et al., 1992). Interposon mutations created in *cpc*E and *cpc*F resulted in low levels of phycocyanin and further analysis showed that bilin attachment at the α82 site was defective (Swanson et al., 1992). These results

Table 2. Bilin types found in cyanobacterial, glaucocystophyte, red algal and cryptomonad algal phycobiliproteins (primary references for these bilins can be found in MacColl and Guard-Friar (1987), Rowan (1989) and Wedemayer (Wedemayer et al., 1991, 1996)

Bilin	Abbreviation	Wavelength (nm)	Phycobiliprotein	Algal Type
Phycocyanobilin	PCB	590–643	Allophycocyanin C-phycocyanin Allophycocyanin-B R-Phycocyanin Phycocyanin 569 Phycocyanin 612 Phycocyanin 645 Phycoerythrocyanin	Cyanobacteria Red algae Glaucocystophytes Cryptomonads
	Cys-PCB	643		
Phycoerythrobilin	PEB	550–568	C-phycoerythrin CU-phycoerythrin b-phycoerythrin B-Phycoerythrin R-Phycoerythrin CU-Phycoerythrin R-Phycocyanin Phycoerythrin 545 Phycoerythrin 566	Cyanobacteria Red algae Cryptomonads
	Cys-PEB DiCys-PEB	550 550		
Cryptoviolin or phycobiliviolin	CV, PXB	540–565	Phycoerythrin 545 Phycocyanin 612 Phycocyanin 645 Phycoerythrocyanin	Cryptomonads Cyanobacteria
Phycourobilin	PUB	about 495	B-phycoerythrin R-phycoerythrin CU-phycoerythrin	Red algae Cyanobacteria
Dihydrobiliverdin	Cys-DBV Di-Cys-DBV	562 562		Cryptomonads
Mesobiliverdin	Cys-MBV 684	684		Cryptomonads
Bilin 584	Cys-Bilin 584	584	Phycocyanin 569 Phycocyanin 569	Cryptomonads
	DiCys-Bilin	584		
Bilin 618	Cys-Bilin 618	618		Cryptomonads
Bilin 697	697B	697	Phycocyanin 645	Cryptomonads

suggested that CpcE and CpcF comprise the enzyme or lyase responsible for the correct attachment of PCB to apophycocyanin at αCys82 (Fairchild and Glazer, 1994).

Two genes that encode a possible phycoerythrocyanin lyase were found in *Anabaena* sp. PCC7120, *pec*E and *pec*F (Swanson et al., 1992). They are located downstream of the genes encoding the structural elements of the phycobilisome and show a high degree of identity to *cpc*E and *cpc*F. Interposon mutants demonstrated a reduction of phycoerythrocyanin. The β^{PEC} subunit was found in reduced

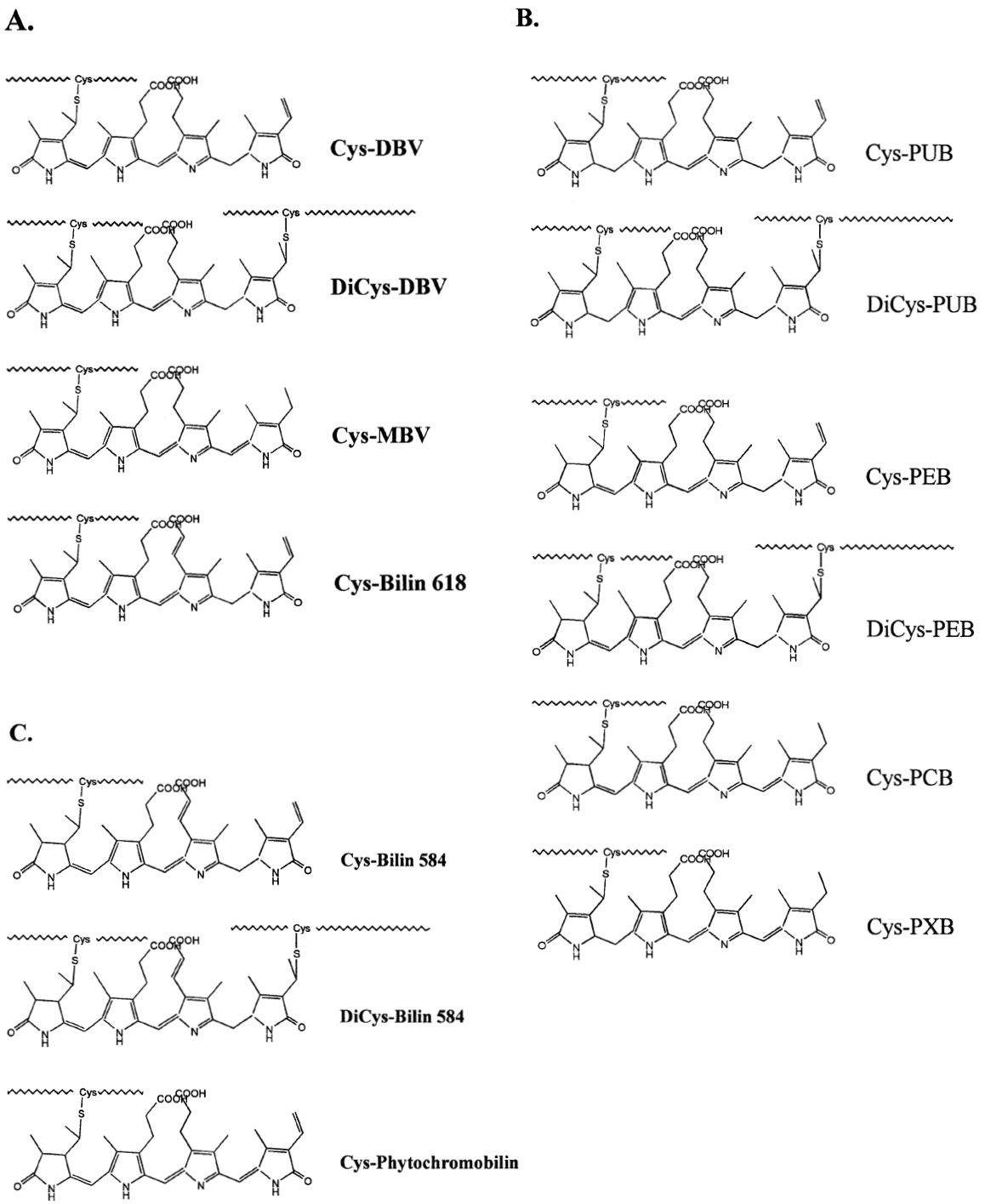

Fig. 6. Structures of phycobiliprotein bilins. Panel A) dihydrobiliverdin (DBV), mesobiliverdin (MBV) and bilin-618 – common cryptomonad bilins and isomers. Panel B) phycourobilin (PUB), phycoerythrobilin (PEB), phycocyanobilin (PCB) and phycobiliviolin (PXB, also known as cryptoviolin or CV) – common red, glaucocystophyte and cyanobacterial bilins and isomers. PXB is also found in cryptomonads. Panel C) Bilin 584 and phytochromobilin – cryptomonad bilin isomer of phytochromobilin.

amounts and the α^{PEC} subunit had phycocyanobilin present instead of the normally attached bilin, phycobiliviolin. These results suggested that in the absence of the appropriate lyase, and because there exists a high degree of identity between the α subunits of PEC and PC, that the phycocyanin lyase encoded by *cpc*E/F could attach phycocyanobilin to the apophycoerythrocyanin α subunit. It was proposed recently that PecE and PecF act in concert to not only in attachment of the phycocyanobilin to the α^{PEC} subunit, but also to catalyze its isomerization to the native phycobiliviolin chromophore (Swanson et al., 1992).

The combined data briefly described above suggest that distinct bilin lyases mediate the correct attachment of bilin to the apoprotein. The three dimensional structure of the apoprotein is necessary for bilin attachment and certain amino acids close to the cysteinyl binding site may play critical role in creating a bilin binding site and orchestrating the correct lyase-mediated attachment of bilin. Little information is available on these processes in red, glaucocystophyte and cryptomonad algal systems.

C. Bilin Synthesis

Bilin biosynthesis in *Cyanidium caldarium* has been proposed to follow the following sequence: 1) conversion of protoheme to biliverdin IXα catalyzed by heme oxygenase, 2) a two-electron reduction to yield 15, 16-dihydrobiliverdin IXα, 3) a two electron reduction to yield (3*Z*)-phycoerythrobilin, 4) isomerized enzymatically to (3*Z*)-phycocyanobilin, 5) enzymatic isomerization to (3*E*)-phycobilins, then 6) ligation into apoproteins by specific lyases (Rhie and Beale, 1994). This is shown schematically in Fig. 7.

The role of heme oxygenase in the unicellular red alga *Rhodella violacea* was recently explored for both its genetics and control (Richard and Zabulon, 1997). These authors isolated the *pbs*A gene, which encodes the heme oxygenase. The chloroplastic gene *pbs*A is split into three distant introns that encode a 27 kDa protein demonstrating strong homology to previously isolated red algal and cyanobacterial heme oxygenases (Cornejo and Beale, 1988). The chloroplastic origin of the *pbs*A gene in *R. violacea* and *Porphyra purpurea* contrasts with the likely nuclear origin of this enzyme in *C. caldarium* (Rhie and Beale, 1994). Light appears to induce heme oxygenase production in *C. caldarium*, resulting in an accumulation of phycocyanin (Rhie and Beale, 1994). The *pbs*A gene from *R. violacea* was under transciptional control responsive to iron-stress (Richard and Zabulon, 1997). Richaud and Zabulon proposed a role for heme oxygenase in red algae in the sequestering of available iron, deduced from the control of the gene at the messenger RNA level by iron-stress (Richard and Zabulon, 1997).

The synthetic pathway for bilins was investigated using crude cell extracts from both red algal and cyanobacterial systems. Extract from the unicellular red alga *Cyanidium caldarium* was found to catalyze the synthesis of the 3*Z* and 3*E* isomers of phycocyanobilin (Beale and Cornejo, 1984). A recent study showed, for the first time that cell-free extracts from Cyanobacteria were capable of conversion of biliverdin IXα to bilins (Cornejo and Beale, 1997). Similarities and differences were observed between extracts from the prokaryotic *Synechocystis* strains PCC6803 and PCC6701 and eukaryotic red algae. All indicate that the enzymes involved in the synthetic pathway are soluble and require two reductants for full activity (in vitro, ferredoxin (reduced) and either ascorbate or a vitamin E analog were used). The cyanobacterial cell-free extracts were more efficient with vitamin E analog, while ascorbate and the vitamin E were equally effective in the red alga. Sensitivity to inhibitors and specificity for the final product produced closely mimicked the physiological activity of the cell.

VIII. Energy Transfer

One of the areas that make phycobiliproteins so fascinating is their ability to efficiently transfer energy down an energy gradient to PS II. Energy transfer has been studied in a variety of different organisms both in situ and in vitro. Low temperature fluorescence measurements have been the method of choice for in vivo work, looking at the effect of uncouplers and other agents on energy transfer. Molecular manipulation has allowed for more precise manipulation around specific bilins leading to further elucidation of the transfer pathway.

The presence of multiple bilins in each phycobiliprotein allows for complex energy transfer within each protein and, subsequently, to adjacent bilins or energy sinks (e.g. PS II). Bilins that absorb higher energy (shorter wavelength) light, known as either donors or sensitizing bilins, transfer energy non-

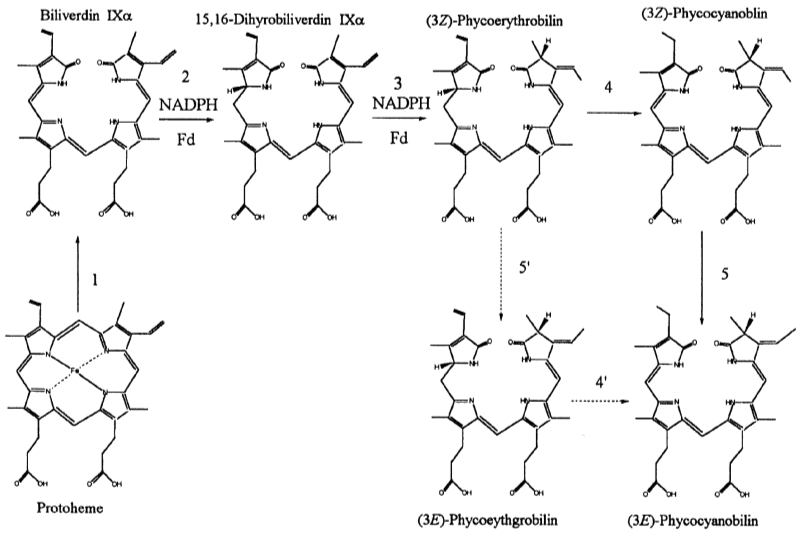

Fig. 7. Schematic representation of phycobilin biosynthetic pathway (Rhie and Beale, 1994). The reactions are catalyzed by (1) heme oxygenase, with nicotinamide dinucleotide phosphate reduced (NADPH) and ferredoxin (Fd) as cofactors (2) biliverdin IXα 15, 16-reductase with NADPH and Fd as cofactors (3) 15, 16-dihyrobiliverdin IXα pyrrole-reductase with NADPH and Fd as cofactors, (4) phycoerythrobilin/phycocyanobilin isomerase then (5) phycobilin ethylidine *cis-trans* isomerase. Reactions 4′ and 5′ are possible alternative pathways.

radiatively to longer wavelength bilins, known as acceptor or fluorescing bilins, that emit absorbed energy as longer wavelength light or pass it non-radiatively to adjacent bilins or chlorophylls. The phycobilisomes of Cyanobacteria, glaucocystophytes and red algae transfer light energy predominately to PS II (Mullineaux, 1999). However, recent research suggests that at least a portion of the energy transferred by the phycobilisome reaches PS I (Mullineaux, 1999; Chapter 13, Larkum).

A. Red Algae

Red algae have simple thylakoid membranes in relation to higher plants, with thylakoids that resemble the stromal lamellae of higher plants (Gantt, 1969). The structure of four marine red algal species was recently elucidated and compared with the more extensive literature on *Porphyridium cruentum* (Tsekos et al., 1996). *Porphyra yezoensis*, *Porphyra leucosticta*, *Erythrocladia subintegra* and *Pterothamnion crispum*, demonstrated a range of characteristics representative of red algal species. *P. yezoensis* and *E. subintegra* have hemiellipsoidal phycobilisomes ranging in size from length of 52.8 nm, thickness 24.4 nm, height 34.6 nm to 45.1, 23.1 29.4 nm, respectively. The phycobilisomes isolated from *Pterothamnion crispum* were hemiellipsoidal. All of the phycobilisomes were arranged in rows that were separated from each other by 60–80 nm. The density of phycobilisomes for *Porphyra yezoensis* on the thylakoid membrane was found to be 770 μm^{-2} of membrane surface. The number of phycobilisomes was smaller than the number of PS II particles observed 1270 per μm^{-2}. This provided a ratio of 0.6 phycobilisomes per PS II complex, which was found to be nearly the same in the other red algal strains studied (range from 0.6–0.7). These observations agreed with prior results with *Porphyridium cruentum* and *Antithamnion glanduliferum*, which reached a ratio of 0.5 (Lichtle and Thomas, 1976). The close order in rows indicates that the PS II particles and phycobilisomes are tightly linked to one another. However, the low ratio of phycobilisome to PS II complex indicates not all PS II complexes are linked to phycobilisomes.

B. Cryptomonads

Energy transfer has been much less studied in cryptomonad algae than in Cyanobacteria and red algae (note that even less is known about glaucocystophytes, although it can be presumed to be like that in Cyanobacteria with phycocyanobilin systems).

The unique bilins present in the cryptomonads (MacColl et al., 1999b) and their characteristic of having a single phycobiliprotein type in each organism (Hill and Rowan, 1989) makes their energy transfer much different to that observed in other phycobiliprotein-containing algae. A proposal for the transfer of energy within the bilins of Cr-PC_{612} was made that incorporates the transfer of energy between the cryptoviolin (CV, also known as phycobiliviolin or PXB) to the phycocyanobilin (PCB) via weak dipole coupling energy transfer events, then transfer for a delocalized PCB pair by a weak dipole coupling event; the photon is then emitted from this delocalized bilin pair (MacColl and Guard-Friar, 1983a). Transient absorption of between 8 to 15 picoseconds (ps) lifetime was observed in Cr-PC_{645} from *Chroomonas* sp. (Holzwarth et al., 1983; Kobayashi et al., 1979). Studies on Cr-PC_{612} from *Hemiselmis* sp. provided relaxation times of 7–10 ps for energy transfer between the short-wavelength (s) and long-wavelength (f) emitting states (Csatorday et al., 1987; Hanzlik et al., 1985). Cr-PE_{566} from *Cryptomonas ovata* was found to have energy kinetics of about 30 ps (Guard-Friar et al., 1989). A study using time-resolved methods concluded that the Förster energy transfer model combined with exciton relaxation on the order of tens of picoseconds was a general method among phycobiliproteins (Malak and MacColl, 1991).

IX. Applications/Industrial Uses

A. Phycobiliproteins

The phycobiliproteins are used by Cyanobacteria and by red, glaucocystophyte and cryptomonad algae to efficiently capture light energy for photosynthesis. However, their uniqueness as pigments has 'interested' other organisms in their possible use, not least, of which is *Homo sapiens*. However, we are not the only non-algal species to make use of phycobiliproteins, as two recent studies report (Coelho et al., 1998; Prince et al., 1998). The aquatic snail *Aplysia californica* utilizes R-phycoerythrin obtained in its diet for the production of defensive ink. *A. californica* digests the phycobilisomes within specialized rhodoplast digestive vacuoles. The vacuole is then the site of further modification of R-PE to produce phycoerythrobilin that is then mixed with a protein secreted by the snail and stored in secretory vesicles used for defense against predators.

Humans have found less physiological uses for phycobiliproteins as fluorescent labels (Fukuda et al., 1998; Corver et al., 2000), food colorants (Akhilender et al., 1999), antioxidants (Rimbau et al., 1999; Tapia et al., 1999; Lissi et al., 2000) and assorted other uses (Glazer, 1999). Phycobiliproteins have been widely applied in immunodiagnostic assays after a series of patents by Stryer et al. outlined the potential promise of these dyes (Stryer and Glazer, 1985; Stryer et al., 1985, 1989). R-PE is the most widely used phycobiliprotein for this purpose, owing to its fairly large absorbance at 498 nm (attributed to phycourobilin in the γ subunit). The phycourobilin absorbance coincided nicely with the 488 nm laser line of the argon laser, which was installed as standard equipment in flow cytometers at the introduction of this dye. As less expensive lasers have been and continue to be introduced that excite at other wavelengths, the other phycobiliproteins have gained an increased presence in flow cytometry. The Helium Neon (HeNe) laser lines at 612 and at 633 nm have been used with allophycocyanin-labeled antibodies with great success (Corver et al., 2000; Schmid et al., 2000). Recent introduction of the ytterbium aluminum garnet laser (YAG) and the green HeNe laser have opened opportunities for B-phycoerythrin and some of the cryptomonad phycobiliproteins that excite well at 545 nm (Telford et al., 2001). To extend the utility of phycobiliproteins in specific binding reactions, they have been conjugated to other fluorescent dyes to extend the emission wavelengths out much farther to the red. Conjugates have been made to other phycobiliproteins such as APC/R-PE (Glazer and Stryer, 1983) and other organic dyes such as R-PE/Cy5 (Waggoner et al., 1993; Sgorbati et al., 2000) and APC/Cy7 (Roederer et al., 1996). Phycobiliproteins remain the most intense direct fluorescent labels available. The recently commercialized use of APC as the acceptor for Europium emission in time-resolved fluorescence assays threatens to unseat R-PE as the most widely utilized phycobiliprotein (Park et al., 1999; Zhang et al., 2000). Both Packard Instruments and Perkin Elmer Lifesciences have high throughput drug screening formats based on time-resolved fluorescent energy transfer from lanthanide chelates or cryptates to conjugates utilizing chemically stabilized APC. These assay platforms, based on the use of APC conjugates, are gaining importance in drug discovery as the need for high sensitivity and rapid turnover increases. Phycoerythrin is being utilized for its high

fluorescence intensity on DNA arrays as a secondary label that is then imaged using charge coupled devices (CCD) or laser scanning imagers (Livache et al., 1998; Morseman et al., 2000). Several recent patent applications indicate a continuing interest in the use of phycobiliproteins for high sensitivity assays. A recent patent was issued on the use of phycobiliproteins in the presence of its corresponding linker polypeptide in specific binding assays with an implied increase in sensitivity (Mathis, 2000). Another invention was described that utilized a large amino dextran polymer on which is immobilized a large number of phycoerythrins in order to achieve large increases in the fluorescent intensity of the phycoerythrin on a binding event basis, essentially by mass action (Siiman et al., 1999).

B. Phycobilisomes

Chemically stabilized phycobilisomes were recently introduced as fluorescent detection reagents (Zoha et al., 1999). They require chemical stabilization in order to withstand the conditions normally used in binding assays (Cubicciotti, 1997). Phycobilisomes potentially have the ability to deliver a huge amount of fluorescence intensity per binding event as well as an enormous Stokes shift. This is reflected in a number of recent application papers on the use of chemically stabilized phycobilisomes (Zoha et al., 1998a,b, 1999; Morseman et al., 1999). The chemically stabilized phycobilisome, designated PBXL dye, was used for detection of thyroid stimulating hormone in microplate format and found to deliver sensitivity equivalent to the chemiluminescent methods currently in use (Zoha et al., 1998a). A recent report indicates that PBXL-1, based on phycobilisomes containing B-PE, R-PC and APC, gave improved sensitivity in Western blot imaging (Gingrich et al., 2000)

C. Bilins

The use of isolated phycobilin chromophores has been demonstrated in a format designed for gene expression detection (Lagarias and Murphy, 1998). This format, termed Phytofluors, utilizes a cassette expressing the phytochrome gene when the inserted gene is expressed. Exogenous addition of phycoerythrobilin to the transformant expressing a target protein, allows a highly fluorescent adduct to form with the coexpressed apophytochrome which can be measured in a fluorometer for quantification of gene expression. This method is currently in development as a commercial alternative to green fluorescent protein use in gene expression quantification. This process offers the advantage of higher sensitivity, but the disadvantage of having to add the bilin exogenously. A recent report of an alternative system being developed that would allow in vivo covalent attachment of the bilin by a lyase also has potential as a gene expression detection format if completed (Schrodeder, 1997).

The area of phycobiliprotein research is still undergoing considerable scrutiny. As the tools for red algal genetic manipulation become available it is certain that additional structure/function relationship studies will be done to confirm the predictions made from a judicious comparison of red algal systems to existing cyanobacterial research. Efforts in the cryptomonad area will also intensify as these unique organisms are carefully compared to results from red algal and cyanobacterial studies, and their unique phycobiliprotein structures promise to reveal new secrets as research continues into this new millennium.

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Chapter 15

Carotenoids of Light Harvesting Systems: Energy Transfer Processes from Fucoxanthin and Peridinin to Chlorophyll

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Summary

The functions of carotenoids in photosynthesis, particularly fucoxanthin and peridin, are considered on the basis of energy diagrams of carotenoid molecules, and the observed kinetic and spectral properties. The energy transfer pathways and mechanisms of energy transfer from carotenoids to (B)Chl *a* are considered from theoretical and experimental points of view, and it is concluded that the transfer mechanism is via a Coulomb interaction. This contradicts previous assumptions based only on a theoretical point of view; however, a complete description of the phenomenon in the pigment-protein complexes is beyond our expectations at present and should drive future studies to explore these phenomena in more detail.

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I. Introduction

Carotenoids are present in all photosynthetic organisms and are particularly diverse in algal photosynthesis. Carotenoid-less organisms (or mutants) can survive only under a very low light intensity, because carotenoids are essential for protection of the light-induced damage to organisms. Carotenoids have at least four kinds of functions in photosynthesis; (1) quenching of the triplet state of chlorophyll (Chl), (2) quenching of singlet oxygen formed by various metabolic and photochemical reactions, (3) quenching of the singlet state of Chl, and (4) sensitizing of the singlet state of Chl (antenna function). Radical scavenging is the fifth function. The first two functions are common to almost all carotenoids; by contrast, the last two functions depend on molecular structure, especially the length of conjugated double bond and substituted atom(s) or group(s) on side chain(s). Functions of carotenoids in photosynthesis are much easier to understand by comparing the energy levels of Chl, carotenoids and oxygen. This will be discussed in Section IV.

In terms of antenna function, the importance of carotenoids is much higher in algae than in land plants because the light environment in the water column is very different from that on land; UV light and red light cannot attenuate deeply into water, therefore blue light is the major light source for photosynthesis in deep water. The pigments responsible for light absorption in this wavelength region are carotenoid(s). In this sense, selection of carotenoid species is critically important for the survival of algae.

In this chapter, we concentrate on fucoxanthin and peridinin that are well known efficient antenna pigments in specific algal classes. Optical properties and energy transfer processes of the two are well understood recently on a molecular basis. Before going onto this point, however, it is very important to understand two points about photosynthetic carotenoids; these are firstly the distribution of carotenoids in algal classes in relation to taxonomy and secondly, the optical properties of carotenoids which are specifically and very closely related to their functions in photosynthesis. Other properties of carotenoids in algal photosynthesis are reviewed in other articles (Rowan, 1989; Frank et al., 1999; Hiller, 1999)

Abbreviations: Chl – chlorophyll; FCP – fucoxanthin chlorophyll protein; LHC – light-harvesting chlorophyll protein; PCP – peridinin chlorophyll protein; rRNA – ribosomal ribose nucleic acid; TAC – time-to-amplitude converter

II. Distribution of Carotenoids in Algae

Figure 1 shows distribution of Chl and carotenoids in relation to algal taxonomy. The phylogenetic tree drawn on the basis of a molecular marker (16s rRNA or 18s rRNA) indicates that eukaryotic algae are classified into at least six different groups. Glaucophyta, Rhodophyta, and Chlorophyta are descendants of the primary symbiosis (Chapter 1, Douglas et al.) and the others come about by the secondary symbioses. The distribution of Chl and carotenoids is known to be a good index for classification of algae. The molecular structure of the four known chlorophyll types is given in Chapter 13 (Larkum). Carotenoids are classified to three categories depending on molecular structures (Fig. 2); one is linear polyenes with or without a ring structure as an end group. The second one includes an allene group in their conjugated double bond (allenic carotenoids). Molecules having an allene group are very rare in biological materials. The third one includes an acetylene group (acetylenic carotenoids). Even though the biosynthetic pathways of allenic and acetylenic are not yet known, it is supposed that acetylenic carotenoids are synthesized from allenic carotenoids. Since the biosynthetic pathways of allene and acetylene groups are mediated by enzymes, the presence of enzymes or genes responsible for individual steps will likely be discovered in the near future. Therefore distribution of carotenoids having different molecular structures and their biosynthetic pathways can be an index for classification of algae.

In prokaryotes, allenic and acetylenic carotenoids are not present (Fig. 1), and this is also the case for Glaucocystophyta, and red algae. Algae in other groups contain allenic or acetylenic carotenoids. In chlorophyta, allenic carotenoids are frequently found but there is no indication for the presence of acetylenic carotenoids, therefore an enzyme(s) for allene formation must have been acquired during the evolutionary stage to Chlorophyta. In chromophyta (Chapter 1, Douglas et al.), allenic carotenoids are major, for example, fucoxanthin in brown algae and diatoms. In this broad group of algae, acetylenic carotenoids are also present.

There is the other classification of carotenoids depending on presence of an oxygen atom, that is,

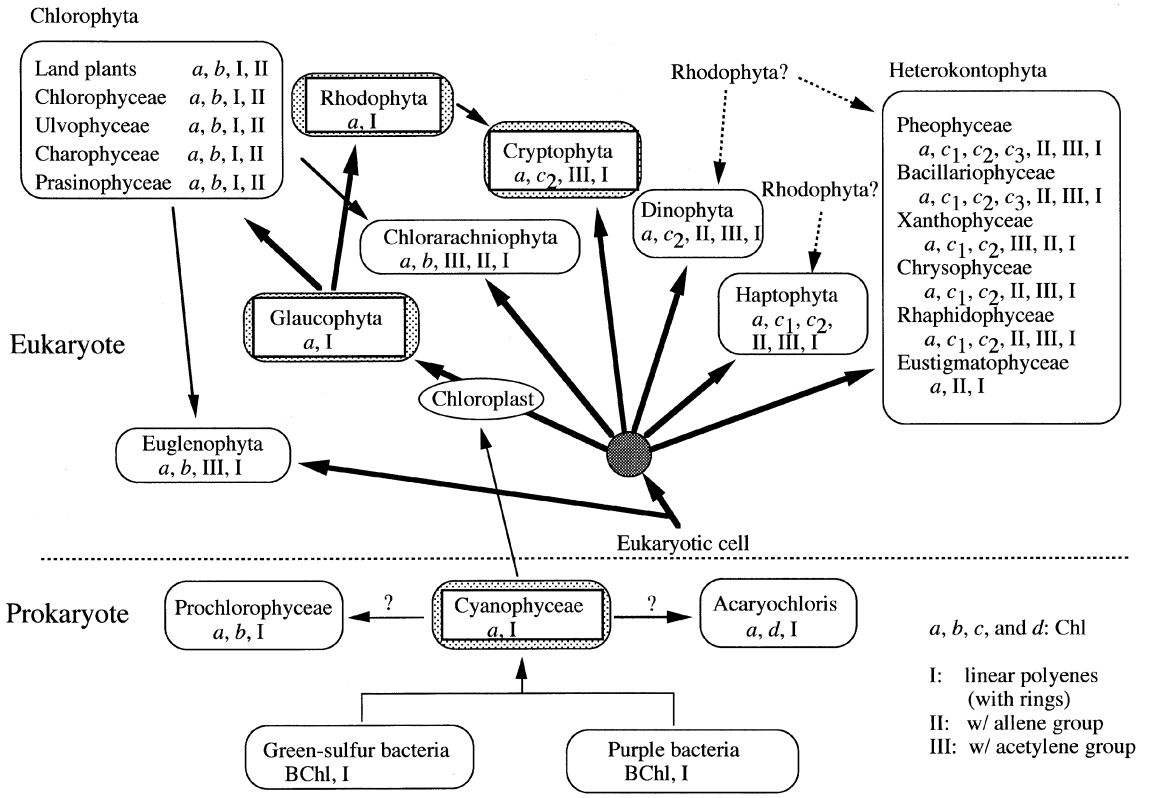

Fig. 1. Distribution of chlorophylls and carotenoids in algae. Characters in italic stand for molecular species of chlorophylls. I, II and III stands for carotenoids of linear polyene, with an allene group and with an acetylenic group, respectively (Fig. 2). 'Rhodophyta?' means a possible origin of symbiont. Cyanophyceae = Cyanobacteria.

carotenes and xanthophylls; the former consists of hydrocarbon and the latter contains an oxygen atom. In all oxygenic photosynthetic organisms, at least one species of carotenes and one species of xanthophylls are present. In general, carotene is bound to reaction center complexes, and xanthophylls, to antenna pigment-protein complexes. β-carotene is a typical example found in RC complexes of almost all oxygenic photosynthetic organisms; only in a limited number of exceptions, is α-carotene associated with RC complexes—see Chapter 3, Partensky and Garczarek. On the other hand, there are several xanthophylls that are specific to respective classes of algae, for example, fucoxanthin in brown algae and diatoms, peridinin in dinoflagellates, diadinoxanthin and/or diatoxanthin in Cryptophyta and Euglenophyta, lutein in green algae. These molecular species are one of the key features for classification. Among these, a few xanthophylls, i.e., fucoxanthin and peridinin, are known to be efficient antenna (quantum yield of energy transfer $\phi_{tr} \cong 1$); however a few kinds of xanthophylls are not necessarily good antenna pigments ($\phi_{tr} \leq \phi\, 0.5$).

III. Optical Properties of Carotenoids in Relation to Functions

Solutions of carotenoids usually look yellow or orange, and sometimes pink. This indicates that these solutions absorb light in the region of violet and blue, that is, in the wavelength region shorter than 500 nm. Absorption of light in this wavelength region leads to transition from the ground (S_0) state to the second singlet excited (S_2) state, because the transition to the first singlet excited (S_1) state is one-photon forbidden due to symmetry of the electronic state (Fig. 3). In the terminology of the group theory, the ground (S_0) state corresponds to the $1A_g$ state, the S_1 state, to the $2A_g$ state, and the S_2 state, to the $1B_u$ state (Hudson et

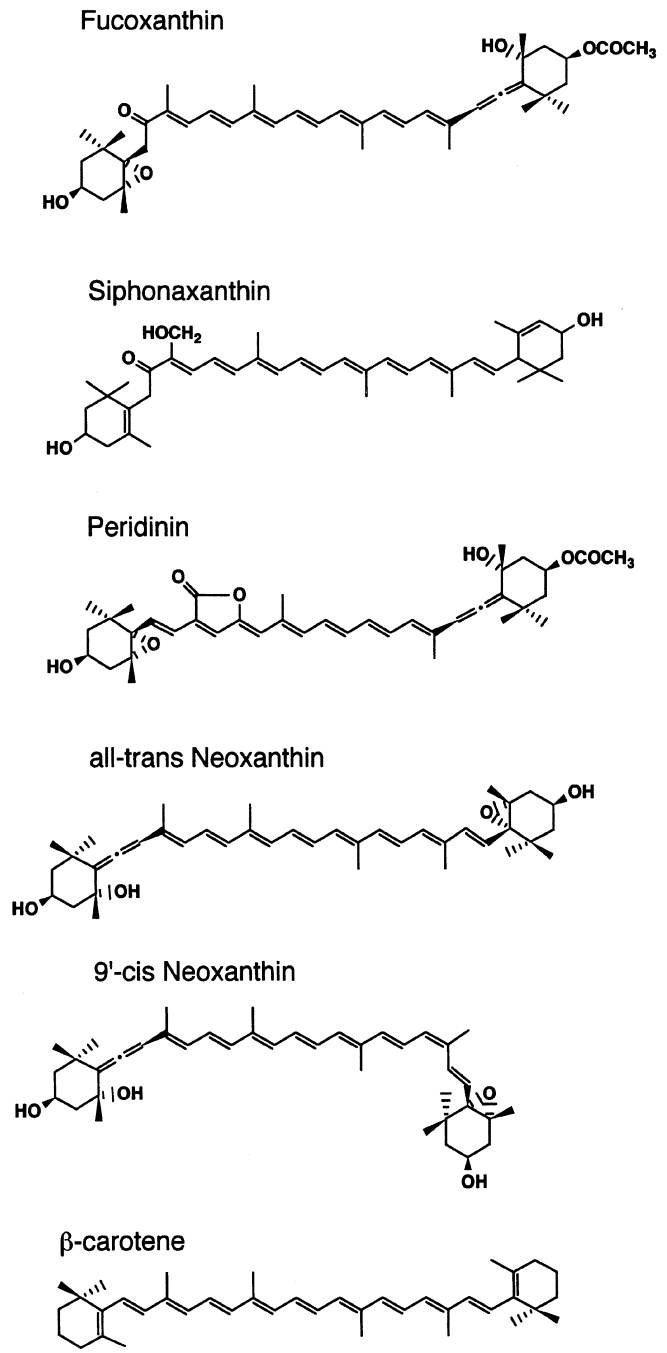

Fig. 2. Molecular structures of several carotenoids characteristic to algae.

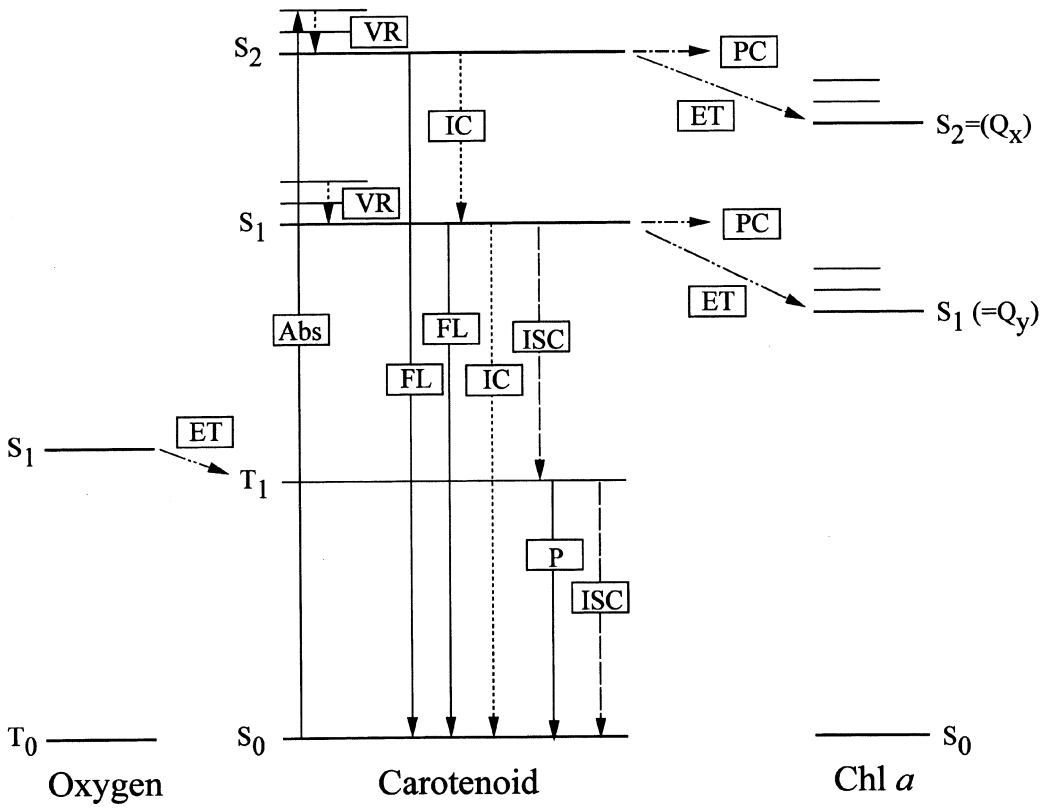

Fig. 3. Energy diagram of carotenoids and relaxation pathways. Arrows indicate excitation and relaxation pathways in carotenoids. Abs, FL, VR, IC, ISC, PC, ET, and P stand for absorption of light, fluorescence, vibrational relaxation, internal conversion, intersystem crossing, photochemistry, energy transfer, and phosphorescence, respectively. Full lines represent radiative processes and broken lines, non-radiative processes. Energy levels of Chl *a* and oxygen are also shown. Arrows with ET indicate the energy transfer pathways from carotenoids to Chl *a* and from the singlet oxygen to carotenoids.

al., 1982). Transition to the same symmetry group is forbidden (from the $1A_g$ state to the $2A_g$ state, for example). Therefore, excitation by visible light induces transition to the S_2 state or a higher state.

A. Absorption Properties

The peak location of absorption maximum correlates primarily with a number of conjugated double-bond (N) (Fig. 4). When N is large, the peak locations shift to the red, and this tendency is the same for S_1 and S_2 states. The peak location is also correlated with the solvent polarizability (α) (Fig. 4). The α is expressed by $(n^2-1)/(n^2+2)$ where n is a refractive index of solvents. When α is large, the absorption maximum shifts to the red (Mimuro et al., 1993b). The absorption spectrum of carotenoids in solutions shows several bands in the visible region, and those correspond to vibronic bands of the S_2 state (Fig. 5). The energy level of the S_2 state is described by the location of the band in the longest wavelength ($S_{2,0}$). As for intensities of vibronic bands, the second band ($S_{2,1}$) from the long wavelength is, in general, strongest. The shapes of the vibronic bands strongly depend on molecular structures of carotenoids and solvents. In general, vibronic bands are clearly seen in linear polyenes, such as oscillaxanthin in some Cyanobacteria and neurosporene in photosynthetic bacteria. When a ring structure is included as an end group, vibronic structure becomes vague. When an oxygen atom is included in the conjugated double bond, such as a keto-carbonyl group, vibronic structure is not necessarily seen. Vibronic structure is clearly seen in non-polar solvents, on the other hand, it is not clearly seen in polar solvents, for example, in alcohol. At low temperature, the vibronic structure becomes

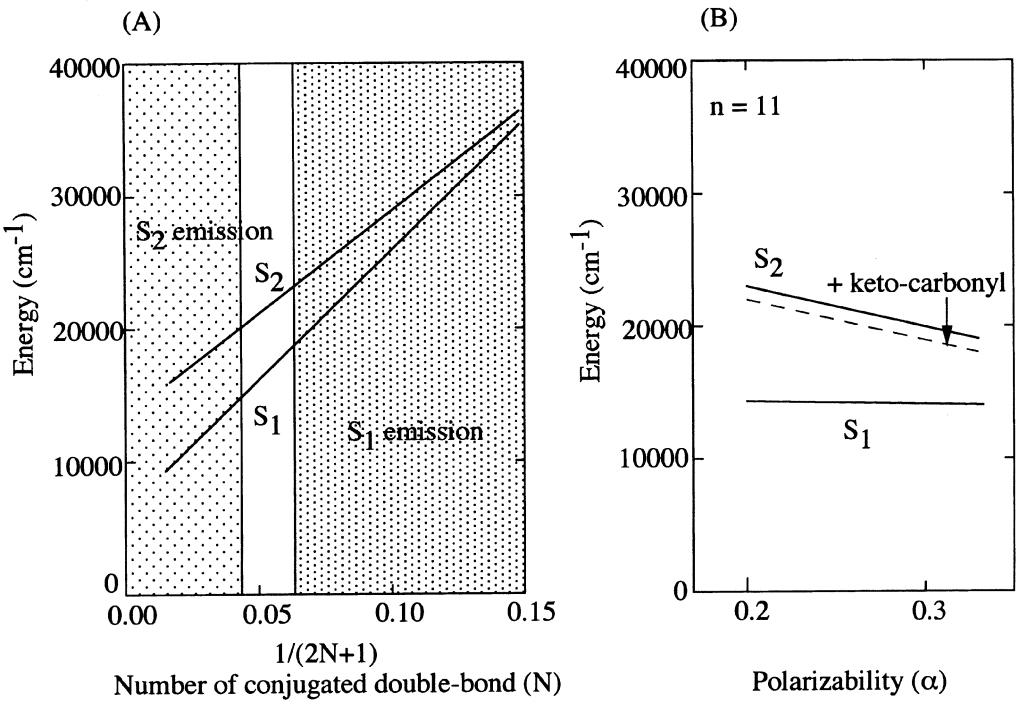

Fig. 4. Energy levels and origins of fluorescence as a function of conjugated double bond (N) of carotenoid molecules. (A) Energy levels are shown by straight lines for the S_1 and S_2 states. Areas with shadow indicate the origins of fluorescence. A clear area between the two indicates that origins of fluorescence are not strictly determined, but are dependent on solvent(s) and other factor(s). (B) Energy levels are given as a function of solvent polarizability (α). Effect of a conjugation with a keto-carbonyl group is also shown.

clear and the ratio of intensities of those vibronic bands will change. In some case, the $S_{2,0}$ band is strongest.

The absorption spectrum of carotenoids in pigment-protein complexes is different from that in organic solvents. In many cases, the maximum is shifted to the red due to a high polarizability of protein moieties. In some cases, a specific absorption band appears only in the pigment-protein complex, i.e., 530 nm band in fucoxanthin-chlorophyll-protein (Fig. 6; Katoh et al., 1989); this might be due to a specific interaction between carotenoid molecules and an amino acid residue(s). A large red shift of neurosporene in the LH2 complex was reproduced by a quinoline solution, aromatic compound, which has a large polarizability (Andersson et al., 1990). On the other hand, absorption of peridinin in the complex (peridinin-chlorophyll *a*-complex, PCP) is very similar to that in methanol, which suggests the local environment of peridinin in the complex is a polar region (Fig. 6).

A transition to the S_1 state is one-photon forbidden, and therefore, in principle it is impossible to detect the absorption band responsible for the transition to the S_1 state. However, the S_1 absorption band is detectable even if its intensity is very low (Mimuro et al., 1993a). For example, in the case of neurosporene, two or three minor bands were observed in the wavelength region from 600 to 700 nm, i.e. very far from the bands of the S_2 state. These were assigned to the S_1 absorption bands. An estimated extinction coefficient of the S_1 band was approximately 10^2, one thousandth of that of the S_2 band (10^5) (Mimuro et al., 1993a). These S_1 bands can also be monitored by fluorescence excitation spectrum (Watanabe et al., 1993) and/or the Raman excitation profile (Thrash et al., 1977). The energy level of the S_1 state is almost insensitive to solvent polarizability. Even though this was not clearly shown experimentally, the peak position of S_1 fluorescence was almost insensitive to solvent polarizability, indicating that the absorption band has the same tendency.

Molecules excited to the S_2 state relax to the S_0 state through the S_1 state or directly (Fig. 3). From

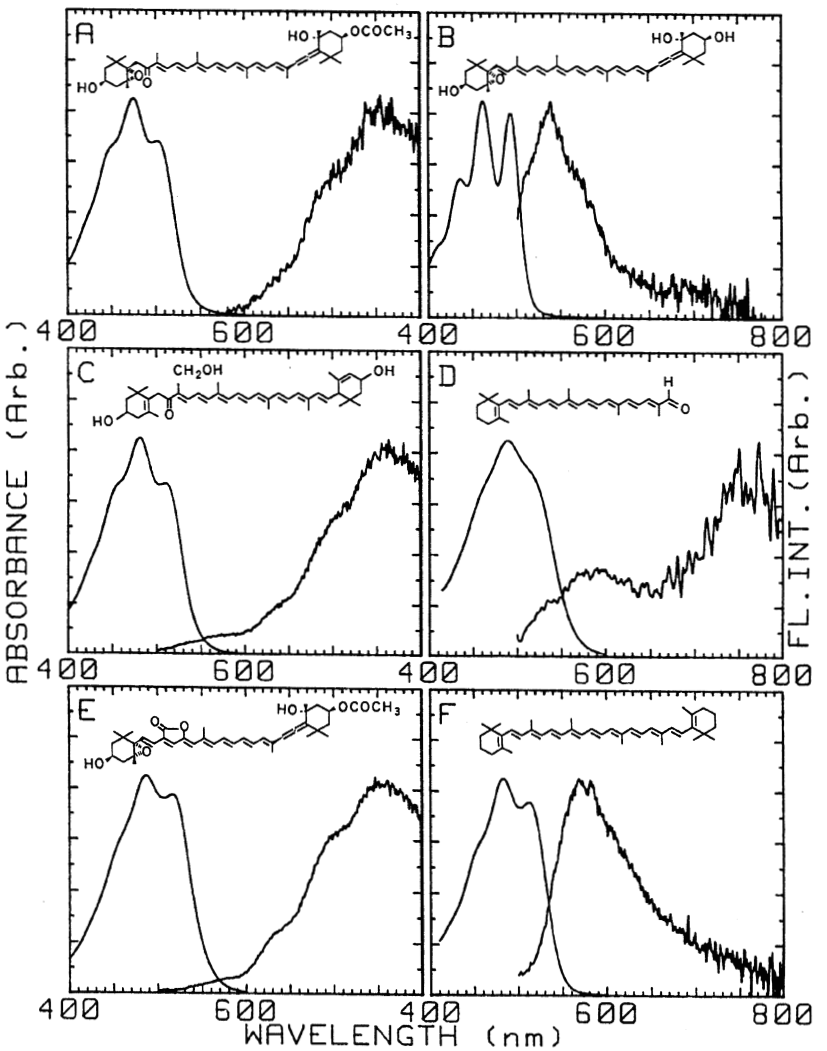

Fig. 5. Optical properties of several kinds of carotenoids in organic solvents. Absorption (left hand side of each graph) and fluorescence (right hand side) spectra in CS_2 at 25 °C. (A) Fucoxanthin, (B) neoxanthin, (C) siphonaxanthin, (D) β-apo-8´-carotenal, (E) peridinin and (F) β-carotene (Mimuro et al., 1992).

the S_2 state, there are at least four relaxation pathways; (1) photochemical reaction, (2) internal conversion to the S_1 state, (3) radiative relaxation (fluorescence) to the S_0 state, and (4) energy transfer if a suitable acceptor is present. To date, radiative relaxation to the S_1 state has not been discovered. It is known that the internal conversion rate is very large, therefore the other decay processes were affected by the internal conversion. Similarly, molecules in the S_1 state relax to the ground state through either of photochemical reactions, internal conversion, radiative relaxation (fluorescence), intersystem crossing (triplet formation), and energy transfer if a suitable acceptor is present. When the triplet state of carotenoids is formed, that can show a very characteristic function due to its long lifetime and energy levels. These energy levels and relaxation pathways from the S_2 and/or S_1 states are very closely related to functions of carotenoids.

B. Fluorescence Properties

Carotenoids are fluorescent; however, their quantum yields of fluorescence are very low, usually less than

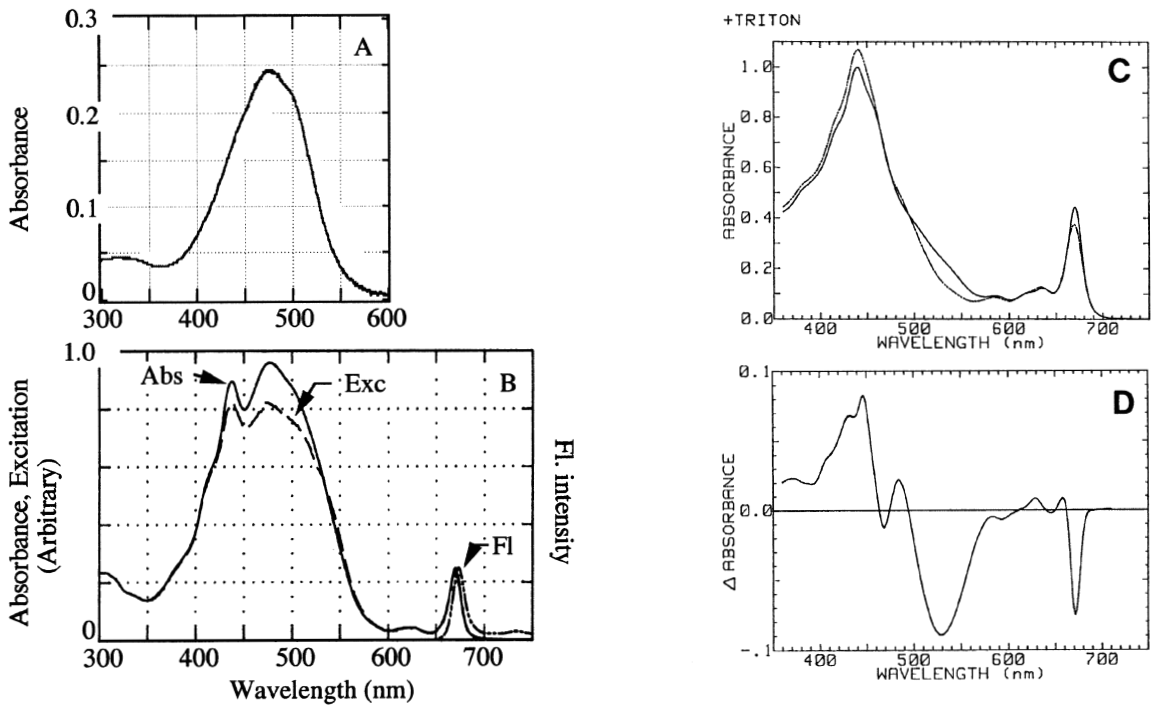

Fig. 6. Changes in absorption spectrum of carotenoid induced by binding to pigment-protein complex. (A) Peridinin in methanol, (B) peridinin in sPCP (Akimoto et al. 1994b), (C) fucoxanthin in FCP (Katoh et al. 1989) with or without Triton X-100, and (D) difference absorption spectrum induced by an addition of Triton X-100 to FCP.

10^{-4}. Therefore, it has been very difficult to detect fluorescence until the middle of 1980s. After an excitation to the optically-allowed S_2 state, carotenoids show dual fluorescence; one is coming from the S_2 state and the other, from the S_1 state (Fig. 3). The contributions of the S_2 and the S_1 fluorescences to the total fluorescence intensity and their quantum yields are affected by several factors, for example, the length of conjugated double bond (Fig. 4), presence of a specific substituted group (Fig. 5), polar property of solvents, and polarizability of solvents. Origins of fluorescence and quantum yield are rather insensitive to temperature.

It is well known that strong fluorescence is observed for short polyenes with a number of conjugated double bond (N) longer than 4. However when N increases, they become less fluorescent. This phenomenon is brought about by two factors; one is the monotonic decrease in the fluorescence quantum yield as a function of N and the other, changes in the origin of fluorescence. When N is shorter than 6, an origin of fluorescence is the S_1 state and a quantum yield is high. When N is larger than 9, the main fluorescence comes from the S_2 state and a quantum yield is very low. When N is 7 or 8, an origin of fluorescence is dual, both from S_1 and S_2 states. N of carotenoids in algal photosynthetic systems are usually between 8 and 11, thus fluorescence from those carotenoids is expected to be weak and come from the S_2 state. However, an origin is drastically changed by molecular structure of carotenoids (Fig. 4). When fluorescence spectra of neoxanthin and fucoxanthin (Fig. 5) were compared, it was shown that the former shows the S_2 fluorescence, on the other hand, the latter, the S_1 fluorescence. The difference in molecular structure between the two is the presence of the keto-carbonyl group conjugated to the conjugated double bond in the latter. The effect of an oxygen atom in the keto-carbonyl group induces a large difference in fluorescence properties (Fig. 5). These data indicate that the electronic states and relaxation pathways of carotenoids are modified by presence of an oxygen atom in the conjugated double bond system, and thus optical properties strongly depend on the molecular structure.

C. Energy Gap Law of Internal Conversion

Relaxation processes of carotenoids are governed by the energy gap law of internal conversion (Englman and Jortner, 1970). The rate constant for the internal conversion (k_{ij}) from the i^{th} to the j^{th} states is written by the following equation.

$$k_{ij} = c \exp(-\gamma \Delta E_{ij}/h\omega_M)$$

where c is a pre-exponential factor related to the electronic coupling matrix element, γ is related to displacement of potential surfaces in individual states, ΔE_{ij} is the energy gap between the i^{th} state and j^{th} state, and $h\omega_M$, the high frequency acceptor mode. For the last term ($h\omega_M$), the C=C stretching mode is known to be the main factor in polyenes (Hudson et al., 1982; Orlandi et al., 1991).

The internal conversion is the fastest relaxation process in the case of polyenes and carotenoids, therefore other processes are governed by the internal conversion. The equation is applicable to the relaxation processes of polyenes from the S_1 state (Fig. 7). Frank and his coworkers showed that the relaxation times of the short polyenes gave a straight line as a function of logarithm of the energy gap between the S_1 state and the ground state (ΔE_{10}) (Frank et al., 1993). They obtained several coefficients for the empirical equation, and applied these to the case of long polyenes. They measured the lifetimes of the S_1 state by transient absorption method, and on the basis of empirical coefficients and measured lifetimes, they estimated the energy gap between the two states that is hard to estimate by absorption spectrum. In 1997, we demonstrated that the energy gap law of internal conversion was applicable to the relaxation from the S_2 state by using β-carotene and its analogues (Fig. 8; Mimuro et al., 1997). The energy gap law of internal conversion, in general, describes the relaxation. However, we also demonstrated that lifetimes of several kinds of carotenoids with different N did not show monotonic decay as a function of N, but the maximum lifetime at approximately N=9 (Fig 8; Akimoto et al., 1999, 2000). This has never been shown before, and therefore it is suggested that the other factor(s) is present, which determines the relaxation processes.

IV. Functions

The functions of carotenoids in photosynthesis are realized mainly through photophysical processes. Radical scavenging is counted for photochemical reaction, and it is not specific to photosynthesis. Figure 3 shows energy levels of carotenoids in relation to chlorophyll and singlet oxygen. On the basis of this energy diagram, it is easy to understand the functions of carotenoids. As has been described, there are at least four functions of carotenoids in photosynthesis; (1) quenching of the triplet state of Chl, (2) quenching of singlet oxygen, (3) quenching of the singlet state of Chl, and (4) sensitizing the singlet state of Chl (antenna function). The first process is the energy transfer from Chl triplet state to Car triplet state, and the second, from singlet oxygen

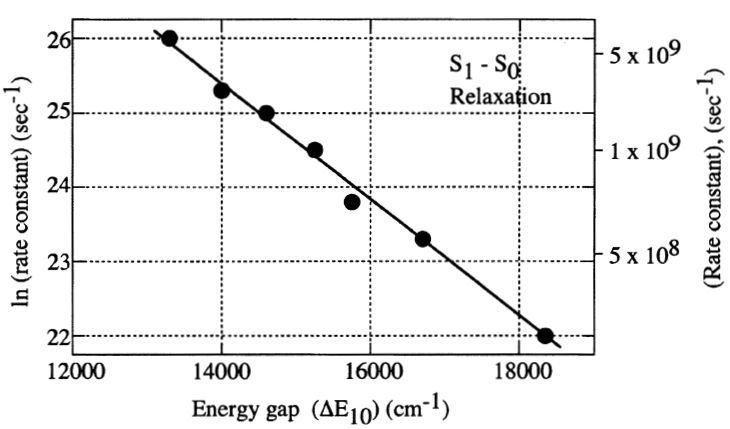

Fig. 7. Rate constants of internal conversion from the S_1 state of carotenoids (k_{10}). Respective points are shown in a natural logarithmic scale as a function of ΔE_{10}. The figure was drawn on the basis of Fig. 3 by Frank et al. (1993).

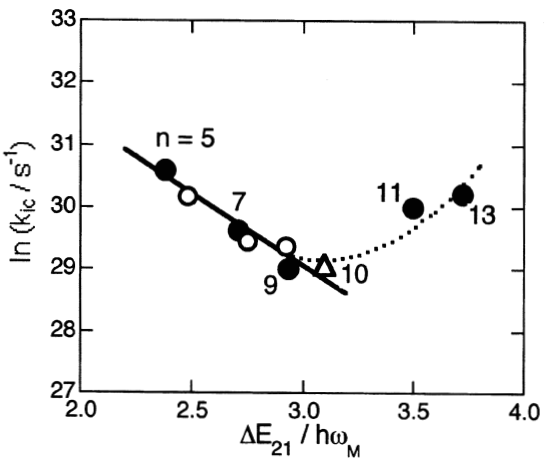

Fig. 8. Rate constants of internal conversion from the S_2 state of carotenoids (k_{21}). Figures are shown in a natural logarithmic scale against $\Delta E_{21} / h\omega_M$. Respective points correspond to linear carotenoids with difference in numbers of conjugated double bond (closed circle, Akimoto et al., 2000), β-carotene and its analogues (open circle, Mimuro et al. 1997), and spheroidene (triangle, Ricci et al. 1996).

to Car triplet state. Since the ground state of oxygen is triplet state, energy transfer from singlet oxygen to Car triplet state is a spin exchange reaction. The third reaction is energy transfer from the Chl singlet state to the Car singlet state, followed by non-radiative relaxation. The fourth function is energy transfer from Car singlet state to Chl singlet state, and there are two possible pathways; one is from the S_2 state of carotenoids to the S_2 state of Chl, and the other, from the S_1 state of carotenoids to the S_1 state of Chl. Both pathways are operative simultaneously depending on molecular species and their spatial geometry. A critical difference between the two singlet excited states is their lifetimes; it is in a range of 200 fs for the S_2 state and in a range from 10 to 100 ps, depending on species, in the S_1 state. Energy transfer is competitive with other relaxation processes, and therefore the transfer efficiency is affected by lifetimes of the excited states. A rather low efficiency is expected for the transfer from the S_2 state, and a high efficiency for the transfer from the S_1 state when it occurs.

Scavenging of radicals is a very important function of carotenoids. Radicals attack unsaturated fatty acid in membranes, which leads to lysis. When carotenoids are present, radicals are scavenged, and thus carotenoids have a protective function. Under high light conditions, carotenoids are synthesized but most of them are not bound to proteins; they are in membranes. These molecules can participate in the scavenging of radicals. This function is not specific to photosynthesis, but is a general function of carotenoids.

V. Antenna Function of Carotenoids in Algae

It is well known that fucoxanthin and peridinin are efficient antenna carotenoids in algae; their energy transfer efficiency is reported to be almost unity. This value is exceptionally high, since for carotenoids it is usually in a range from 50 to 60%. Fucoxanthin is a major carotenoid in brown algae, diatoms, haptophytes, and chrysophytes. Since the primary production of these kinds of organisms is very large in the ocean, they are ecologically very important. Peridinin is a major carotenoid of dinoflagellates that are known to form red tides in the sea and cause toxic blooms harmful to fish and to humans. Recently, considerable data have been accumulated on these two kinds of carotenoids, which has led to understanding of the function and reaction mechanism of carotenoids in general.

A. Static Properties of Pigments

The molecular structures of fucoxanthin and peridinin are unique among carotenoids in plants and algae in terms of an additional conjugation of a keto-carbonyl group (Fig. 2). In fucoxanthin, an allene group is located at one end of a conjugated double bond, and at the opposite end, a keto-carbonyl group is present. An allene group is found only in the carotenoids in biological materials. The plane of the conjugated double bond is rotated by 90° at the site of the allene bond. Thus the planarity of the conjugated double bond system is broken at this site. A keto-carbonyl group is the s-*trans* configuration to the conjugated double bonds, therefore the conjugation is prolonged to the keto site. However, differences in the configuration do not induce significant differences in the optical properties, as shown by a systematic survey on analogues of keto-carotenoids (Yamano et al., 1997). In the case of peridinin, a ring structure at the both ends is very similar to those of fucoxanthin. An allene group is also present. One oxygen atom is located in the furanic ring. This oxygen atom is included in the conjugated double bond system, and the optical properties of peridinin are modified by

the oxygen atom (see the next paragraph). Planarity of these two molecules is broken due to the presence of an allene group and the end group at the opposite side. The π-electron systems of the two carotenoids are asymmetric. These properties are very important when molecular structure in the pigment-protein complexes are considered.

Neoxanthin is a good reference for fucoxanthin, because both molecular structures are similar to each other except for the presence of a keto-carbonyl group only in fucoxanthin. Two molecular forms of neoxanthin are known, all-*trans* and 9′-*cis* forms. Optical properties of these two, however, are similar to each other (Mimuro et al., 1998, Takaichi and Mimuro, 1998); thus comparison between neoxanthin and fucoxanthin will provide essential information of the electronic state (Fig. 5) and thus of the optical properties (see the next paragraph).

B. Pigment-Protein Complex

Fucoxanthin and peridinin are associated with characteristic binding proteins to form pigment-protein complexes (Chapter 4, Durnford). These complexes are not directly associated with reaction center complex and thus are called peripheral antennae. Fucoxanthin is associated with so called FCP (fucoxanthin-Chl *a*/*c*-protein) in thylakoid membranes (Katoh et al., 1989) and FCP belongs to LHC superfamily, an antenna protein of algae and land plants (Durnford et al., 1989); a typical example is LHC II isolated from spinach chloroplasts (Kühlbrandt et al., 1994).

FCP is a major antenna protein in brown algae and diatoms. Its protein-chemical properties and stoichiometry of pigments are not well resolved. A molecular weight of the FCP is approximately 20,000, as similar to LHC II, thus its molecular structure is also expected to be similar to LHC II. A number of pigments associated with FCP is reported to be 4 Chl *a*, 1 Chl *c* and 4 fucoxanthin (Caron et al., 1995).

Peridinin binds to peridinin-Chl *a*-protein, so called PCP (Haidak et al., 1966), and two types of PCP are known in dinoflagellates; one is a water-soluble protein (sPCP) and the other, membrane bound protein (iPCP). The iPCP is reported to be the LHC superfamily protein (Green et al., 1996). Thus, binding site and pigment stoichiometry is conserved in this protein. The sPCP isolated from *Amphidinium carterae* was has a molecular weight of approximately 30 kDa (Hiller et al., 1995), and was crystallized and its three-dimensional structure was resolved (Hoffman et al., 1996).

C. Crystal Structure and Optical Properties of sPCP

The three-dimensional structure of sPCP isolated from the dinoflagellate *A. carterae* was resolved by Hoffman et al. in 1996 (Fig. 9; Hoffman et al., 1996). Three-identical units form a trimer, and each monomer shows a very unique protein structure. In the monomer, a pseudo C_2 symmetry axis is present, and N-terminal half and C-terminal half are similar to each other. Each part consists of 8 α-helices and forms a jellyroll fold, a very unique structure. In the central part, there is a large hole which is occupied by pigments, 2 Chl *a* and 8 peridinins. Mutual distance between Chl and peridinin is close to each other, almost the van der Waals contact. One lipid molecule is closely located to Chl *a*.

The absorption spectrum of sPCP has a strong Q_y band of Chl *a* at 675 nm and several peaks of peridinin in the wavelength region between 550 to 450 nm. The latter bands are classified to several groups, each of which corresponds to molecular forms on the basis of vibronic structure of peridinin (Akimoto et al., 1996). The absorption spectrum of peridinin in sPCP was very similar to that in methanol, indicating the local environment is polar. This is not common in the pigment-protein complexes in photosynthesis.

VI. Electronic States and Dynamic Properties of Molecules

Absorption and fluorescence spectra of fucoxanthin and 9′-*cis* neoxanthin are shown in Fig. 5. The absorption spectrum of 9′-*cis* neoxanthin in CS_2, a non-polar solvent, shows a clear vibronic structure in the S_2 state. The fluorescence spectrum also shows a maximum at 550 nm with a clear vibronic structure; its shape is a mirror image of the absorption spectrum with the strongest intensity of the 0–1 transition. The origin of fluorescence is assigned to the S_2 state by the location of the band. On the other hand, fucoxanthin in CS_2 shows the absorption maximum at approximately the same wavelength as that of 9′-*cis* neoxanthin, but fluorescence maximum is observed at 650 nm, very far from the absorption band. The origin of fluorescence is assigned to the S_1

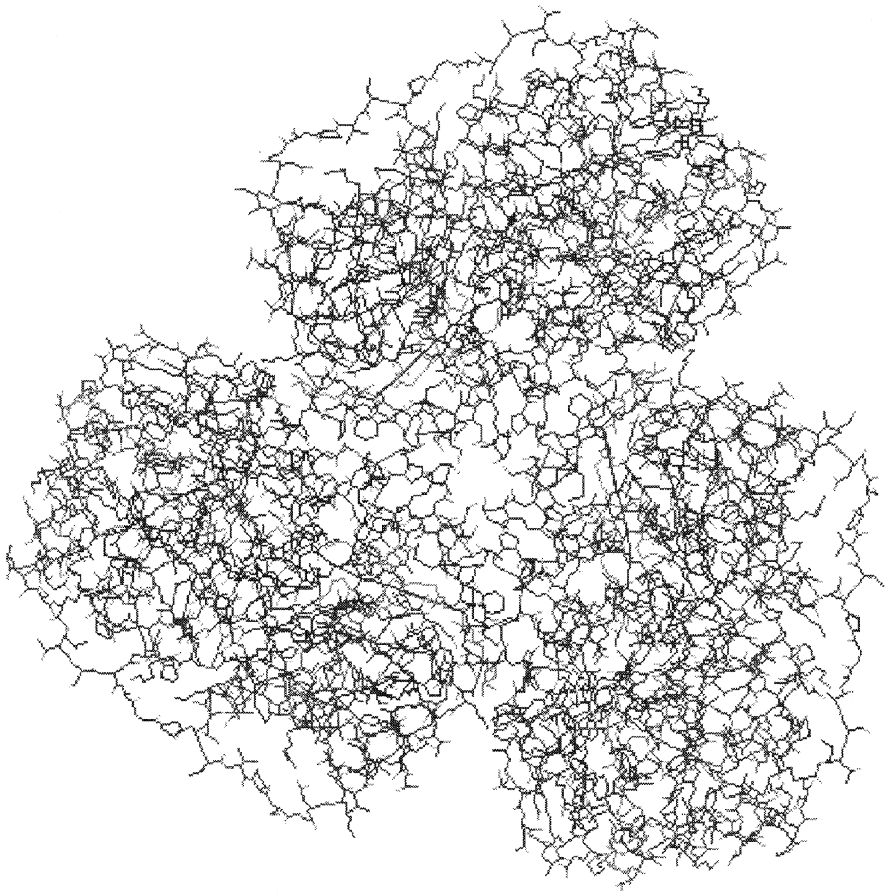

Fig. 9. Crystal structure of PCP. Structures were drawn on the basis of PDB data (1pcp; Hoffman et al. 1996) by using a Ras-Mol program.

state. These results indicate that the absorption peak is determined by the length of the conjugated double bond; however the relaxation processes are determined by other factors. We synthesized several analogues of fucoxanthin to systematically study the relaxation processes of keto-carotenoids (Yamano et al., 1997), and found that substitution of side chain and configuration of a keto carbonyl group to conjugated double bond is not the main factor in relaxation, rather the energy gap and additional factor are essential for relaxation.

As stated previously, relaxation processes of carotenoids are determined by the energy gap law of internal conversion. This is because internal conversion rate is very fast compared with other relaxation processes, and thus the whole relaxation process is influenced by this internal conversion. The energy gap between the S_2 and S_1 states (ΔE_{21}) of fucoxanthin was approximately 3500 cm^{-1}. This small energy gap explains a very fast internal conversion from the S_2 state to the S_1 state in fucoxanthin.

VII. Energy Transfer Processes and Mechanism

The energy transfer mechanism from carotenoids to Chl depends on the energy transfer pathway (Mimuro, 1990a). The transition to the lowest singlet excited (S_1) state of carotenoids is shown to be one-photon forbidden. Therefore when energy transfer occurs from the S_1state, a dipole-dipole interaction is not applicable (Mimuro and Katoh, 1991), because a transition dipole moment of the S_1 state is not

expected. Thus another type(s) of interaction must be involved. It has been suggested that the electron exchange interaction (Dexter mechanism) is likely to occur (Dexter, 1953). When transfer occurs from the S_2 state, a dipole-dipole interaction is applicable.

A. Theoretical Considerations of Energy Transfer Mechanism from Carotenoids to (B)Chl

Energy transfer processes can be considered from a theoretical standpoint. In 1993, Nagae et al. formulated the excitation transfer matrix element applicable to any multiconfigurational wave function (Nagae et al., 1993). On the basis of resultant formulas, the excitation transfer matrix element between the S_2 and S_1 states of neurosporene and the S_2 and S_1 states of BChl *a* was calculated. They showed that the energy transfer between the S_2 states occurs very efficiently, and the transfer between the S_1 states occurs via the Coulomb mechanism including multipole-multipole interaction at a speed more rapid than that via the electron exchange mechanism. Furthermore, they also inferred that when the symmetry of the π-electron system is broken, a small (1%) perturbation of the S_1 state induces a 10% transition dipole moment of the S_2 state, and the expected transfer time would become 180 fs from the asymmetric S_1 state of carotenoid to the S_1 state of BChl *a*. Recently, a few other groups also published on the transfer mechanism (Krueger et al., 1998, Damjanović et al., 1999, Damjanović et al., 2000), and Coulomb interaction is postulated to be the transfer mechanism.

B. The Actual Energy Transfer Process

The energy transfer process from peridinin to Chl *a* was investigated on sPCP isolated from the dinoflagellate *Alexandrium tamarense* by fluorescence up-conversion technique (Akimoto et al., 1996). This technique ensures a time resolution down to 50 fs, because the major optical system is very similar to the transient absorption change method (Shah, 1988). The use of a Ti:Sapphire laser as an excitation source enables us to examine the relaxation dynamics of carotenoids after an excitation to their S_2 state (Kandori et al., 1994). Occurrence of energy transfer can be monitored by comparing fluorescence lifetimes between that in a free form and that in the sPCP; when the energy transfer occurs, the lifetime becomes short. Therefore, fluorescence lifetimes of peridinin in organic solvents were first investigated. In methanol, the lifetimes of the S_2 and S_1 state of peridinin were 190 ± 5 fs and 100 ps (Akimoto et al., 1996), respectively. The S_1 lifetime was very long, compared with other carotenoids, for example, the S_1 lifetime of β-carotene is in about 9 ps (Wasielewski et al., 1986, Mimuro et al., 1997). On the other hand, the lifetime of peridinin in sPCP was measured to be 185 ± 5 fs, indicating that energy transfer does not occur from the S_2 state of peridinin (Akimoto et al., 1996). The possible energy transfer pathway was, thus, to be limited to the case from the S_1 state. This was ensured by the shortening of the S_1 fluorescence lifetime of peridinin. We have tried to estimate the S_1 lifetime. However, due to a severe overlap of the S_1 fluorescence of peridinin with the fluorescence of Chl *a*, we could not measure the peridinin fluorescence. Instead, we measured a rise of Chl *a* fluorescence with a time resolution of 3 ps by the TAC (Time-to-amplitude) system. The rise of Chl *a* fluorescence was not resolved by our laser system, indicating that the rise was shorter than 3 ps. Compared with a lifetime of peridinin in methanol, the rise time was less than 3%; thus the energy transfer from peridinin to Chl *a* was shown to occur from the S_1 state of peridinin to the S_1 state of Chl *a* (Fig. 10). This efficient energy transfer via the S_1 states was confirmed by a transient absorption experiment (Bautista et al., 1999).

The configuration of peridinin molecules in sPCP has been shown to be non-planar (see above). The presence of a keto-carbonyl group induces a localization of electrons. These observations clearly suggest that the π-electron system of peridinin is very different from that of β-carotene in solutions. Symmetry of the π-electron system is broken down, and thus the forbidden S_0-S_1 transition is modified to partially allowed properties, even though we could not detect the absorption band responsible for the S_1 state of peridinin.

In the case of bacterial antenna, the energy transfer from carotenoid to BChl *a* was proved to occur through two routes; from S_2 to S_2 states and from S_1 to S_1 states (Ricci et al., 1996).

Based on the above several experimental data, it is most probable that the energy transfer mechanism from carotenoids to (B)Chl is via the Coulomb interaction, and this is consistent with theoretical considerations.

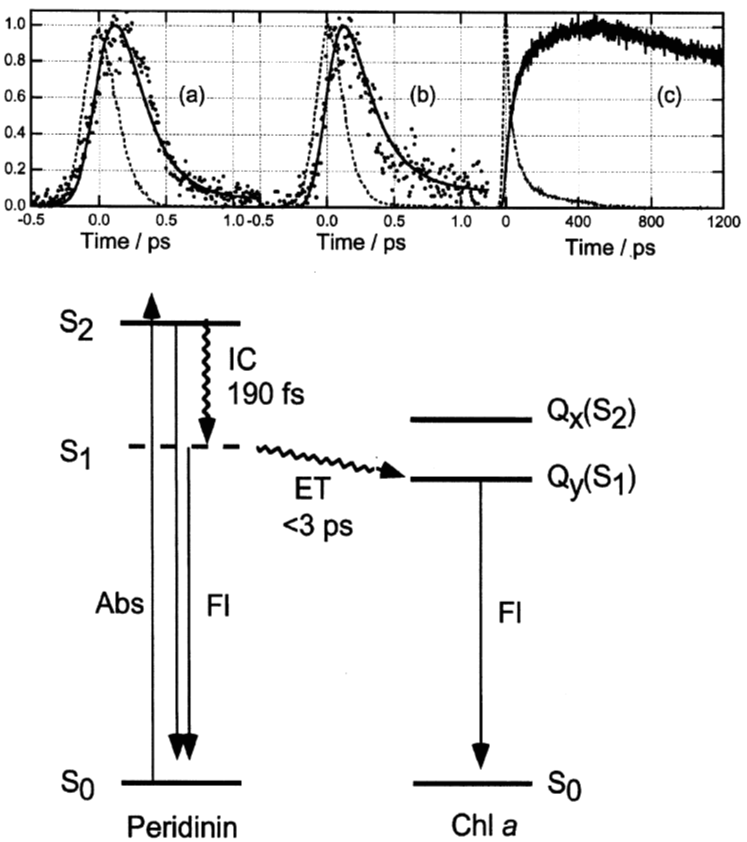

Fig. 10. Relaxation kinetics of peridinin and the energy transfer pathway in PCP. (a) and (b) shows the fluorescence kinetics from the S_2 state of peridinin in methanol and in PCP, respectively. (c) Fluorescence rise and decay kinetics of Chl *a* region in PCP. (a) and (b) were measured with a fluorescence up-conversion system and (c), with a TAC system (Akimoto et al., 1996).

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Chapter 16

Photoinhibition, UV-B and Algal Photosynthesis

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Summary

Photoautotrophic algae inhabit a dynamic photic zone that changes both in light quantity and quality. Algae possess adaptive mechanisms to maximize light harvesting and to limit damage by excessive amounts of photosynthetically active radiation (PAR) and UV radiation in this environment. Strategies for optimizing photosynthetic performance reflect the high degree of diversity in the photosynthetic apparatus of these organisms and go beyond those typically seen in higher plants.

Photoinhibition can be summarized as the net outcome of photoprotective energy dissipation and photoinactivation/photodamage to the D1 protein of PS II reaction centers under conditions of excess PAR. Photoprotection and photoinactivation are highly complex, interactive processes, both of which reduce the efficiency of light utilization, but through markedly different mechanisms. A range of photoprotective reactions involving many different xanthophyll pigments and alternative energy sinks can occur in algae, but the mechanisms of repair and reactivation have not been studied in most divisions. UV radiation also reduces maximum photosynthetic rates and efficiency, but through processes differing from photoinhibition. Targets for damage by UV radiation are more numerous than for photoinhibition, ranging across light harvesting and PS II reaction centers, to carbon reduction and partitioning. As for photoinhibition, relative susceptibility varies widely amongst organisms. At the organismal level, adaptive responses to high PAR and UV involve changes in gene regulation, the molar concentrations of enzymes, photosynthetic and nonphotosynthetic pigments, and perhaps anatomy. At the community level, changes in species composition under enhanced UVB have been observed. Detailed spectral and molecular analyses on primary effects obtained under ecologically relevant conditions are still lacking.

Additional research on responses to high light and UV radiation should be beneficial at many levels. Photosynthesis evolved in a high UV environment, therefore molecular analysis of responses to excess PAR and UV radiation may provide insight into evolutionary relationships among these ancient and diverse organisms. Our inability to project the effect of reduced carbon fixation by current levels of UV-B to conditions of higher UV-B has implications for analyses of global carbon budgets at higher CO_2 levels and increased temperature. We are hampered by lack of knowledge in the following areas: action spectra for UV responses at all biological levels, interaction between responses to UV and to other environmental factors including nutrient availability, temperature, and photoinhibition, and community level responses including recruitment and herbivory.

I. Introduction

The importance of photosynthetically active radiation (PAR, 400-700 nm) in determining how deeply underwater photoautotrophic algae can grow was recognized in the late 1890s (Oltmanns, 1892), and has been recently reviewed (Raven et al., 2000, Chapter 19, Yellowlees). Viewing the limitation of photosynthesis by PAR at the upper end, Steeman Nielsen (1952, 1962) noted that the photosynthetic efficiency of a number of macroalgae declined after a short period of time at light levels that normally just saturated oxygen evolution. Therefore, bathymetric distributions could be bounded on either extreme by PAR: at depth, photoautotrophic growth was limited by insufficient light, but in shallow water, photosynthesis, and by extension growth, might be inhibited when more light was absorbed than could be used to drive photochemistry. Anticipating modern interpretation by some 40 years, Steeman Nielsen

Abbreviations: A – antheraxanthin; a – absorption coefficient, in m^{-1}; BWF – biological weighting function; CA – carbonic anhydrase; CCM – carbon concentrating mechanism; cDOM – chromophoric dissolved organic matter; Dd – diadinoxanthin; DPS – de-epoxidation status, equals A+Z/V+A+Z; Dt – diatoxanthin; DTT – dithiothreitol; E^* – net downward irradiance; E* – effective irradiance; E_o – scalar irradiance; E_d – downwelling irradiance; $E(\lambda)$ – spectral exposure; $\epsilon(\lambda)$ – scaling coefficient; F_o, F_m, F_v – intrinsic chlorophyll fluorescence in weak light, maximal fluorescence during a saturating flash, and variable fluorescence (F_m-F_o), respectively; HL – high light; K_{back} – attenuation coefficient for background scattering; K_d – vertical attenuation coefficient; K_E – vertical attenuation coefficient for net downward irradiance; LHCII – light harvesting complex of PS II; LSU – large subunit of Rubisco; MAP – Mehler asorbate peroxidase; NPQ – nonphotochemical quenching; PAR – photosynthetically active radiation, 400-700 nm; PCR – photosynthetic carbon reduction; PS I, PS II – Photosystems I and II, respectively; PUR – photosynthetically utilized radiation; qP – photochemical quenching; qN – nonphotochemical quenching; Rubisco – ribulose bisphosphate carboxylase oxygenase; UVR – ultraviolet radiation, UV-A: 320-400 nm, UV-B: 280-320 nm, high energy UV-C (200–280 nm); V – violaxanthin; VHL – very high light; Z – zeaxanthin; z – depth in meters

hypothesized that the reversible downregulation of photosynthetic efficiency in excess light was a strategy to protect the photosynthetic machinery from costly damage by excess radiation. Recent advances have revealed this strategy to be a multifaceted biochemical and biophysical process and, along with damage and repair of photosynthetic components, it has been collected under the umbrella of photoinhibition.

At the shallow endpoint of limitation by PAR comes the potential for algae to be exposed to ultraviolet radiation (UVR, UV-A: 320-400 nm, UV-B: 280-320 nm[1]; high energy UV-C (200–280 nm) is effectively removed from the incident solar spectrum by stratospheric ozone). Aquatic algae are exposed to UV depending on their bathymetric position and on water clarity, and a lower photosynthetic rate under ambient UV-B conditions has been recorded for many species. This is in contrast to terrestrial algae and higher plants, which are always exposed to UV-A and UV-B, and whose photosynthetic apparatus appears well-protected from UV-B (Day and Neale, 2002). The differential attenuation of light under water means that UVR may be quickly removed from the water column. But UVR has always been a factor for micro- and macroalgae and microbial mats in the eulittoral zone and phytoplankton in upper photic zone of clear oceanic water. In these cases, UV exposure occurs in combination with high PAR. Furthermore, as ozone is formed from oxygen, itself the result of evolution of oxygenic photosynthetic organisms on the Earth, the earliest oxygenic photosynthetic organisms evolved under extremely high UV conditions that exerted strong selective pressure (Cockell, 1998).

Theoretically, photosynthesis can be driven by wavelengths down to 300 nm, but the efficiency of UV energy transfer to reaction centers is very low (reviewed in Holm-Hansen et al., 1993), and PS II fluorescence emission due to UV excitation is rarely observed (Halldal and Taube, 1972). High-energy UV-B radiation impairs photosynthetic performance and causes direct or indirect damage to a range of other molecules (see reviews by Tevini, 1993; Vincent and Neale, 2000). Despite well-documented damage to PS II by PAR and UV-A during photoinhibition, these wavebands are generally associated with the 'positive' processes of photosynthesis, repair of UV-B damage by photoactivation, and photoregulation of biosynthetic pathways. Thus, PAR, UV-A, and UV-B are inexorably linked through a continuum of photosynthesis, damage, and repair. Nevertheless, many aquatic productivity models have not included the effects of UV radiation, since data were either collected in the absence of UVR, or UV-B effects were not specifically separated from PAR. The lack of appreciation of UV-B effects is particularly problematic now that anthropogenic ozone reduction has led to an increase in the amount of UV-B radiation relative to UV-A radiation and PAR striking the Earth's surface. How might the balance along the photosynthetic continuum be maintained or shifted?

A select few algae have played a major role as model organisms for elucidating higher plant mechanisms for avoiding long term injury by light, i.e. the adjustments to light harvesting and processing capacity and the repair of damage to Photosystem II reaction centers (Chapter 7, Nugent et al.). They have been especially valued for their potential for genetic manipulation. But the responses of algae in general to high irradiance are of interest also from the points of view of their position as the primary producers of aquatic habitats and their inherent photosynthetic diversity. While the basic components of photosynthesis are highly conserved among algal divisions, diversity of accessory pigments, thylakoid arrangement, light harvesting and chloroplast structures, and forms of Rubisco far exceeds that among all other photosynthetic organisms combined (Chapters 2, Larkum and Vesk and 11, Raven and Beardall). As models for higher plants, the Chl *a/b* containing-Chlorophyta make up less than 40% of the known algal species, with as many as 90% of algal species, probably diatoms, yet to be described (Anderson, 1992). Even within the Chlorophyta, diversity in chloroplast structure, thylakoid arrangement and accessory pigmentation is greater than in all of the more advance lower and higher Chl *a/b* containing groups (Chapter 2, Larkum and Vesk; Chapter 13, Larkum). Clearly there is the potential for a variety of ways to downregulate and protect photosynthesis in a controlled response to optimize photosynthetic performance in a changing light field.

Various aspects of photoinhibition of photosynthesis have been extensively reviewed (Prásil et al., 1992; Baker and Boyer, 1994; Long et al., 1994; Osmond et al., 1999; Marshall et al., 2000), including absorption and processing of excitation energy, the role of the xanthophyll cycle in the harmless

[1] By CIE (Commission Internationale de l'Eclairage) definition, UV-B spans 280-315 nm and UV-A, 315-400 nm. Most biological and environmental researchers define UV-B as 280-320 nm, due to the practical availability of filtering material.

dissipation of energy as heat, and mechanisms of degradation and resynthesis of reaction center proteins. Given the broad spectrum of biological compounds that absorb UV-B, the effects of UV-B on wide-ranging aspects of higher plant physiology have been studied and reviewed, particularly with respect to crop species (Allen et al., 1998; Caldwell et al., 1998; Jansen et al., 1998). UV-B can directly affect a wide range of aquatic geochemical and biochemical processes besides photosynthesis (de Mora et al., 2000), and these effects can flow on to alter algal productivity (for reviews, Cullen and Neale, 1994; Wängberg et al., 1996; Franklin and Forster, 1997; Häder and Figueroa, 1997; de Mora et al., 2000, Day and Neale, 2002). In this chapter, we focus on those aspects of photoinhibition and UV-B radiation which are particularly related to algal photosynthesis in the context of natural ecosystems, pointing out specific differences to paradigms established for higher plants and emphasizing the important part that diversity plays in broadening our understanding of photosynthesis.

II. The Algal Light Climate

A. Incident Irradiance

As light penetrates any medium except a vacuum, it is absorbed and deflected (scattered) by the molecules in its path. While the spectral dependence of absorption depends on the molecule present, scattering is most effective at shorter wavelengths. A comprehensive summary of the fate of solar radiation striking the Earth's atmosphere, and the effect of scattering by air and dust particles and absorption by water vapor, oxygen, carbon dioxide and ozone is given by Kirk (1994a). Particularly relevant to this discussion is the relationship between ozone concentration and the amount of UV-B reaching the Earth's surface. Although UV-C and UV-B are specifically absorbed by ozone, from a global perspective the amount of UV radiation reaching the Earth's surface depends most strongly on solar angle. This is because short wavelength light is more effectively scattered at low solar elevation, due to the longer pathlength through the atmosphere. Thus the highest levels of surface UV-B measured occur in the tropical regions, in the summer, and at high altitude. The effect of solar angle can even outweigh the effect of springtime ozone holes such that the UV-B level recorded in the direct solar beam under areas of ozone depletion is less than that recorded in the tropics in the summer at higher ozone concentrations. However, the contribution of diffuse skylight to total irradiance is greater at lower solar elevation, so the incident spectrum becomes relatively enriched in short wavelengths. Therefore, when the amount of direct light from the solar beam is reduced, either in the morning and evening, or on dull days or high latitudes, the skylight is enriched in UV relative to PAR (Fig. 1) (Dring et al., 2001).

B. Underwater Spectral Attenuation

As light penetrates water, irradiance falls in a manner approximated by the exponential function:

$$E_d(z) = E_d(0)\, e^{-K_d}$$

where $E_d(z)$ and $E_d(0)$ are the values of downwelling irradiance at z meters and just below the surface, respectively, and K_d is the vertical attenuation coefficient. (Note that the international symbol for irradiance is E, not I, which is reserved for radiant intensity, the radiant flux per unit solid angle). This effect is due to absorption and deflection (scattering) by dissolved compounds and particulates in the light path, and varies across the spectrum as in the atmosphere. Pure water itself has an absorption coefficient, a (m^{-1}), for light that increases with wavelength (Table 1), with maximum transmission at 480 nm. Absorbance is greater at longer wavelengths, increasing nearly exponentially after 600 nm. The vertical attenuation coefficient for net downward irradiance, K_E, which is often used to describe water clarity is related to the absorption coefficient as follows:

$$K_E = (aE_o)/\, E^*$$

where E_o is the scalar irradiance and E^* is the net downward irradiance (Kirk, 1994a). Natural waters always contain some dissolved chemicals, which increase the absorption of light, i.e. increase K_d. If these chemicals are strong pigments, such as the breakdown products of plant pigment, especially humic substances or chromophoric dissolved organic matter (cDOM, yellow substance, gilvin or Gelbstoff), then the absorption of these chemicals far outstrips the contribution of water itself. In particular, penetration of UV-B in the water column depends

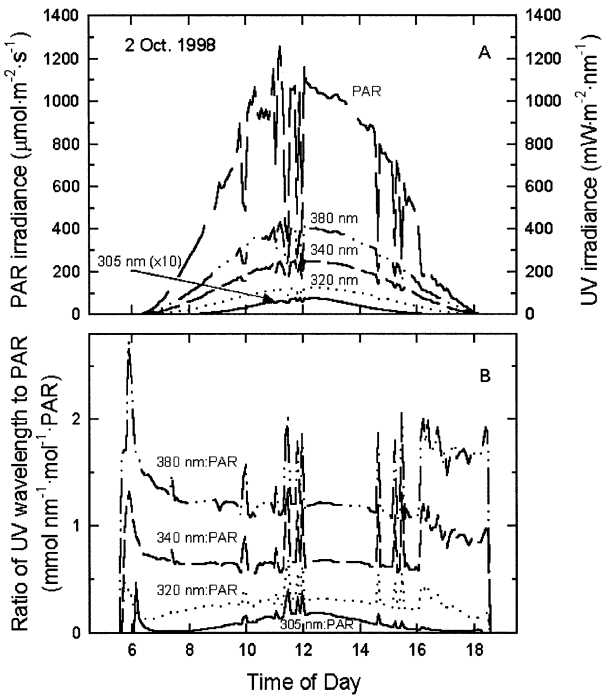

Fig. 1. (A) Diurnal variation in irradiance in each of four UV wavelengths (305, 320, 340 and 380 nm; right-hand axis) and of PAR (left-hand axis) at the North Sea island of Helgoland (54°12′N, 7°54′E) on 2 October, 1998. (B) Diurnal variation in the ratio of each UV wavelength (305, 320, 340 and 380 nm) to PAR calculated from (A). From Dring et al. (2001).

strongly on the concentration of cDOM (Kirk, 1994b; Laurion et al., 1997). Therefore, climate-induced changes in planktonic or allochthonous sources of cDOM, either through changes in vegetation cover, decomposition, or quantity of glacial meltwater may have a higher impact on the underwater UV-B regime than ozone depletion (Sommaruga et al., 1999; Gibson et al., 2000; Pienitz and Vincent, 2000; Dixit et al., 2001; Hanelt et al., 2001; Williamson et al., 2001).

Pure water also scatters light, to a greater degree at shorter wavelengths. This means that photons are deflected from their initial path, and a portion take a path diametrically opposite to their original one. This has two effects: it decreases the amount of light reaching greater depths and, because the pathlength is greatly increased, it enhances absorption. For these reasons, the equation for downward irradiance given above is only approximate. Living and nonliving particles that tend to accumulate in natural waters enhance light scattering and thus, as we intuitively know, turbid waters attenuate light very rapidly. Light scattering also complicates attempts to measure light attenuation and means that the use of simple formulae is not possible (Kirk, 1994a). The role of these two factors in various water bodies has been assessed in a number of studies (Morel, 1997), and numerical models are becoming available (Stramska et al., 2000).

Table 1. Absorption coefficients, a, (m^{-1}) for pure water (Kirk, 1994a), as a function of wavelength

Wavelength (nm)	Absorption coefficient, a (m^{-1})
300	0.0085
400	0.018
500	0.026
600	0.245
650	0.350
700	0.650
750	2.47

Thus, the degree of suspended solids and dissolved substances, especially gilvin, affect very strongly the quantity and quality of light in natural waters. In the, 1950s and 1960s, Jerlov (1976) proposed the most

widely used optical classification of oceanic and coastal water (Fig. 2). In the Oceanic category he distinguished three types (I, II and III) on the basis of increasing amounts of dissolved chemicals and solid particles. Even Type III was relatively clear with an absorption coefficient at 440 nm of greater than 0.02 m^{-1} (Kirk, 1994a) and a maximum irradiance at depth between 480 nm to 500 nm. In the Coastal Class, suspended solids, gilvin and other chemicals increase until waters become turbid and very colored (usually yellow/brown). The types here range from 1 to 9 with 9 being the most turbid. As shown by Kirk (1994a), the proportion of absorption in a Type III oceanic water may be: water itself, 68%; soluble fraction, 24%; particulate fraction, 8%. In comparison, a coastal water of type 7 (estuarine water) has corresponding values of: 18%, 28% and 54% and an inland water body with high gilvin concentrations has values of: 22%, 60% an 18%. One of the most notable effects of the increase in gilvin and turbidity (due to particulates) is the change in light quality over a very small depth interval. Compared to oceanic waters, attenuation of wavelengths less than 550 nm is particularly strong in coastal waters. For example in estuarine waters, e.g. in Australia or the Baltic, or in regions where large quantities of meltwater enter the oceans, e.g. in Antarctica, red and blue light cannot be measured at depths greater than a meter. Below this the light is mainly green, and, although it is possible to detect it by a Secchi depth reading (because the eye is most sensitive to green-yellow light), there is almost no photosynthetically usable radiation (PUR) and therefore almost no photosynthesis in the phytoplankton at these depths. In such conditions, light harvesting by phycobilin-, fucoxanthin- or peridinin-containing algae can be very important (Robinson et al., 1995).

Jerlov's categories are still widely used. Morel and others have continued to improve and fine-tune the categories and this work is discussed in a number of general books and reviews (Morel, 1991, 1997; Kirk, 1994a). Recent attempts have been made to model the light qualities of water bodies (Anderson, 1993; Vila et al., 1996; Ciotti et al., 1999), including the use of Monte Carlo models to simulate light scattering (Kirk, 1994a; Bowers and Mitchelson Jacob, 1996). Other advances have come with attention to wave conditions, which can strongly affect the underwater light climate by producing light flecks. Algal photosynthesis under intermittent light can be more efficient than that under the equivalent exposure to continuous light (Kubler and Raven, 1996). Krause-Jensen and Sand-Jensen (1998) have reviewed the literature and concluded that generally there is a good correlation between light attenuation and photosynthesis in aquatic plant communities.

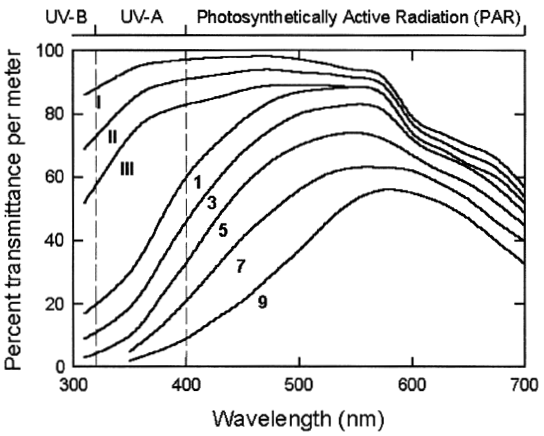

Fig. 2. Transmittance per meter of downward irradiance through the surface layer of various types of oceanic (types I-III) and coastal (types 1-9) waters. From Jerlov (1976).

Although ultraviolet radiation is more strongly attenuated and scattered than PAR in coastal waters, it remains present in the spectrum seen by microbial mats, intertidal, and upper subtidal macroalgae at low tides. In clear oceanic water, the depth for 90% attenuation of UV-B can be as great as 30 m and for UV-A, 60 m (Smith and Baker, 1979; Gieskes and Kraay, 1990, Kirk, 1994b). Though not as energetic as UV-B, the relatively greater amount of UV-A and its weaker attenuation render it a significant component in the underwater light field. UV-B has been shown to improve water column clarity by the photo-oxidation of gilvin (Morris and Hargreaves, 1997), increasing the potential for biological effects of both UV-A and UV-B. UV-B is difficult to measure, though it is possible to estimate UV-B by modeling PAR attenuation (Kirk, 1994b; Shooter et al., 1998). Summaries of some historical data are found in Kirk (1994b) and Franklin and Forster (1997).

C. Lakes, Rivers and Special Aqueous Environments

Lakes and wetlands are highly variable bodies of water; their attenuation properties depend strongly on their latitude, altitude, phytoplankton content, age, and surrounding vegetation. As in oceanic water,

cDOM concentration controls attenuation. If there is much vegetation in the watershed then large amounts of gilvin can accumulate, leading to a very shallow photic zone (Bukaveckas and Robbins-Forbes, 2000; Laurion et al., 2000; Williamson et al., 2001). In tropical regions the breakdown of organic matter may be relatively rapid and these waters may be more affected by particulates (Havens, 1995; Graneli et al., 1998). Lakes are also subject to stratification caused by density changes, largely due to temperature differences, but sometimes caused by salinity differences. In some cases the strata are permanent while in others, seasonal changes cause the strata to break up or turn over, with dramatic effects on turbidity and nutrients (Kirk, 1994a; Markager et al., 1999; Wetzel, 1999; Arts et al., 2000). The spectral pattern of light attenuation in the UV and blue regions (360-500 nm) was analyzed for seven high Arctic lakes (Markager and Vincent, 2000). The best description of K_d versus wavelength was obtained with an exponential model similar to the model used for absorption by cDOM, but with an additional parameter (K_{back}) that accounts for background scattering. While gilvin may be important as an environmental UV screen, Kaczmarsha et al. (2000) showed that highly attenuating waters do not always provide the best protection for phytoplankton at depth.

Rivers are similarly very varied in their attenuation properties, dependent on their flow rate, their drainage source and their geological substrata. Thus they can range from crystal clear to extremely turbid and brownish-yellow with a photic zone of only a few cm (Kirk, 1994a; Kelly et al., 2001).

Photosynthesis occurs in algae in a wide number of other light conditions, such as algal mats in hot springs, or under surface of sea-ice or pack ice. The algae involved here are either thermophiles or psychrophiles, and the light conditions are modified strongly. Kühl et al. (1994) have analyzed the light climate in temperate algal mats. The upper layers of the mat usually contain high concentrations of algae that filter out PUR leaving a light field enriched in infrared and greenish yellow light. Apart from some cyanobacteria, few algae can live in such a light climate, and photosynthetic bacteria usually populate the lower layers of 'algal' mats. The light climate within and under ice, which may be several meters thick, is also specially modified. Snow cover attenuates PAR more than ice, especially at longer wavelengths (Schubert et al., 2001), and the development of an ice algal community specifically removes photosynthetically usable radiation from the under-ice spectrum (Palmisano et al., 1987). Transmission of UVR through sea ice depends strongly on the concentration of both cDOM and particulate organic matter (POM), including ice algae, as well as the spectral albedo and scattering by snowcover, within the ice, and interstitial brine. Transmission of UV-B through ice cover changes during the season, either declining by an order of magnitude as a highly scattering turbid brine layer develops on top of the ice, and as ice algal communities bloom (Trodahl and Buckley, 1990; Perovich et al., 1998), or increasing if snow melt ponds form on top of the ice, thereby reducing albedo and scattering (Belzile et al., 2000). In the latter case, the under ice spectrum becomes enriched in UV-B relative to PAR, and may exacerbate UV-B effects (see below).

Other light climates may be generated by biological symbioses (e.g. Sand-Jensen and Pedersen, 1994). For example, the Chl-*d*-containing *Acaryochloris marina* exists as a second symbiont with the symbiotic, Chl-*b*-containing *Prochloron didemni* in didemnid ascidians (Miyashita et al., 1996). Chl *d* absorbs light in its long-wavelength band at >700 nm. It can therefore be assumed that *A. marina* exists in a niche within the ascidian where it can harvest light that is not absorbed by the more plentiful *P. didemni* (although the exact location of *A. marina* has not been documented). Another well-known example is the special light environment inside coral tissues. Halldal (1970) described the acclimation of the green alga *Ostreobium* to conditions with its host coral *Favia pallide*, where only light longer than 680 nm reaches the alga. An increase in the concentration of long wavelength-absorbing forms of Chl *a* was observed, such that photosynthetic rates in far red light were similar to those at 675 nm (discussed in detail in Chapter 12, Trissl). This increase was reversible in dim, full spectrum PAR.

II. Photoinhibition by PAR

A. Definitions and Component Mechanisms

Concepts of photoinhibition emerged over a century ago through the intricate experiments of, among others, Ewart (1896) who used Englemann's bacterial method to determine, under the microscope, whether

chloroplasts in leaves of shade plants retained their capacity to evolve O_2. Algal ecophysiological studies in the 1950s rekindled interest in photoinhibition, and model green algal systems such as *Ulva rotundata* (Henley et al., 1991b; Franklin et al., 1992; Osmond et al., 1993) and *Chlamydomonas reinhardtii* (Heifetz et al., 1997; Keren and Ohad, 1998; Förster et al., 1999) have contributed much to elucidating component processes. Early definitions put it quite simply: photoinhibition was the loss of photosynthetic efficiency, whatever the reason. Our understanding of photoinhibition, and our vocabulary, have expanded greatly in the last few decades, so much so that previous attempts to find paths through the semantic minefield of photoinhibition while preserving a historical perspective (Osmond, 1994; Osmond and Grace, 1995) should now be abandoned (Osmond et al., 1999). We will limit ourselves to the following terms: *photoinhibition*, *photoacclimation*, *photoprotection*, *photoinactivation*, and *photorepair*. *Photoinhibition* is the generic outcome of the failure of photoprotection to mitigate photoinactivation, which is itself the outcome of situations in which damage of reaction center proteins exceeds *photorepair* in the complex molecular ecosystem of PS II. *Photoacclimation* is the generic outcome of adjustments in PS II structure and function and in the rest of the photosynthetic apparatus that avoid or rapidly repair damage and thus mitigate photoinhibition. Photoprotection and photoinactivation both reduce the efficiency of light utilization, but through markedly different mechanisms. *Photoprotection* principally involves events in the antenna complexes of PS II that decrease excitation transfer to the reaction center, whereas *photoinactivation* involves loss of PS II reaction center function, usually accompanied by loss of the reaction center protein, D1, which is subsequently *(photo)repaired* at a maximum rate in dim light. Dissipative processes in reaction centers may also contribute to photoprotection (Barber and De Las Rivas, 1993; Schweitzer and Brudvig, 1997) and there are also situations in which photoinactivation itself can be considered photoprotective!

Functionally, photoprotection refers to a light dependent, often rapidly reversible decline in the quantum efficiency of primary photochemistry indicated by a decline in the ratio of variable to maximal chlorophyll fluorescence (F_v/F_m), accompanied by a decline in intrinsic fluorescence in weak light (F_o). Also signaled by an increase in the coefficients of non-photochemical quenching (q_N or NPQ) measured by pulse modulated chlorophyll fluorometers during illumination, photoprotection mitigates photoinactivation by relieving 'excitation pressure' on the reaction center of PS II. Excess light leading to 'excitation pressure' in PS II is indicated by the coefficients of photochemical quenching (a decrease in q_P or increase in $1-q_P$) measured with pulse modulated fluorometers during illumination. This signals the presence of highly reduced electron carriers between the photosystems, conditions that promote photoinactivation and lead to damage.

Photoinactivation refers to a light dependent, slowly reversible decline in primary photochemistry (F_v/F_m), often accompanied by an increase in F_o, and well-correlated with a decline in the population of functional PS II reaction centers. The rise in F_o has been thought to indicate an increase in fluorescence lifetimes in the antennae where excitation builds up when reaction center function is impaired during prolonged exposure to excess light. Interpretation of F_o changes is complicated by the fact that, at room temperature, a large part of this fluorescence signal arises from PS I (Pfündel, 1998; Gilmore et al., 2000). Moreover, interpretation is further complicated when the net change in F_o is the sum of quenching due to photoprotection and an increase due to photoinactivation, as was observed in the light stress responses of *Ulva* (Franklin et al., 1992).

The balance between these processes of protection and inactivation defines the limits within which adjustments in the photosynthetic apparatus, i.e., photoacclimation, take place (Osmond, 1994; Anderson et al., 1997; Melis, 1999; Osmond et al., 2000).

B. Energy Dissipation and Photoprotection

Excitation energy within PS II and its associated light-harvesting complex is partitioned between dissipation as heat, mostly in the antennae, and utilization for photochemistry. Nearly all absorbed photon energy that is not used in photochemistry is dissipated as heat, whether excitation is 'grounded' (quenched) in the antennae, in functional PS II reaction centers, or in nonfunctional, photoinactivated PS II centers. Photoprotection from PAR principally involves changes in PS II antenna complexes and the regulation of excitation transfer to the PS II reaction centers (Table 2). The major outer light-harvesting antennae of higher plants and chlorophytic algae (LHCII trimers) deliver exciton energy via the minor

Table 2. Compounds which provide active (energy dissipation) or passive (screening) protection from damaging radiation and their cellular locations. λ_{max} is the wavelength of maximum absorption

Protective agent	Location	Effective wavelengths	Protective strategy	Organism
xanthophyll cycle; violaxanthin antheraxanthin: zeaxanthin	chloroplast, PS II antenna	PAR	reversible energy dissipation	VAZ: most Chlorophyta, Phaeophyceae, Chrysophyceae, Bacillariophyceae
diadinoxanthin: diatoxanthin				DdDt: Bacillariophyceae, Haptophyta, Dinophyta, Euglenophyta
non-photosynthetically active accessory pigments: carotenes and xanthophylls	membranes (segregated from thylakoids), cytoplasm, periplasmic space in Cyanophyta	PAR, UV	screening; de-activating excited states of O_2 and Chl; antioxidant	Most algae, including Cyano-bacteria
mycosporine-like amino acids (MAAs)	intracellular, water soluble	309-360 nm	screening	Esp. Rhodophyta, surface-blooming Dinophyta, Haptophyta, Chrysophyceae, Cryptophyta; to various degrees in many other alga classes, Cyanobacteria
phlorotannins	physodes, around nucleus	UV-B	screening	Phaeophyceae
biopterin glucoside[1]	Intracellular, water soluble	320-390 nm	screening	Marine planktonic *Oscillatoria* sp.
sporopollenin	cell wall	UV-A + UV-B	screening	Chlorophyceae, Trentepohliophyceae, Zygnematophyceae, Dinophyceae
scytonemin	extracellular sheath	280-450 nm, λ_{max} 370 nm	screening	Cyanobacteria

[1] (Matsunaga et al., 1993)

monomeric LHCs (CP29, CP26 and CP24) to the reaction centers. Light-dependent, ΔpH-driven changes in the composition of xanthophyll pigments associated with these minor chlorophyll protein complexes appear to lower the efficiency of excitation transfer to PS II by quenching singlet excited chlorophyll molecules. This leads to the dissipation of excess excitation energy as heat and confers photoprotection on the reaction centers. Dissipation of excitation energy in the antennae (Xanthophyll cycle) is likely to be the major process contributing to photoprotection (Chapter 13, Larkum).

'Excitation pressure' builds up in PS II whenever photon absorption and delivery exceed the capacity of metabolism to consume ATP and reduced NADP. To some extent, excess electrons arriving at the reducing side of PS I can be sequestered either through the Mehler-Ascorbate-Peroxidase (MAP) pathway, through alternate electron sinks, or through photorespiration (Chapters 9, Miyake and Asada and 10, Raven and Beardall). The build up of ATP produced by the electrochemical gradient established across the thylakoid membranes as a consequence of photosynthetic electron transport is more difficult to accommodate. Such a build up leads to an increase in the H^+ concentration in the thylakoid lumen (Schoenknecht et al., 1995) which promotes conversion of specific xanthophyll pigments from an epoxidated form to a de-epoxidated form in a xanthophyll cycle (Chapter 13, Larkum). Effectively, the change makes the antennae more competitive with respect to the reaction centers so far as grounding of excitation is concerned, hence preventing the build-up of excitation pressure and prolonging the life of functional PS II centers. The mechanism by which the xanthophyll cycle promotes photoprotection has been studied most thoroughly in higher plants and green algae, involving the conversion of violaxanthin (V) to antheraxanthin (A) and zeaxanthin (Z) . There is a strong correlation between the accumulation of Z +

A and q_N or NPQ (Gilmore and Yamamoto, 1993). Upon binding Z or A, LHCII appear to be transformed into energy traps with about the same half-time for fluorescence decay (inner antenna +Z, $t_{1/2} \approx 10$ ps) as the PS II reaction center itself (PS II reaction center, $t_{1/2}$ 8–20ps) (Gilmore et al., 1995). Although genetic analysis has confirmed an essential role for Z in thermal dissipation (Niyogi et al., 1997), the xanthophylls do not differ as much as was previously thought in their ability to accept excitation from chlorophyll (Frank et al., 2000).

Formation of de-epoxidated xanthophylls in non-green algae has also been associated with the development of nonradiative energy dissipation (Demers et al., 1991; Arsalane et al., 1994; Olaizola et al, 1994). Presumably this occurs by the same mechanism (Frank et al., 1994), despite great differences in thylakoid membrane arrangement. However, the specific epoxidated/de-epoxidated xanthophyll pairs that are responsible for this process vary with algal group, and the kinetic relationship between NPQ and de-epoxidation can vary from that seen in higher plants (Casper-Lindley and Björkman, 1998). Analogous cycling of diadinoxanthin (Dd) and diatoxanthin (Dt), by a single de-epoxidation step, is found in diatoms, dinoflagellates, and haptophyte algae (Stransky and Hager, 1970; Chapter 13, Larkum), which contain Chl *c* and fucoxanthin or peridinin instead of lutein bound in their light-harvesting chlorophyll-protein complexes. However, members of the Phaeophyceae, the Chrysophyceae, and the diatom *Phaeodactylum* are reported to contain the VAZ cycle (Stransky and Hager, 1970; Arsalane et al., 1994; Benet et al, 1994; Urmacher et al., 1995; Lichtlé et al., 1995), and both VAZ and Dd/Dt cycles seem to occur in several Chl *a/c*-containing microalgae under prolonged high light stress (Lohr and Wilhelm, 1999). Additional roles for V and VAZ cycling have been proposed; these are as a common precursor for the major light harvesting carotenoids in Chl *a/c*-containing algae (fucoxanthin and peridinin), and to optimize synthesis of these carotenoids under fluctuating light conditions (Lohr and Wilhelm, 1999, 2001). Conversely, it appears that not all taxa containing V or Dd undergo de-epoxidation reactions in high light, e.g. *Chlorodesmis fastigiata* and *Euglena gracilis*, respectively (Franklin et al., 1996; Casper-Lindley and Björkman, 1998). Rhodophytic algae contain phycobilisomes as the major light harvesting antennae and lack xanthophyll cycling, but may contain zeaxanthin (Rmiki et al., 1996). A similar process of ΔpH-driven energy dissipation has been reported for several red algae (Ritz et al., 1999). In this respect red algae may be similar to the prokaryotic Cyanobacteria, which do not have a xanthophyll cycle and have zeaxanthin as a major xanthophyll. It will therefore be interesting to investigate the situation in cryptophyte algae where the plastids are descended from a close relative of red algal plastids (Chapter 1, Douglas et al.).

Other short-term, photoprotective processes involve scavenging of reactive oxygen species (Chapters 9, Miyake and Asada and 10, Raven and Beardall), also facilitated by Z (Niyogi, 1999). The role of chloroplast movement in algae in relation to photoinhibition and photoprotection is discussed in Chapter 18 (Hanelt et al.). Longer-term adjustments in the stoichiometries of antenna and reaction center complexes, and increases in the concentrations of other non-photosynthetically active accessory pigments (Table 2) reflect acclimation to higher irradiance so as to limit the potential for photoinactivation.

C. Photoinactivation, Damage, and Repair

The weakest link in PS II reaction centers is the D1 or 32 kDa protein (Keren and Ohad, 1984; Kyle et al., 1984; Aro et al., 1993; Zer et al., 1994). The D1 and D2 proteins form heterodimeric PS II reaction centers that bind all the redox components involved in photosynthetic charge separation. D1 also contains the plastoquinone receptor site on the stromal side of the thylakoids and stabilizes the water-splitting complex on the lumenal side. The D1 protein is located in one of the most energetically charged environments of all living systems, which causes its rapid turn over (it has been dubbed 'the suicide polypeptide'). In vascular plants most PS II complexes assemble into dimers (Hankamer et al., 1997; Rhee et al., 1998) and these complexes, composed of a 'molecular ecosystem' of more than 25 different polypeptides, facilitate thylakoid membrane appression leading to the formation of grana stacks (Chapter 2, Larkum and Vesk). A few PS II complexes with relatively small light-harvesting antennae (probably PS II monomers) are located, along with PS I, in the stroma-exposed grana margins and in unappressed thylakoids that link granal stacks (Förster et al., 1999). The majority of PS II complexes is not in direct contact with PS I, which limits energy spillover (Chapter 13, Larkum) from the slower PS II electron transfer reactions to the faster electron

transfer reactions of PS I (Trissl and Wilhelm, 1993). The arrangement of these complexes plays a role in regenerating functional PS II centers after photoinactivation.

Molecular mechanisms controlling the initial steps of photoinactivation in vivo are diverse (Aro et al., 1993). Processes that regulate accumulation of, and charge recombination between, the primary radical pair, $P680^+$ $Pheo^-$, are probably critical determinants of the extent of photoinactivation (Anderson et al., 1998; Melis, 1999); Oxborough and Baker (2000) have evaluated a number of potential triggers for photoinactivation in light of current theories of photoprotection. When plants absorb excitation energy beyond light saturation of photosynthesis and dissipation of excitation energy in the antennae, the normal, ongoing photorepair of PS II may no longer be able to keep up with damage; as a result nonfunctional PS II complexes accumulate in the grana. Granal stacks that accumulate in chloroplasts of deeply shaded leaves exposed to excess excitation may serve as repositories for nonfunctional PS II centers (Anderson and Aro, 1994). Such nonfunctional centers may continue to dissipate excitation energy as heat and serve a photoprotective role (Öquist et al., 1992). In low-temperature acclimated spinach (Öttander et al., 1993) and *Ulva* (Franklin, 1994), where enzymatically mediated processes of photoprotection and repair are inhibited, nonfunctional but undamaged centers appear to provide the greatest proportion of protection. Damaged D1 polypeptides are recognized and repaired after the reaction centers migrate (possibly as monomers) to the non-appressed stromal lamellae. The repair process involves degradation of the damaged D1, its de novo synthesis on chloroplast ribosomes followed by proteolytic processing, and its re-association with chlorophyll, D2, and other PS II reaction center proteins (Van Wijk et al., 1997; Baena-Gonzalez et al., 1999). Although photorepair requires light, the process saturates at very low irradiance, regardless of the irradiance to which the plant is acclimated (Park et al., 1996, Anderson et al., 1997). Much of the current work on D1 turnover during photoinhibition has relied on very high light (VHL) exposure of shade grown *Chlamydomonas reinhardtii* in high CO_2 cultures supplemented with acetate (Keren and Ohad, 1998). We now know that these growth conditions minimize photoprotection, and predispose the cells to more extensive photoinactivation (Heifetz et al., 2000).

Not surprisingly, PS II reaction centers seem to have a finite functional life in higher plants. Some nonfunctional centers are always present in the cell (Chow et al., 1991) and a subpopulation of centers seems to be more susceptible to photoinactivation than others (Park et al., 1996a). The rule of reciprocity appears to apply to photoinhibition: rate of decline of PS II function depends upon the photon dose (intensity × duration). Measurements of the repetitive flash yield of O_2 evolution showed that, even at subsaturating light levels (100 μmol photons m^{-2} s^{-1}), about 10^6 PS II complexes per mm^2 of leaf surface are inactivated every second (Park et al., 1995). A similar rate of inactivation has been calculated for the tropical turf-forming green alga *Chlorodesmis*, which curiously lacks a xanthophyll cycle (Franklin and Larkum, 1997). In both higher plants and algae, the decline in functional centers is greatly accelerated by inhibitors of chloroplast protein synthesis. Hence, the rate of photoinactivation reflects a balance between reaction center damage and photorepair (Park et al., 1995).

Compared to vascular plants, there is much greater diversity in the structural organization of alga chloroplast membranes (Gunning and Schwartz, 1999 for chlorophytes; and Chapter 2, Larkum and Vesk), with unknown consequences with respect to PS II functions in excess light. Thylakoid arrangement in algae varies from single lamellae (Rhodophyta) to groups of two or three lamellae, with only the most evolutionarily advanced taxa containing plastids with significant thylakoid stacks (Chapter 2, Larkum and Vesk). Nor is there any compelling evidence for the lateral heterogeneity of photosynthetic components that occurs in higher plants (Bertos and Gibbs, 1998; Chapter 13, Larkum). The relationship between thylakoid architecture and the exchange of functional and nonfunctional PS II centers has not been addressed, despite the widespread use of algal model organisms.

D. Photoprotection, Photoinactivation, and Fitness in Algae

Photosynthesis (including photo-oxidative processes such as the MAP Pathway—Chapters 9, Miyake and Asada and 10, Raven and Beardall) is the best protection against photoinhibition, and both electron transport and carbon metabolism can be photoprotective (Park et al., 1996b), in that their capacities largely determine the irradiance at which 'excess light' is perceived in the photosynthetic apparatus.

Table 3. Effect of CO_2 supply on the de-epoxidation status (DPS = A+Z/V+A+Z) of xanthophyll pools in VHL^R mutants of *Chlamydomonas* (Förster and Osmond, unpublished).

Strain and growth light	DPS of xanthophylls		Chlorophyll fluorescence quenching parameters		
	air	5% CO_2	NPQ	1-qP	1-qP/NPQ
Wild-type, HL	0.41	0.04	0.82	0.85	1.05
S4, HL	0.49	0.09	2.32	0.91	0.39
S4, VHL	0.85	0.78	1.71	0.74	0.43
Parent, *A251L**, HL	0.46	0.01	0.43	0.99	2.30
L5, HL	0.34	0.03	0.27	0.99	3.67
L5, VHL	0.85	0.63	0.18	0.94	5.22

Coefficients of chlorophyll fluorescence quenching indicating potential for photoinactivation (1-qP; Q_A pool reduction state; 1=fully reduced), and for photoprotection (NPQ) in VHL^R mutants and their parents. Cells were grown photoautotrophically in 5% CO_2 and fluorescence quenching parameters were measured under excitation pressure of 1700 μmol photons $m^{-2}s^{-1}$ (n = 2-7; Osmond, unpublished). VHL, 1500 (L5) and, 2000 (S9) μmol photons $m^{-2}s^{-1}$; HL, 600 μmol photons $m^{-2}s^{-1}$.

Compared to CO_2-free seawater, exposure to high light in natural seawater slows the onset of photoinhibition in *Ulva rotundata* (Osmond et al., 1993). In spite of the induction of a CO_2 concentrating mechanism in *Chlamydomonas*, cells grown in air have a much higher xanthophyll de-epoxidation status (greater photoprotection) than those grown in 5% CO_2, presumably because the high CO_2 sustains higher rates of photosynthesis (Förster et al., 1999; Table 3). Significant protection is possible in 2% O_2 by photosynthetic electron transport to O_2 via the MAP pathway, but the degree to which this is important varies widely in algae (Sültemeyer et al., 1986; Badger et al., 2000). Alternatively, cyclic electron flow around PS II via PQH_2, Cyt b_{559}, and Chl Z may act as a 'clutch' to maintain charge separation capacity when photosynthesis is limited by other factors (Falkowski et al., 1986; Prásil et al., 1996; Franklin and Badger, 2001; Longstaff et al., 2002). In higher plants, photorespiratory carbon metabolism can also reduce the rate and extent of formation of non-functional PS II centers in leaves exposed to strong light (Park et al., 1996a), but the situation in algae is not clear. However, metabolic sinks rarely account for more than 20 to 30% of photoprotection at light saturation in air (Badger et al., 2000). In cases where photosynthesis is limited by low temperature or dehydration, photoprotective responses are enhanced, although these interactions are not well studied (Huppertz et al., 1990; Franklin, 1994; Maxwell et al., 1994; Patterson et al., 1994; Pena et al., 1999).

Xanthophyll- and ΔpH-dependent photoprotection are thought to be the dominant processes under most natural conditions, but as alluded to above, photoprotection does not always prevent photoinactivation. In *Arabidopsis*, functional PS II centers declined by 50% during the period of xanthophyll interconversion, and by 80% after 1 h in strong light, in spite of a high A + Z pool (Russell et al., 1995). Furthermore, Z-enriched ABA-mutants of *Arabidopsis* are no better photoprotected than wild-type plants (Hurry et al., 1997), suggesting the importance of stoichiometries of the pigments and their subcellular locations. Generally, these relationships remain to be investigated in algal systems. However, it is clear that inhibition of photoprotective mechanisms promotes photoinactivation. Thus, in *Ulva*, DTT (an inhibitor of V → A+Z) and in peas and the red alga *Rhodella*, nigericin (an uncoupler that prevents build-up of high ΔpH) increases the rate at which nonfunctional PS II centers form and accumulate (Franklin et al., 1992; Osmond et al., 1993; Park et al., 1996a; Ritz et al., 1999).

Very high light resistant (VHL^R) mutants of *Chlamydomonas* show just how complex the relationships between photoprotection and photoinactivation can be. These VHL^R mutants that are grown in 5% CO_2 or air, at VHL (1500 to 2000 μmol photons $m^{-2}s^{-1}$) were isolated from both the wild-type strain and from a chloroplast *psbA* mutant *A251L** with an Ala to Leu substitution in the D1 protein at residue 251. Neither of the parental strains survive long-term exposure to these light intensities,

but the *VHL*^{R} mutants grow photoautotrophically in 5% CO_2 or air at rates similar to their respective parental strains under HL (600 μmol photons $m^{-2}s^{-1}$) and maintain high growth rates under VHL. The *A251L** mutant exhibits a 10-fold reduction in the rate of PS II electron transfer, accelerated D1 turnover, reduced growth rate, reduced photosynthetic efficiency and resistance to high concentrations of herbicides (Förster et al., 1997; Lardans et al., 1997). That such severe lesions in PS II functions do not prevent the selection of *VHL*^{R} mutants suggests that optimum PS II function is not essential to survival in VHL.

At the maximal 'excitation pressure' that still allows survival, the *VHL*^{R} mutant from wild-type (*S4*) have highly reduced Q_A pools and show greater NPQ than wild-type cells after growth in HL or VHL (Table 3). In contrast, the *VHL*^{R} mutants from *A251L** (*L5*) exhibited low NPQ under all conditions, presumably because slow PS II electron transfer in these mutants was insufficient to sustain the ΔpH needed to effectively generate high NPQ. Neither NPQ nor DPS provide a complete picture of photoprotection in these mutants. The ratio of quenching coefficients (1-qP/NPQ) implies that the *S4* should experience much less photoinactivation than *L5*, but in fact at VHL, *S4* shows about the same decline in F_v/F_m and all other parameters of PS II function that have been examined (Förster et al., 2001). All *VHL*^{R} mutants showed lower chlorophyll content at VHL on a cell and biomass basis, suggesting that their survival may involve acclimation through changes in antenna-reaction center relationships. Other results indicate that the slower PS II electron transfer and low connectivity among PS II centers, characteristics of the D1 protein-impaired mutants, appears in the *VHL*^{R} mutants from wild type at VHL (Förster et al., 2001).

The two classes of *VHL*^{R} mutants show that although growth at VHL is inevitably associated with increased de-epoxidation status (DPS = A+Z/V+A+Z) of the xanthophylls, this does not automatically confer high NPQ, or translate into photoprotection and prevent impairment of PS II functions. Growth at near maximal rates in VHL with impaired PS II activities is common to these 'gain of function' mutants. Clearly low PS II efficiency does not limit growth in excess light. Previous studies showed that *Chlamydomonas reinhardtii* transformed with the herbicide resistant mutation *S264A* had slower photosynthesis and growth in HL than wild type, as did a streptomycin- and spectinomycin-resistant double mutant with impaired chloroplast protein synthesis. Both 'loss of function' mutants, either with impaired D1 function or impaired D1 synthesis, showed reduced competitive ability when grown in chemostats with wild-type at 300 μmol photons m^{-2} s^{-1} (Heifetz et al., 1997).

All *VHL*^{R} mutants showed lower chlorophyll content at VHL on a cell and biomass basis, suggesting that their survival may involve acclimation through changes in antenna-reaction center relationships. Other results indicate that the low connectivity among PS II centers, characteristic of the D1 protein-impaired mutants, appears in the *S4* and *S9* strains at VHL (Förster et al., 2001). It is also possible that the lesions alter differential regulation of gene expression in response to the highly reduced state of the Q_A pool or electron transport pathway. The nature of the loci mutated in these strains is not known but it is possible that they encode regulatory proteins involved in orchestrating the diverse adjustments in antenna, reaction center and metabolic responses to 'excitation pressure,' in directing photoacclimation.

E. Photoacclimation

Photoacclimation and the avoidance of photoinhibition is the ultimate indicator of fitness in relation to high light. It is the result of a strategy of photoprotection that minimizes photoinactivation and facilitates a balance between photodamage and photorepair. Increased photosynthetic capacity is an important component of photoprotection in the course of photoacclimation in higher plants, reflected in large increases in Rubisco and ATPase activities when shade leaves are transferred to strong light (Chow, 1994). Likewise, algae growing along a light gradient, or transferred from low light environments respond to higher irradiance with decreased synthesis of light harvesting pigments, increased synthesis of photoprotective carotenoids, including xanthophylls (Henley et al., 1991b; Willemoes and Monas, 1991; Rmiki et al., 1996), changes in the pool sizes of electron transport components and Rubisco, and higher photosynthetic rates (Falkowski and Raven, 1997). Surprisingly, the *VHL*^{R} mutants of *Chlamydomonas* described above have lower light- and CO_2-saturated rates of photosynthetic O_2 evolution per total chlorophyll than the wild-type or *A251L** strains

(Förster et al., 1999). Acclimation from low light to high light in wild-type *Chlamydomonas* is accompanied by complete reduction of Q_A and a transient decline in F_v/F_m as the message levels for the *psbA* gene encoding D1 protein, and D1 protein synthesis itself increase five- to ten-fold (Shapira et al., 1998). At the same time, translation of the *rbcL* gene encoding Rubisco LSU protein ceases and does not resume for about 12 h, when cell division resumes. A picture is emerging in which acclimation processes depend on 'a nested series of photo-acclimatory responses' (Durnford and Falkowski, 1997) that may sequentially develop and be most responsive to electron transport rates, but in algae the adjustments in the stoichiometry of components may differ from that in higher plants.

Durnford and Falkowski (1997) have discussed three main hypotheses to account for changes in LHC accumulation in response to irradiance (Chapter 4, Durnford). These involve photoreceptors, a feedback effect of chlorophyll synthesis intermediates, and a feedback by metabolites/reductants that reflect photosynthetic activity. Evidence is accumulating suggesting that the redox state of the photosynthetic electron transport chain controls the expression of genes that encode polypeptides important for PS II function. Maxwell et al. (1994) showed that *Chlorella* cells sensed 'excitation pressure' in PS II whenever the rate of photon delivery exceeded electron transport to PS I and suggested a feedback mechanism mediated by the reduction status of the Q_A pool. This hypothesis has strong support from work with *Dunaliella salina* (Baroli and Melis, 1996, 1998; Melis, 1999). Fujita et al. (1987) proposed that photosystem stoichiometries in cyanobacteria were regulated by the redox state of inter-photosystem electron transport pathway components and Escoubas et al. (1995) showed that *Lhc* gene expression responded to the redox state of the plastoquinone pool. Murakami et al. (1997) and Pfannschmidt et al. (1999) demonstrated redox regulation of chloroplast gene expression in *Chlamydomonas* and higher plants, respectively. Montané et al. (1998) also concluded that the redox status of the Q_A pool regulated acclimation processes in barley. Anderson et al (1997) suggested that genes encoding components of the photosynthetic apparatus might be controlled by redox-influenced interactions between PQ and the Cyt b_6f complex.

III. Effects of UV Radiation

A. Spectral Weighting Functions

1. General Concepts

The effectiveness of light to cause apparent photoinhibition increases dramatically at wavelengths less than 400 nm, but the mechanistic basis for reduced photosynthetic activity due to UV radiation differs from that described above for PAR. Since the solar spectrum in the aquatic environment contains variable amount of UV relative to PAR, and ozone depletion only affects the amount of UV-B reaching the Earth's surface, one needs a way of describing the relative effectiveness of all wavelengths in photosynthesis and damage, and to be able to separate responses to UV from those induced by excessive PAR. This is achieved through the use of action spectra, particularly **biological weighting functions** (BWFs). BWFs are essential for determining the effect of current and additional UV-B in the context of the natural spectrum, or for comparing results of studies using various artificial radiation sources.

Weighting functions for the effectiveness, E* (dimensionless), of a radiation treatment follow the general formula:

$$E^* = \sum_{\lambda = 280\,nm}^{400\,nm} \varepsilon(\lambda)E(\lambda)\Delta\lambda$$

where the product of a spectral exposure, $E(\lambda)$, and a scaling coefficient, $\epsilon(\lambda)$, are summed over a spectral range, the UV-B and UV-A spectrum in this example. Detailed descriptions of the creation and application of exposure response curves and weighting functions to UV-B studies on plants and algae, and BWFs especially related to modeling photosynthesis, have been presented (Caldwell et al., 1986; Cullen and Neale, 1993, 1994, 1997; Suzuki et al., 1995; Holmes, 1997; Neale, 2000). A brief summary of key points is presented here.

There are two basic ways to create a spectral response curve, depending on the question to be asked. Monochromatic light can be used to elicit a particular response from the system being investigated, assuming that all wavelengths contribute independently to the process in question. The resulting monochromatic action spectra can be used, for example, to identify potential targets or chromophores for damage by UV radiation, or to determine the

efficacy of screening pigments in protecting specific molecules or processes (Peak and Peak, 1983). However, organisms experience in vivo a combination of UV-B and longer wavelengths that stimulate repair or protective processes (see below). Indeed, the relative effectiveness of UV-B depends inversely on the quantity of concurrent UV-A exposure (Madronich, 1992). A BWF takes into account the contributions of longer wavelengths. It is determined using a number of longpass filters to achieve a series of polychromatic treatments (BWFs are sometimes referred to as polychromatic action spectra). These may simply demonstrate a change in function in the presence or absence of UV (two treatments), or explore the fine structure of responses by cutting off the UV spectrum in 10–20 nm slices. In the most basic form of a polychromatic action spectrum, Steeman Nielsen (1964) showed that ^{14}C uptake by phytoplankton was higher when samples incubated in quartz bottles were shielded from natural UV.

Several important points related to the creation and use of BWFs should be emphasized. Clearly, damage to molecules or processes increases at shorter wavelengths, so knowledge of the spectral composition of radiation sources is critical to interpreting results among experiments and with respect to natural radiation. Although BWFs are usually created using artificial radiation, even the best solar simulators are not perfect. This raises questions about the applicability of these BWFs to natural conditions where ratios of UV-B to UV-A and PAR vary and UV-A and PAR may be higher. The response of a particular molecule to UV in vitro is likely to be quite different in vivo, due to the different probability of photons striking their targets, a change in sensitivity in the context of the cellular milieu, or the opportunity for repair. Therefore, BWFs created using thylakoid membranes or chloroplasts are unlikely to be applicable to whole organisms for predictive purposes. It is also critical that treatments are applied for various periods of time: many responses are not dose dependent, due to simultaneous repair of damaged molecules or the ability of the organism to acclimate with enhanced repair or screening. In these cases, irradiance and time cannot be interchanged in treatments, i.e. reciprocity is not obeyed. Neale (2000) has illustrated and discussed several general models for net damage to photosynthesis given a range of repair activities.

2. Action Spectra and Biological Weighting Functions for UV Damage to Algal Photosynthesis

The majority of action spectra and BWFs available describe the inhibition of photosynthesis by UV (Smith et al., 1980; Cullen and Lesser, 1991; Behrenfeld et al., 1993; Cullen and Neale, 1994; Boucher and Prézelin, 1996; Neale et al., 1998b; Banaszak and Neale, 2001). While the spectra all share the general feature of increasing effectiveness at shorter UVR, they vary dramatically in overall shape and relative effectiveness at a given wavelength, especially in the UV-A region. Both biological and methodological factors are likely to contribute to this variation (Neale, 2000). Biological factors leading to differences in inherent sensitivity could include differences in species composition of the samples, and the presence of screening compounds like mycosporine-like amino acids (MAAs), below. Repair may or may not occur concomitant with damage, thus the overall effect may be dose-rate dependent (reciprocity law fails) or dose-dependent (reciprocity law applies), respectively. Responses might vary seasonally due to changes in species composition or in temperature dependent repair (Pakker et al., 2000b). The effect of repair rate and MAA content are particularly important to variation in response to UV-A, and are significant because there is always a greater proportion of UV-A (and PAR) in the natural spectrum relative to UV-B. Methodological factors that could contribute to BWF diversity include the effect of using artificial light sources with different spectra that may enhance or diminish other light-dependent processes, the number of filter combinations used, and the computational methods for deriving the BWF.

An example of the degree of variability in BWFs and its consequences can be seen in a recent comparison of seasonally averaged functions for the inhibition of photosynthesis by UV in phytoplankton from temperate, estuarine environments to those for Antarctic populations. On the one hand, the seasonally averaged BWFs for inhibition of photosynthesis in estuarine phytoplankton populations were nearly identical to each other and to the average BWF for Antarctic populations, despite differences in species composition (Banaszak and Neale, 2001). Yet, a high degree of within season variability was observed, equivalent to the most and least sensitive assemblages found in Antarctica. In all cases, natural assemblages were more sensitive than cultured organisms. Within

the estuary, variability in estimates for changes in water column productivity due to UV could be attributed primarily to variation in water column optical properties (particularly the ratio of transparency to UV and to PAR) and differences in BWF, but the source of variation in the BWF remains to be determined (Neale, 2001).

Action spectra and weighting functions for inhibition of macroalgal photosynthesis by UV-B are limited, but support strong effects of both UV-A and UV-B (Nultsch et al., 1990; Forster and Lüning, 1996). No functions are available for algal/invertebrate or algal/fungal symbioses. Given the variation in BWFs that is known to exist for phytoplankton, it is likely that the responses of organisms with more complex optical pathlengths, e.g. macroalgae, microbial mats, ice algae, and corals, will differ significantly from phytoplankton. Until a greater understanding of the biological significance of changes in BWFs is achieved, BWFs constructed in short-term experiments for chloroplasts, phytoplankton, or general UV damage to higher plants (Caldwell, 1971) should be used with caution to predict long-term responses of other organisms to ozone depletion.

B. Direct Effects of UV Radiation on Photosynthesis

Photosynthesis can be conceptually and structurally compartmentalized: the harvesting of light energy, the production of reducing equivalents and ATP, the acquisition of carbon, and its reduction into simple sugars in the photosynthetic carbon reduction (PCR) cycle. Since a wide range of biological molecules absorb UV-B, components of each of these processes are potential targets for damage by UV-B, either directly or indirectly by the production of reactive O_2 species (Tevini, 1993; Lesser, 1996b,c; Chapters 9, Miyake and Asada and 10, Raven and Beardall). When UV radiation is removed from the natural spectrum, the photosynthetic performance of whole organisms usually improves. However, there is ongoing debate whether increased UV-B will be directly responsible for lower productivity in nature, and what that decline might mean for other trophic levels (Fiscus and Booker, 1995; Allen et al., 1998; Vincent and Neale, 2000). Much effort has gone into identifying individual primary photosynthetic targets using artificial irradiance, but it is difficult to extrapolate effects to the field because of the variety of light sources used and the heavy reliance on analyses at the sub-cellular level. Studies of integrated effects on whole organisms, as done on higher plants (Nogués and Baker, 1995), and on whole communities are yielding more comprehensive data for predicting total UV effects on primary productivity. In this section, we discuss briefly experimental techniques, followed by potential direct cellular targets and protective mechanisms.

Experiments carried out in the laboratory, growth cabinets or glasshouses with artificial radiation and UV-B supplementation, or using natural radiation supplemented by non-modulated UV-B lamps often employ unnatural ratios of PAR and UV radiation. This experimental design leads to the overestimation of UV-B effects (Fiscus and Booker, 1995; Allen et al., 1998; Vincent and Neale, 2000), and should be treated cautiously for predicting the effect of ozone depletion. Experiments with natural radiation often employed the polychromatic approach of removing UV-B or all UVR with filters, or increasing UV-B levels by moving samples to more shallow depths and changing the entire spectrum. While these studies have the advantage of natural spectral ratios, they still cannot answer the question of how photosynthesis might change if UV-B is increased at a given level of UV-A and PAR in a climate change scenario. They also do not replicate the effect of mixing, if applicable, as the usual approach is to confine samples in bottles at a defined irradiance or fixed depth for a number of hours. This prevents assessment of the contribution of repair, which theoretically could dominate once the cells are moved out of the UV-B zone but are still receiving UV-A and PAR. Neither does it address the effect on total water column productivity of moving new, undamaged cells into the photic zone (Helbling et al., 1994; Neale et al., 1998c).

Like photoinactivation by PAR, PS II is also implicated as a primary target of UV-B damage since reduced photosynthetic efficiency of higher plants and algae is generally observed in the presence of UV-B. Photosystem I seems relatively insensitive to UV-B (Strid et al., 1990, 1994). Experiments with thylakoid membrane fragments from spinach have indicated damage both to the donation of electrons from the water-splitting complex to a site on the D1 protein of PS II and to the quinone electron acceptors (Renger et al., 1989; Post et al., 1996; Vass et al., 1996). These results have been confirmed in vivo for *Synechocystis* sp. 6803, and a model for inactivation of PS II by UV-B has been proposed (Vass et al.,

1999; Larkum et al., 2001). UV-B irradiation also led to loss of the D1 and D2 proteins and Cyt b_{559}, which make up the core complex of PS II (Jansen et al., 1996; Mackerness et al., 1996; Vass et al., 1999). Despite being initiated by different photosensitizers, degradation of D1 in the aquatic plant *Spirodela* by UV-B and PAR proceeded through a common 23.5 kDa intermediate (Greenberg et al., 1989). As in the case for photoinhibition by PAR, UV-B-damaged PS II centers are constantly replaced by de novo protein synthesis in the chloroplast, and the inhibition of chloroplast protein turnover by antibiotics stops recovery from photoinhibition by UV-B in a number of algae (Lesser et al., 1994; Ekelund, 1996; Xiong et al., 1997; Franklin and Lüning, 1998). Moderate levels of PAR during the UV-B exposure decrease the net loss of D1 and D2 by further increasing the rate of de novo protein synthesis within the chloroplast (Vass et al., 1999). This demonstrates the importance of polychromatic approaches and consideration of ongoing repair in studying UV-B sensitivity.

When both the light reactions and carbon fixation are considered together, carbon fixation is often reduced at lower dosages than are required to affect photosynthetic efficiency. This implies that there are other, more sensitive photosynthetic targets than PS II, and that the loss of photosynthetic efficiency may be (initially) a response to higher excitation pressure on electron transport, and therefore a reflection of photoprotective downregulation to avoid photoinhibition by PAR. These other, more sensitive targets may be impaired uptake of inorganic carbon, or impaired capacity to reduce CO_2.

UV-B affects membrane transport function in general (Murphy, 1983; Tevini, 1993). Thus it is reasonable to expect that any damage to photochemistry or membranes by UV-B which alters membrane integrity, the supply of ATP for a transporter or protons to carbonic anhydrase (CA) will also affect the carbon concentrating mechanism (CCM) employed by many algae to increase the amount of CO_2 available to Rubisco (Chapter 11, Raven and Beardall). Inhibition of HCO_3^- transport has been suggested to occur in the green nannoplankter *Nannochloropsis gaditana*, but not in *Nannochloris atomus*, which has active CO_2 uptake (Sobrina et al., 2001). More detailed studies are required to determine the direct effects of UV-B on CCMs, including inhibition of CA, changing the affinity for the HCO_3^- transporter, or changing membrane permeability.

Carbon reduction appears to be even more susceptible to UV-B damage than PS II electron transport. Several studies on peas demonstrated that Rubisco content and activity were reduced under enhanced UV-B with some (Strid et al., 1990) or no reduction of PS II efficiency (Nogués and Baker, 1995). In the later case, prolonged exposure led to reversible reductions in efficiency with no recovery of the saturated rate, suggesting that electron transport was downregulated to match reduced PCR cycle activity. Antarctic diatoms (Neale et al., 1992) and the marine dinoflagellate *Prorocentrum micans* (Lesser, 1996c; Lesser, 1996b) responded to a gradual increase in ambient PAR containing UVR with reduced Chl *a*-specific Rubisco content (loss of the LSU fraction) and a lower maximum rate of photosynthesis with little change in photosynthetic efficiency. In the latter case, reduced photosynthesis was correlated with an overall 30% decline in growth rate. Measurements of reduced maximum rate of O_2 evolution by both UV-A and UV-B suggested that the same process may occur in the kelp *Laminaria digitata* (Forster and Lüning, 1996). However, acclimation to long term exposure to ambient UV can occur. Hazzard et al. (1997) showed that, after an initial period of inhibition, rates of carbon fixation and cell specific Rubisco activity in cultures of the subtropical marine diatom *Chaetoceros gracilis* exposed to ambient UV were similar to those not exposed. In cases where algae are already acclimated to intertidal UV conditions, Rubisco activity may be more resistant. In a five-day laboratory experiment, Bischof et al. (2000) exposed intertidal samples of *Chondrus crispus* and *Mastocarpus stellatus* daily to 6 h of typical levels of UV-A and UV-B with low background PAR. They found that Rubisco activity did not change from initial levels, despite 20 to 40% reduction in photosynthetic efficiency as measured by chlorophyll fluorescence.

A mechanism for the damage by UV-B has been proposed for higher plant Rubisco, which involves the oxidation of tryptophan by singlet O_2 at the enzyme active site and/or between the subunits of the holoenzyme. Its reaction with adjacent amino acids will cross-link the large and small subunits of the holoenzyme into new 66 kDa particles (Wilson et al., 1995; Gerhardt et al., 1999), disrupting function. The synthesis of new Rubisco may be affected as well, since Rubisco mRNA levels are reduced after UV-B treatment (Jordan et al., 1992). Two major forms of Rubisco have been described in algae:

Type 1 with eight large subunits and eight small subunits, and Type 2 with just two large subunits (summarized in Chapter 11, Raven and Beardall; Raven, 1997). Molecular genetic evidence shows that all types evolved from a common ancestral form, resulting in five distinct subfamilies that can be separated on kinetic characteristics. It would be interesting to know if functional differences also exist with respect to UV-B sensitivity.

Additional targets for UV-B are found throughout the photosynthetic apparatus. Porphyrins act as photosensitizers for UV-B, leading to the production of singlet oxygen and photo-oxidation of pigments and membranes (Tevini, 1993). A number of studies on algae report a reduction of pigment content as a consequence of long-term UV-B exposure (Wood, 1987, 1989; Altamirano et al., 2000a). Lesser et al. (1994) reported that the destruction of chlorophyll as a function of UV-B exposure was secondary to the loss of chlorophyll-specific carbon uptake in *Thalassiosira pseudonana*. The pigment content of a periphyton community exposed for two weeks to UV-B treatments declined only when the level of UV-B was greater than ambient (McNamara and Hill, 2000). Similarly, the Chl *a* concentration in phytoplankton in a stratified lake declined by 65% during a natural ozone-thinning event (Xenopoulos et al., 2000). Phycobiliproteins and phycobilisomes are also reported to be sensitive to very high levels of UV-B (Sinha et al., 1995; Lorenz et al., 1997; Rajagopal et al., 1998). However, natural levels of UV-B applied with high background levels of PAR (1100 μmol photons m^{-2} s^{-1}) did not lead to a loss of light harvesting and transfer efficiency by phycobilisomes in the red alga *Palmaria palmata* (Lorenz et al., 1997). On the other hand, a certain amount of UV radiation may be required for pigment biosynthesis (Araoz and Häder, 1999). Rao et al. (1995) reported an increase in the content of Chl, carotenoids, and UV screening compounds in *Arabidopsis thaliana* during a five-day exposure to UV-B, and Altamirano et al. (2000b) found that the chlorophyll and carotenoid concentration in *Ulva rigida* declined over a two month period when ambient UV-B was filtered out of the natural spectrum.

Electrons move from PS II to PS I via a series of (variably) diffusible redox intermediates, plastoquinone, cytochrome b_6f, and plastocyanin or cytochrome c_6 (or both), resulting in an electrochemical gradient across the thylakoid membranes. The extent to which UV-B damages any of these components directly or indirectly is at present only suggestive. Damage to membrane structure and function occurs when UV-B induces the formation of lipid free radicals and peroxide radicals, each of which continues to react with other fatty acids and to form lipid peroxides in a chain reaction sequence (Murphy, 1983; Malanga and Puntarulo, 1995). Severe structural changes in thylakoid membranes have been reported in *Micrasterias denticulata* exposed to 275 and 280 nm radiation (Lutz et al., 1997). The ability of pea thylakoids to maintain a pH gradient declined with much higher than normal UV-B exposure (Strid et al., 1994; Fiscus and Booker, 1995). Quinones absorb strongly in the UV-B region, and the plastosemiquinone anion radical Q_A^- has been proposed to be one of the photoreceptors for the combined UV-B and PAR-induced D1 degradation (Babu et al., 1999). Reduced levels of ATP due to UV-B exposure have been reported for plankton assemblages in the Weddell Sea (Vosjan et al., 1990) and for *Dunaliella tertiolecta* (Döhler et al., 1997).

In contrast to expectations of negative impacts of UV-B on PS II function and thylakoid membrane integrity, the interconversion of xanthophyll cycle pigments may be either enhanced or diminished in the presence of UV-B. Pfündel et al. (1992) reported that violaxanthin de-epoxidase activity in higher plants decreased during UV-B treatments. However, when examined in the presence of 300 μmol photons m^{-2} s^{-1} PAR, the conversion of Dd to Dt in *Phaeodactylum tricornutum* was stimulated by the addition of 3 μmol photons m^{-2} s^{-1} UV-B and a parallel increase in the level of nonphotochemical quenching was observed (Goss et al., 1999). The same polychromatic UV-B treatment that inhibited HCO_3^- uptake in *Nannochloropsis gaditana* (above) caused 24 percent out of 60 percent de-epoxidation of V (Neale et al., 2001). Several possible mechanisms were proposed, including inhibition of ATP synthase resulting in an enhanced electrochemical gradient across the thylakoid membranes, or inhibition of concomitant epoxidation by the epoxidase, but these have not yet been confirmed. In any case, the pigments themselves appeared to be rather resistant UV-B (Lutz et al., 1997).

Assimilation of CO_2 into simple sugars provides basic carbon skeletons for synthesis of other macromolecules: proteins, carbohydrates, lipids, and low molecular weight compounds. Changes in the partitioning of carbon among these pools as a result of UV-B is not thoroughly studied (reviewed by

Hessen et al., 1997), but is likely to be significant, considering the number of possible targets of UV-B damage in the cell. Effects are likely to be both dosage- and species-dependent (Arts and Rai, 1997). Allocation to protein seems to be the most sensitive component, but reduced protein synthesis could also be the result of inhibition of N uptake and incorporation into free amino acid pools, observed in Antarctic and temperate microalgae and macroalgae exposed to artificial radiation (Döhler et al., 1995; Döhler, 1997a,b; Döhler and Hagmeier, 1997). On the other hand, the N and P content of a periphyton community exposed to ambient, two or four times enhanced UV-B did not change (McNamara and Hill, 2000). Allocation of C to proteins and lipids in a marine benthic diatom mat increased at the expense of allocation to polysaccharides and low molecular weight compounds after four days exposure to approximately three times ambient UV-B (Sundback et al., 1997). A consensus on downstream changes in cellular biochemistry is difficult to reach due not only to differences among species in their inherent susceptibility to damage, their ability to redirect energy to enhanced photosynthetic protein turnover, to alter pigment content, and to synthesize protective compounds, but also to different treatment conditions. Clearly more work in this important area is justified.

C. Protective Strategies and Acclimation

In common with photoinhibition by PAR, photosynthesis provides crucial protection against UV damage by providing energy for acclimation or to repair damage (Adamse and Britz, 1992). Many species can acclimate to greater rates of UV-B damage to reaction center proteins and can limit net protein loss. Transcription of multiple genes encoding for alternative forms of D1 and D2 in cyanobacteria (*psb*A1-3, *psb*D1-2, respectively) was differentially up-regulated during UV-B exposure (Campbell et al., 1998; Mate et al., 1998; Viczian et al., 1998; Vass et al., 1999, 2000); in *Synechocystis* the gene products were identical, while in *Synechococcus*, the D1:2 was more resistant to UV-B. Up-regulation of photosynthetic genes may be considered an indirect effect of UV-B, since reactive O_2 species are required for the response in *Arabidopsis* (Surplus et al., 1998; Mackerness et al., 1999). The ability of cells to scavenge active O_2 species is also enhanced (Malanga and Puntarulo, 1995; Malanga et al., 1999). When combined with greater synthesis of protective compounds, effective acclimation may well reduce damage to PS II in nature to negligible levels (Vincent and Neale, 2000), but will have an energetic cost that has yet to be quantified.

In contrast to the active photoprotection from exposure to excess PAR provided by antenna-based energy quenching, extracellular and intracellular UV-absorbing compounds, most of which are transparent to PAR, provide passive protection from UV damage (Table 2). A number of types have been identified in algae, and many species contain several. The various compounds described thus far are related to UV-B absorbing secondary metabolites in higher plants, the flavonoids, in that precursor molecules are all synthesized by the shikimate pathway. These compounds were originally linked to UV protection by correlation with the probability for UV-B exposure. For example, species growing in surface blooms, shallow water or high altitude lakes tend to have more UV absorbing compounds than those at depth. Fewer studies have demonstrated the specific degree to which presence of screening compounds actually enhances protection.

Scytonemin, a yellow-brown, lipid-soluble compound found in the extracellular sheath of many cyanobacteria is composed of tryptophan- and phenylpropenoid-derived subunits and has a broad in vivo absorption maximum (λ_{max}) at 370 nm. Its sunscreen role has been demonstrated by García-Pichel and co-workers (García-Pichel and Castenholz, 1991; García-Pichel et al., 1992) who found that Chl bleaching by UV-A increased dramatically when the extracellular sheath was removed from *Chlorogloeopsis*.

The polymer sporopollenin is found in the cell walls of a number coccoid green microalgae and the subaerial members of the Trentepohliales, in the pellicle of the Peridiniales, as well as in walls of resting zygotes of the Peridiniales and the Zygnematophyceae (van den Hoek et al., 1995). Made of variable amounts of isoprenoid and aromatic components, it is usually associated with protecting cells from desiccation. But it also absorbs broadly across the UV region, and increasingly at shorter wavelengths. Extensive screening of more than 60 species of microalgae identified the presence of sporopollenin and effective repair of PS II damage by turnover of the D1 protein as being the two key factors conferring tolerance to moderately high levels of UV-B (Xiong et al., 1996, 1997), although sporopollenin absorption relative to Chl *a* was much less than that reported for

intracellular UV-absorbing compounds in other organisms.

Intracellular UV-absorbing compounds include phenolic substances and mycosporine-like amino acids (MAAs). Phenolic or polyphenolic compounds (phlorotannins) play a number of important roles in algae beside absorption of UV-B (summarized in Lobban et al., 1985). Thus, the accumulation of phlorotannins in response to UV-B may impact algal biology at multiple levels (or vice versa), as in higher plants. High levels of phenolpropanoids reduced UV-B penetration into leaves (Mazza et al., 2000), and the leaves became more sensitive to UV-B when phenolpropanoid synthesis was inhibited (Gitz et al., 1998). However, their nutritional value was reduced when phenolpropanoid synthesis was induced by UV-B (Grammatikopoulos et al., 1998). In brown algae, phlorotannins are sequestered in vacuole-like bodies called physodes that occur in very high numbers near meristematic tissue and in sporophylls where screening of UV-B would be important to maintaining cell division and reproduction. The response of phlorotannin biosynthesis to a number of environmental factors including UV-B has been investigated in one brown alga, *Ascophyllum nodosum* (Pavia et al., 1997; Pavia and Brock, 2000). Induction of phlorotannin biosynthesis by UV-B required two to several weeks, but a 30% increase in phlorotannin content, induced by a 50% increase in UV-B, did not lead to lower preference by the invertebrate (isopod) grazer *Idotea granulosa*.

The most actively studied of the intercellular UV-B screens are the highly stable MAAs (reviewed by Dunlap and Shick, 1998, Karentz, 2001), derivatives of a mycosporine cyclohexenone chromophore conjugated with one or two of several different amines. At least, 19 different compounds have been described, each with a slightly different λ_{max} between 310 and 360 nm (Shibata, 1969; Tsujino et al., 1980; Nakamura et al., 1982; Karentz et al., 1991). MAAs occur in taxonomically diverse organisms across terrestrial, marine and freshwater habitats at all latitudes, and particularly in corals (Dunlap et al., 1986; Dunlap and Shick, 1998), freshwater, terrestrial, and marine microalgae (Jeffrey et al., 1999; Sommaruga and García-Pichel, 1999; Xiong et al., 1999), and rhodophyte macroalgae (Sivalingam et al., 1974; Karentz et al., 1991; Karsten et al., 1998b; Hoyer et al., 2001). Although heterotrophic organisms are unable to synthesize MAAs, these compounds are also found in high concentration in the tissues of zooxanthellate and azooxanthellate clams (Ishikura et al., 1997) and a number of nonsymbiotic invertebrates (Dunlap and Shick, 1998), presumably through grazing. Furthermore, they can be transferred to grazers and incorporated into reproductive structures, providing a high degree of protection from UV-B (Adams and Shick, 1996; Karentz et al., 1997). The concentration of MAAs in algal tissues is correlated with total irradiance (Sivalingam et al., 1974; Beach and Smith, 1996; Karsten and Wiencke, 1999; Karsten et al., 1999; Hoyer et al., 2001). Cellular components and physiological processes in algae that contain MAAs are generally more resistant to UV-B-induced damage than those that do not, though the degree of protection is variable and depends on cell size (García-Pichel, 1994; Lesser, 1996a; Neale et al., 1998a; Franklin et al., 1999). Besides having a role in UV screening, a few MAAs also demonstrate antioxidant properties (Dunlap and Shick, 1998).

Synthesis of MAAs proceeds via early steps of the shikimate pathway, the general pathway for the synthesis of aromatic amino acids in photoautotrophic organisms and fungi (Shick et al., 1999). The conditions required to induce synthesis appear quite variable, including either UV-B, UV-A, or PAR, a combination of these, or osmotic stress in darkness (Carreto et al., 1990; Riegger and Robinson, 1997; Hannach and Sigleo, 1998; Karsten et al., 1998a, 1999; Franklin et al., 1999; Karsten and Wiencke, 1999; Portwich and García-Pichel, 1999; Shick et al., 1999). Alternatively, MAAs can be constitutively expressed in some species for generations under very low light, UV-free culture conditions (Jeffrey et al., 1999). A third group of species does not appear to have the capacity to synthesize MAAs at all (Hoyer et al., 2001). This makes it difficult to come to a consensus on triggering mechanisms. Blue light and UV-A appear to be particularly important in some species (Carreto et al., 1990; Riegger and Robinson, 1997; Franklin et al., 2001), while UV-B is required in others. Furthermore, the final concentrations and possibly the particular composition of MAAs found reflect the quantity (duration) of radiation applied. Recently, Litchman et al. (2002) demonstrated that MAA concentration depended on the level of N availability, with low N cells containing similar quantities of MAAs as those grown in low light but high N. Multiple signal transduction pathways may be responsible for MAA induction (Franklin et al., 2001), as demonstrated for the activity of enzymes

responsible for flavin biosynthesis in higher plants (Fuglevand et al., 1996). The cue for induction may be a more general trigger related to environmental stress, for example the presence of active O_2. Thus, MAAs do not reliably indicate the presence of UV-B in the environment.

IV. Photoinhibition and UV Stress in the Field

We have discussed photoinhibition and damage by UV radiation independent of each other, and mainly under controlled laboratory conditions. What are the relative contributions of these processes to reduced productivity under field conditions, where these processes work in concert and other environmental factors also affect photosynthetic performance?

A. Responses to Altered Light Climates

The study of sensitivity to excess PAR or UV in the field often involves increasing the irradiance in a sudden, step-wise fashion, as would occur if the canopy were removed, or if water quality changed suddenly. In these cases, low light-acclimated benthic algae transferred to more shallow or exposed sites with higher light are almost invariably more photoinhibited by PAR and/or damaged by UV radiation than the same or similar species collected from high light habitats (Dring et al., 1996b; Franklin et al., 1996; Häder et al., 1996c; Hanelt et al., 1997a; Franklin et al., 1999; Brouwer et al., 2000). Typically, there is a greater loss of efficiency, and (where measured) a reduced maximum rate of photosynthesis, less so if UV is removed from the spectrum. Recovery in subsequent low light periods is also reduced. Thus, sensitivity to PAR and UV measured over the short-term reflects the typical bathymetric distribution of algae (Dring et al., 1996b; Hanelt et al., 1997a; Sagert et al., 1997; Hanelt, 1998) and their acclimation status.

Over the longer term, acclimation to the new light condition may proceed by the mechanisms discussed above, within the constraints of genotype and limitation by other environmental factors (e.g. temperature or nutrient availability). A high degree of photoinactivation, photodamage, and inhibition of carbon fixation that lessen over time can accompany the acclimation process (Henley et al., 1991b; Hazzard et al., 1997; Franklin et al., 1999). A change in species composition to more resistant species, as reported for phytoplankton communities (Wängberg et al., 1996, 1998) might also 'mitigate' reduced community productivity.

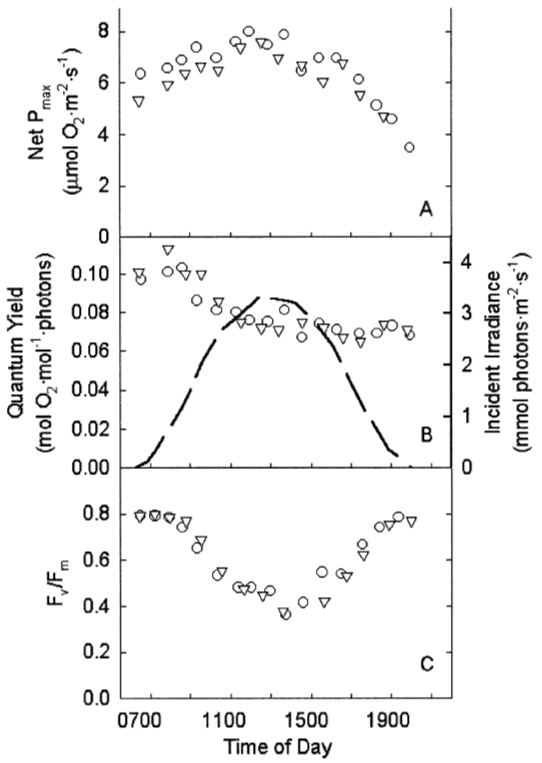

Fig. 3. Diurnal patterns of areal (one-sided) (A) net photosynthetic capacity, (B) quantum yield of O_2 evolution, and (C) the chlorophyll fluorescence parameter F_v/F_m of *Ulva rotundata* growing at 100% surface irradiance. Circles and squares represent consecutive days. Dashed line in (B) represents the diurnal pattern of incident irradiance. Note that the efficiency of O_2 evolution does not recover from the midday depression on the same time scale as F_v/F_m, even though both parameters are taken to indicate photosynthetic efficiency. From Henley et al. (1991a).

B. Diurnal Patterns of Photosynthesis

A sustained step-change in irradiance is not usually experienced in the field. Rather, incident light changes gradually over the day, or suddenly but relatively briefly with passing clouds, movement of the canopy, or tides. Implicit from our discussion of photoprotection, acclimation to a particular light climate does not mean a constant rate of photosynthesis. In situ measurements reveal daily changes in both the efficiency (quantum yield) and maximum rate of

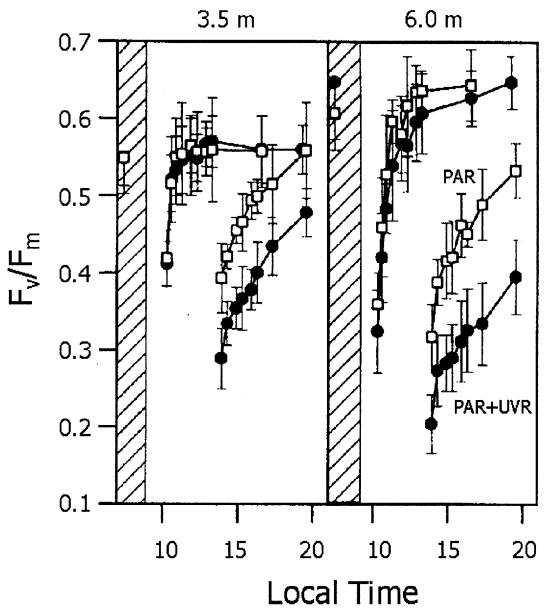

Fig. 4. Recovery of photosynthetic efficiency (F_v/F_m) after exposure to PAR (open symbols) or PAR+UVR (closed symbols) in the field, plotted against time of day. In the early morning, populations of the red alga *Chondrus crispus* were selectively screened in the upper (3.5 m) and lower (8.5 m) intertidal zone at Roscoff, France. Recovery in low light was measured periodically starting after moderate (1000 hours) and maximum (1430 hours) photoinhibition was observed. From Sagert et al. (1997).

photosynthesis that are related to incident light and strongly dependent on previous light history. Typically, efficiency declines as irradiance increases due to either the diel solar pattern, clearing of the skies, falling tides, or transport into the upper layer of the photic zone (Henley et al., 1991a; Hanelt et al., 1993, 1994; Häder et al., 1996a,b,c; Longstaff et al., 2002) (Fig. 3). As irradiance falls, efficiency recovers almost immediately. These dynamic changes in efficiency are typical of what is expected in high light-acclimated algae when photoprotection is engaged to limit photoinactivation and photodamage, and have been demonstrated at a range of latitudes in numerous species. Experiments that selectively remove UV-B and UV-A from the solar spectrum show that diurnal changes in photosynthetic efficiency of macroalgae are primarily due to PAR. However, both UV-A and UV-B can further reduce photosynthetic efficiency of thalli measured in situ and slow the recovery of photosynthetic efficiency once the light stress is removed (Fig. 4) (Häder et al., 1996c; Hanelt et al., 1997c; Sagert et al., 1997; Cockell and Rothschild, 1999). UV-A is often (but not always) more effective than UV-B, reflecting the greater total energy of UV-A in the solar spectrum. While UV-B may reduce maximum photosynthetic rates more than photosynthetic efficiency (see above), this has not been well-studied under field conditions, and deserves further attention.

Mixing strongly affects the extent of PAR and UV-B damage to phytoplankton photosynthesis integrated over the photic zone in both limnic and marine systems. While mixing provides an ever changing pattern of irradiance to an individual cell as it moves into inhibitory levels of PAR and UVR and then to lower irradiance where repair is enhanced, it also moves new, undamaged cells into a damaging climate and increases the overall proportion of damaged cells in the water column. Simulations of mixing regimes with Antarctic phytoplankton demonstrated an overall reduction in total photosynthesis compared to static incubations (Helbling et al., 1994), but modeling studies using data from the Weddell-Scotia Confluence revealed that inhibition could be enhanced or reduced, depending on both the depth and rate of vertical mixing (Neale et al., 1998c). Mixing within the photic zone led to enhanced inhibition relative to a static condition, even at relatively slow turnover times of 8 h. In contrast, mixing below the photic zone reduced overall inhibition as damaged cells were carried out of the photic zone and replaced with uninhibited ones. In a stratified system, phytoplankton may be able to move to optimal irradiances within the chemical and temperature constraints of the system, but in mesohumic lakes those boundaries can be quite narrow (Xenopoulos et al., 2000). Although dinoflagellates show positive phototaxis in humic lakes which is essential for obtaining light for photosynthesis, this behavior also brings them into high UV-B conditions. UV-B has been shown to affect negatively both motility and orientation of several flagellates (Ekelund, 1993, 1994), and may affect the ability of phytoplankton to alter their position to limit both photodamage and UV damage.

We should point out that much of our information on photoinhibition and UV damage in the field comes from the analysis of chlorophyll fluorescence data for PS II. Few studies have attempted to measure simultaneously the changes in chlorophyll fluorescence and gas exchange, which together give a more complete picture of photosynthetic energy flow (Häder et al., 1996a,b; Beer et al., 1998; Beer et al., 2000; Franklin and Badger, 2001; Longstaff et al.,

2002). It is possible that efficient charge separation at PS II may be supplying electrons to oxidants other than CO_2 (Falkowski and Raven, 1997). For example, photosynthetic efficiency measured as F_v/F_m recovered more rapidly during a diurnal time course than efficiency measured by O_2 evolution (Fig. 3) (Henley et al., 1991a). Furthermore, maximal rates of O_2 evolution in high PAR+UVR can be obtained at the same time that efficiency is minimal (Fig. 3) (Henley et al., 1991a; Bruhn and Gerard, 1996). This illustrates that photoinhibition would not necessarily limit productivity in cases where acclimation to the given light regime has occurred, unless UV-B damage has suppressed Rubisco content or activity.

C. Interactions of Photoinhibition and UV Stress with Other Environmental Factors

Photosynthetic energy flow is regulated to maintain balance under the particular set of co-occurring environmental conditions (Maxwell et al., 1994; Dealda et al., 1996; Kana et al., 1997). Thus a given light exposure may be more or less damaging depending on other variable and co-occurring factors, like N limitation, temperature stress, and desiccation. Field data on interactions between irradiance and other potentially limiting factors are sparse, but suggestive of the great difficulty in separating PAR and UV effects from other limitations. This was clearly demonstrated by the temporal variation in effects on primary productivity of taxonomically diverse microbial mats observed when UV-A and UV-B were selectively removed from the natural spectrum (Cockell and Rothschild, 1999).

Separating N uptake and photosynthesis as the primary targets of UV radiation may be particularly difficult. Under laboratory culture conditions, low N availability limited photoacclimation of *Ulva* to high light, leading to sustained photodamage (Henley et al., 1991b). Marshall et al. (2000) have presented a model for photoinhibition by PAR incorporating damage to the D1 protein and NPQ under various photoacclimation and N conditions, but the possible effects of UV radiation on carbon fixation were not considered. Litchman et al. (2002) found that dinoflagellates grown at low N were more sensitive to the entire UV spectrum, presumably as a result of smaller cell size and slower rates of damage repair. Altamirano et al. (2000a) estimated that seasonal variation in the relative growth rate of *Ulva olivascens* in the field in southern Spain was primarily controlled by N availability (70%), rather than PAR (15%), UV-B (5%), or temperature (4%). The combination of high irradiance with UV-B can induce N limitation in natural phytoplankton populations by reducing NO_3^- uptake and reduction (Behrenfeld et al., 1995; Döhler et al., 1995; Kristiansen et al., 1998), regardless of prevailing N availability. However, addition of N, P, and Si ameliorated the effects of enhanced UV-B in a diatom-dominated benthic community (Wulff et al., 2000).

High temperature in combination with saturating PAR can be highly damaging (Henley et al., 1992; Bruhn and Gerard, 1996), and photo-oxidation due to a combination of UV-B and high temperature stress is implicated in the loss of photosynthetic performance of zooxanthellae during coral bleaching (Lesser, 1997; Chapter 19, Yellowlees and Warner). On the other hand, acclimation to low temperature enhances photoprotective strategies for high PAR, similar to growth at high light (Franklin, 1994; Maxwell et al., 1994). Desiccation during low tide may provide a measure of protection to PS II from simultaneous exposure to high PAR and UV (Huppertz et al., 1990).

D. Inhibition of Recruitment

Both PAR and UV may limit community productivity by preventing the successful establishment of spores and the recruitment of new organisms (Dring et al., 1996a; Santas et al., 1998; Yakovleva et al., 1998; Swanson and Druehl, 2000; Wiencke et al., 2000). Indeed, spores, gametophytes and young sporophytes may differ markedly in their susceptibility to PAR and UV-B (Dring et al., 1996a and Hanelt et al., 1997b for *Laminaria* spp.). Some might considered this a 'non-effect', in that the productivity of an individual is not reduced, because UV-B or PAR has already excluded it from the habitat. An equally valid argument can be made that this represents photoinhibition or UV-B damage at their most effective. In either case, the extent to which propagules are susceptible to radiation needs to be determined in order to assess the potential community-wide effects of ozone depletion.

V. Scope for Further Research

While both PAR and UV-B directly influence photosynthesis and thus the upper boundary of algal

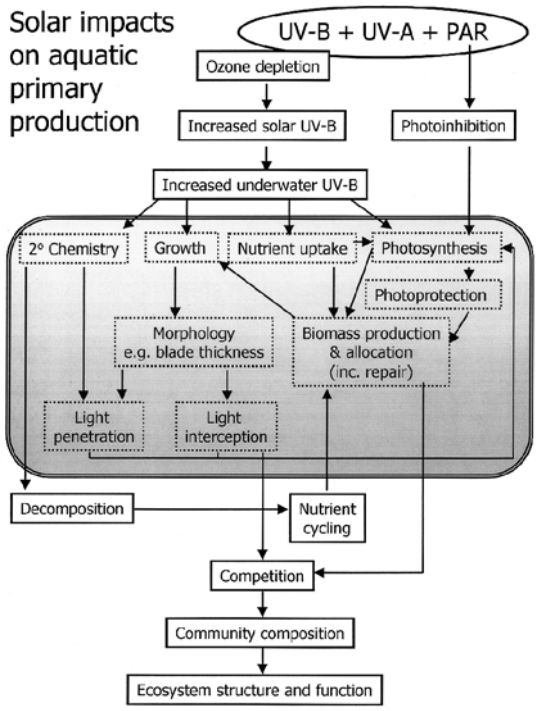

Fig. 5. Potential impacts of solar radiation on aquatic primary productivity. At the level of the organism, responses to photoinhibition (by PAR) can ameliorate damage by UV-B, while changes in response to UV-B may feedback to alter the way in which algae can respond to PAR. Responses of organisms need to be viewed in the context of the whole community, since successional patterns may change with no discernible change in primary production.

distributions, our current understanding indicates distinct differences in the mechanisms by which this occurs. At the level of the organism and within communities, Fig. 5 summarizes some general areas where the impacts of high levels of PAR and UV-B on photosynthesis occur and might be seen to flow on. This figure may seem to be all-encompassing, yet we still must speculate at many of these points, as discussed above. Our understanding of the ways in which photoinhibition and UV stress influence primary productivity will be enhanced with research focused in the following directions.

Chloroplast structure in algae is highly variable, yet our knowledge of photoprotection, photoinactivation and the repair of photodamage is based almost exclusively on higher plants and a few green algae, analyzed outside the community context. It is likely that there are more discoveries to be made at the molecular level in other algal groups which will contribute to the understanding of evolutionary relationships among these groups, as well as how total communities are optimizing photosynthetic performance. It is also necessary to expand our study of changes in photosynthetic efficiency to the full photosynthetic process, as it is clear that algal productivity cannot be predicted exclusively from changes in photosynthetic efficiency.

Likewise, in order to understand the potential impact of a global increase in UV-B, a better understanding of the biochemical and physiological responses to UV radiation is needed in the context of the whole organism. In particular, changes in the whole of photosynthesis, growth rate and success of recruitment will have major impacts on community productivity, succession, and species ranges in an environment that includes higher CO_2 and temperature. BWFs for processes at all physical levels (gene to membrane) and all process levels (nutrient acquisition to growth and recruitment) will be critical in this regard. Perhaps the most important of these steps is the effect on growth, as growth integrates damage to nutrient acquisition, photosynthesis, DNA replication and cell division (Pakker et al., 2000a), and the cost of repair and acclimation (Franklin et al., 1999). Recent studies with higher plants, using natural radiation supplemented with UV-B to simulate a given ozone depletion scenario, suggest that indirect effects on photosynthesis from changes in morphology, growth rate, and secondary chemistry are more significant than direct damage to photosynthetic structures (Caldwell et al., 1998). A focus on parts of photosynthetic process, using techniques limited to only one aspect of this multifaceted whole, may well over- or underestimate the ability of algae to adjust their photosynthetic capacity to maintain optimal productivity in the face of global change.

More studies of photoinhibition and UV responses need to be carried out in ecologically relevant contexts, using realistic treatments of appropriate spectral composition. Analyses of productivity need to consider effects of both excess PAR and UVR. Temperature clearly affects responses to excess PAR and UV, but there are almost no ecophysiological data on the combined effects of temperature, nutrient (including carbon) availability, desiccation, and salinity stress, any of which might be expected to co-occur with light stress in aquatic habitats. Nor are there many studies of the effect of light on community composition and herbivory. Given the seasonal nature

of algal populations and exposure to light, studies need to be carried out over a range of time scales. Information on responses of early settlement stages will be particularly useful for ecosystem analysis. While the immediacy of ozone thinning, CO_2 enrichment, and rising temperature lend impetus to ecophysiological studies of photosynthesis, we will also gain an understanding of the evolutionary pressures on these ancient organisms.

Acknowledgment

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Chapter 17

Adaptation, Acclimation and Regulation in Algal Photosynthesis

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Summary

Relating the photosynthetic processes of algae to their environments requires that the responses are considered over time-scales of seconds to minutes (regulation), hours to days (acclimation) and up to thousands of millions of years (adaptation). All of these responses are genetically determined, with so-called adaptations reflecting genetic changes which distinguish taxa from the strain (ecotype), i.e. infraspecific level, up to the Division (Phylum) level. Tempting as it is to assign the establishment of these genetic differences to natural selection, the genetic differences at the higher taxonomic levels should be related to the environments at the time at which they evolved. Genetic differences limit the responses that algal genotypes can make to their immediate environment (i.e. during a single generation). Using photosynthetic pigments as an example, the content per unit biomass of photosynthetic light-harvesting pigment-protein complexes, and, where they occur, of the energy-dissipating xanthophyll cycle pigments change with the photon flux density for growth; light-harvesting pigments decrease with increasing light, while xanthophyll cycle pigments increase. There are cost-benefit considerations not only in the extent of such acclimation, but also of the rate at which acclimation occurs. Regulation involves allosteric or covalent modification of pre-existing catalysts, e.g. ribulose bisphosphate carboxylase-oxygenase, xanthophyll cycle pigments, and the pigment-protein complexes involved in state transitions. Much remains to be done to not only understand adaptation, acclimation and regulation in algae, but also to understand how the three processes interact.

I. Introduction

Algal photosynthesis proceeds according to the (minimal) equation

$$CO_2 + 2H_2{}^*O + 8\ \text{photons} \xrightarrow{\text{catalysts}} (CH_2O) + {}^*O_2 + H_2O$$

The adaptation and acclimation of the photosynthetic machinery in algae concerns the responses of the organisms to variability in the availability of the substrates (CO_2, H_2O and photons), and variability in the build-up of the products (O_2 and (CH_2O)), of photosynthesis. It also concerns variability of the availability of the resources that are required to produce the catalysts used in the photosynthetic reactions. Among these requirements for producing the catalytic apparatus of photosynthesis are the carbohydrates produced by photosynthesis, and the nutrients obtained from the environment. These nutrients include N (immediately as NH_4^+), P (as HPO_4^{2-}), Mg^{2+}, Cl^-, Mn^{2+}, Fe^{2+}/Fe^{3+}, Zn^{2+} and (often) Cu^{2+}. The availability of these resources in algal habitats varies widely, in some cases by at least four orders of magnitude (Table 1).

At the outset we need to differentiate clearly amongst the terms adaptation, acclimation and regulation[1]. Adaptation will be used to describe an outcome of evolution as recorded in the gene pool of a species. It also refers to changes in gene frequency within a gene pool as a result of selection with or without recombination. Acclimation will be used to describe changes of the macromolecular composition of an organism that occurs in response to variation of environmental conditions. According to this usage, photoacclimation occurs via synthesis or breakdown of specific components of the photosynthetic apparatus. Acclimation operates within constraints set by the genetic make-up of the species (or, more often, the clonal population) under investigation. Regulation

Abbreviations: CAM – Crassulacean acid metabolism; CF_o-CF_1 – chloroplast or cyanobacterial ATP synthetase; dd-dt – diadinoxanthin-diatoxanthin cycle; I – incident photon flux density; I_k – saturation function of photosynthesis; photon flux density at which the extrapolation of the initial slope of the photosynthesis:incident photon flux density relationship interects the extrapolation of the light-saturated rate of photosynthesis; K_m – Michaelis-Menten constant; L_2 – form II Rubisco with two large subunits; L_8S_8 – form I Rubisco with eight large and eight small subunits; NADH – nicotinamide adenine dinucleotide (reduced form); $NADP^+$ – nicotinamide adenine dinucleotide phosphate (oxidized form); PQ – plastoquinone; $PQ^{\cdot-}$ – plastosemiquinone; PQH_2 – plastoquinol; PS I – Photosystem I; PS II – Photosystem II; Ψ_p – pressure component of water potential; Ψ_π – osmotic component of water potential; Ψ_w – water potential; RCII – reaction center of Photosystem II; Rubisco – ribulose bisphosphate carboxylase-oxygenase; UQ – ubiquinone; $UQ^{\cdot-}$ – ubisemiquinone; UQH_2 – ubiquinol; v-a-z – violaxanthin-antheraxanthin-zeaxanthin cycle

[1] The term adaptation has been employed loosely in studies of algal photosynthesis and ecology to refer to genetic adaptation, physiological acclimation and physiological regulation. We feel that it is necessary to differentiate between these processes. The term behavior can be reserved for activities such as swimming or other movements/migrations.

Table 1. Range of external conditions in which at least some algae can grow photolithotrophically. Natural conditions unless otherwise specified.

Environmental factor	Minimum value at which photolithotrophic growth can occur	Maximum value at which photolithotrophic growth can occur	References
Photon flux density of photosynthetically active radiation 400–700 nm	20 nmol photon m^{-2} s^{-1} (crustose coralline marine red alga growing at 274 m) Usually at least 0.5 μmol photon m^{-2} s^{-1}	≥ 2 mmol photon m^{-2} s^{-1} (full sunlight) As little as 1 μmol photon m^{-2} s^{-1} for deep-water crustose coralline red alga	Raven, Kübler and Beardall (2000)
UV-B flux density 280–320 nm	0	?	Franklin and Forster (1997)
Inorganic carbon concentration	1 mmol m^{-3} (?) in some	100 mol m^{-3} (cyanobacteria in soda lakes) Lower for some other algae	Raven (1990)
CO_2 concentration	1 mmol m^{-3} (?) in some	≥ 80 mmol m^{-3} (*Cyanidium coldarium* s.l. in vitro) ≥ 30 mol m^{-3} (*Chlorella* in vitro)	Raven (1990)
pH	0.5 (*Dunaliella acidophila*) 1.0 (Cyanidiophyceae)	11 (some cyanobacteria, Chlorophyta)	Raven (1990)
O_2 concentration	~ 0 (laboratory)	≥ 1 mol m^{-3} (*Enteromorpha*, *Ulva*) Lower for some other algae	Raven et al. (1994)
Temperature	~ 1.8°C (cyanobacteria, eukaryotes in Antarctic)	+ 45 °C (Cyanidiophyceae) + 55 °C (cyanobacteria)	Raven and Geider (1988)
Water potential (Ψ_w) components Ψ_p and Ψ_π; $\Psi_w = \Psi_p + \Psi_\pi$	–38 MPa (Ψ_π) (*Dunaliella* in Dead Sea; *Apatococcus* on tree trunks) Ψ_p + 0.1 MPa: atmospheric pressure at sea level; higher in Dead Sea, lower at high altitudes)	+ 2.84 MPa (Ψ_p) (crustose coralline marine red alga growing at 274 m, with atmospheric pressure of + 0.1 MPa at the surface added to 2.74 MPa due to 274 m depth of water) (Ψ_π–2.46: seawater) +1. MPa (Ψ_p) (benthic algae growing at 150 m in Lake Tahoe, with atmospheric pressure of MPa at the surface added to 1.50 MPa due to 150 m of water Ψ_π – 0.005 MPa; very low osmoloarity fresh water)	Raven (1984a, 1993), Raven, Kübler and Beardall (2000)

will be used to describe the adjustments of catalytic efficiency that occur without net synthesis or breakdown of macromolecules. Adaptation, acclimation and regulation are generally thought to act according to rules that optimize performance (maximize evolutionary success or fitness) within constraints set by the environmental conditions. In photosynthesis optimization involves a trade-off among:

- maximizing the rate of photosynthesis per unit of resource (energy, carbon, nitrogen, etc. used) in constructing the photosynthetic apparatus.
- maximizing the quantity of CO_2 fixed per incident photon when light is limiting photosynthesis, and
- minimizing the damage that can arise from excess light, UV radiation, and oxygen radicals.

Regulation operates on a time scale of seconds to minutes. Acclimation operates on a time scale of hours to days. Adaptation operates on a wide range of time scales from days for shifts in gene frequency, to seasons or years for species replacement during succession, to hundreds of millions of years for the evolution of new species. Together, the processes of adaptation, acclimation and regulation determine the niche that a population occupies. Adaptation refers to the hard-wired genetic information within an organism, whereas acclimation and regulation refer to the implementation of this information within specific environmental contexts.

The genetic basis for photosynthesis by algae *sensu lato* involves thousands of (morphologically defined) species of Cyanobacteria, and of the tens of thousands of species of species of algal eukaryotes derived from them and a protist host by endosymbioses (Chapter 1, Douglas et al. and Chapter 2, Larkum and Vesk). These include primary endosymbiosis of a cyanobacterium-like ancestor to produce the Chlorophyta and Rhodophyta (and Glaucocystophyta), and secondary endosymbiosis of photosynthetic eukaryotic cells to yield the Euglenophyta and Chlorarachniophyta (endosymbiosis of a green alga) and the Heterokontophyta, Haptophyta, Cryptophyta and (probably) Dinophyta (endosymbiosis of a red alga). This diversification of algae involves not only the evolution of the Cyanobacteria and the plastids which are derived from ancestral Cyanobacteria, but also the genetic variability in the chemoorganotrophic 'host' eukaryotes. This involves seven chemoorganotrophic eukaryotic taxa as the hosts for the photosynthetically active symbionts which ultimately became the plastids that we know today (van den Hoek et al., 1995; Chapter 1, Douglas et al.). This genetic 'mixing and matching' in the evolution of the algae provides the basis for evolutionary adaptation of algae to particular photosynthetic environments.

The occurrence of tens of thousands of species of algae *sensu lato* implies, via classic niche theory, the occurrence of a corresponding number of niches which may not, of course, all relate directly or indirectly to photosynthetic reactions. However, G. E. Hutchinson has pointed out that there are more morphologically defined species of phytoplankton algae than there are obvious niches. This is Hutchinson's 'paradox of the plankton', and it can be applied to algal habitats other than the plankton (as well as to many animal habitats). One way round this paradox is the involvement of more environmental (biotic and abiotic) factors in the definition of niches than have previously been used. These include the quantitative use of factors in the form of resource availability ratios and clone-specific or species-specific disease (viruses, protista and fungi) and/or grazing pressure. A further means of resolving the paradox is to invoke temporal variability in the habitats such that competitive exclusion of one (or more) species by another species that is better adapted to that habitat does not go to completion before the combination of environmental conditions changes and alters the selective balance. Such 'explanations' become even more necessary as molecular genetic methods and redefinitions of the criteria for morphologically defined species (e.g. of diatoms), increase the number of species (or ecotypes) to be accommodated in the niches.

Complementary to adaptation is acclimation, i.e. phenotypic changes in an organism within a generation and without any genetic change contrasting with adaptation which involves genetic change over a number of generations. Acclimation increases the niche width for a given genotype. The occurrence of so many species of algae shows that acclimation has significant constraints. Presumably the constraints on the extent of acclimation involve the greater fitness of a genotype when growing in a

habitat close to its optimum than when acclimated to a very different habitat. In other words, there are genotypes of other species that have greater fitness in this environment that is far from optimal for our acclimating species.

Acclimation operates within the genetic constraints set by adaptation. Although not yet available for any alga *sensu stricto* (i.e. a non-embryophyte eukaryotic phototroph), complete nucleotide sequences of genomes should be very useful in examining the limits of acclimation within genotypes. Not all genes are expressed simultaneously. Thus, complete gene sequences will show the range of traits that can be exhibited by the genotype. Complete nucleotide sequences for the cyanobacterium *Synechocystis* sp. PCC 6803 (Kaneko et al., 1996) and other Cyanobacteria (Hess et al., 2001) are proving very helpful in probing adaptation and acclimation, especially if the constitutive or inducible nature of the enzymes (or other proteins) encoded can be determined. A (non-algal) example of the complete genome sequence of an organism that can tolerate very extreme conditions (by the standards of the majority of organisms) is *Deinococcus radiodurans* R1 (White et al., 1999). This gram-positive non-photosynthetic bacterium has the greatest resistance to ionizing radiation of any known organism, as well as great tolerance of UV-B radiation and desiccation. The first two attributes (and possible the third) relate to the very wide range of DNA repair mechanisms available, often with multiple gene copies, and the 5–10 copies of the genome in growing cells. Resistance to ionizing radiation also correlates with desiccation tolerance in Cyanobacteria (Potts, 1999). Again, the extent to which the mechanisms are constitutively expressed and the extent to which some of them are inducible deserves exploration.

This chapter deals with adaptation, acclimation and regulation as they relate to algal photosynthesis. We shall see that the outcomes of adaptation and of acclimation to particular environments are frequently similar, presumably as a result of constraints due to physics and chemistry and to evolutionary history. Before considering adaptation and acclimation, we consider the range of environments in which the photosynthetic apparatus can function, taking photosynthetic algae as a whole.

II. The Range of Resource Availabilities and Other Environmental Factors within Which Algae Can Photosynthesize

Table 1 summarizes the range of resource availabilities and other environmental factors which are consistent with photosynthetic growth of extant algae. Of the factors which can be expressed as ratios of maximum to minimum values, the ratio for photosynthetically active radiation is at least 10^5, for inorganic carbon the concentration ratio is 10^5, for CO_2 the concentration ratio is almost 10^5, while for (external) H^+ activity the ratio is 10^{10}.

Some of the extremes of the ranges of environmental factors in Table 1, e.g. the 80 mmol m^{-3} CO_2 tolerated by growing *Cyanidium* may seem laboratory artifacts, irrelevant to the present environments in which algae grow, while others (photon flux density, pH, O_2) do reflect environments in which algal growth occurs today. However, even the extremely high CO_2 levels tolerated by *Cyanidium* have natural parallels in the past. Thus, the Earth's atmosphere at the time of the evolution of photosynthesis (3.5–3.8 million years ago) probably had CO_2 partial pressure of several atmospheres (i.e. a significant fraction of 1 MPa) (Falkowski and Raven, 1997; Cockell, 2000), with a decreasing trend (but with significant variations) subsequently; ocean pH would correspondingly have been lower than the present value at earlier times. O_2 was at about 10^{-8} of the present atmospheric level, i.e. at the partial pressure generated by atmospheric photochemistry, until just before 2 billion years ago when global accumulation of photosynthetically produced O_2 began (Falkowski and Raven, 1997), with an increasing trend (but with significant variations) subsequently. Solar energy output has increased, as has the maximum wavelength and total energy of solar emission, over the 4.5 billion years of the Earth's existence (Falkowski and Raven, 1997). The increase in photosynthetically active radiation over the last 4.5 billion years is some 25%, while the flux of UV-B radiation has decreased (Falkowski and Raven, 1997). The current (Pleistocene) glaciations are an exception to the general occurrence of a warmer Earth with smaller equator to pole temperature gradients, so sea surface waters cooler than 5 °C or so have been the exception during the time that algae have existed (Falkowski and Raven, 1997).

Extant algae have a day of 24 h and a year of 365

days. With increasing latitude the photoperiod shows increasing seasonal variation. The phasing of cellular activities to the diel cycle of light and darkness relates to circadian rhythms as reset by light (and temperature) cycles. In strongly seasonal environments a number of macroalgae are 'seasonal anticipators' (Kain, 1989), i.e. change their behavior in a manner which relates to the 'expected' environmental conditions in the following weeks or months. This effect is probably related to circadian rhythms (Lüning, 1993). While the length of the year has not changed over the time (~3.8 billion years) for which life has existed on earth, the daylength has almost doubled and the number of days per year has almost halved as the lunar orbit has receded from the Earth with retention of angular momentum (Walker et al., 1983), so presumably circadian rhythms have almost doubled their free-running periods. Without the moon the Earth's obliquity would not have been stabilized at $23.3^{\circ} \pm 1.3^{\circ}$ (Laskar et al., 1993), and the resulting chaotic variations in obliquity would have had profound photoperiodic consequences (Cockell, 2000).

III. Adaptation of the Photosynthetic Apparatus

A. Background

Some of the putative adaptations of the photosynthetic machinery concern the occurrence of different catalysts or ratio of catalysts of particular reactions among taxa. The processes that will be considered are (1) light-harvesting pigments and associated proteins, (2) substitutions within the photosynthetic electron transport chain, (3) xanthophyll cycle pigments, (4) state transitions, (5) variations in the main carboxylating enzyme, ribulose bisphosphate carboxylase/oxygenase (Rubisco), (6) enzymes involved in glycolate metabolism and (7) enzymes involved in protection from photo-oxidative stress.

B. Light Harvesting

The rate of light absorption sets an upper limit on algal productivity. There are large differences in light absorption spectra, as well as in the distribution of excitation energy between Photosystems I and II, among the higher alga taxa (Fig. 1; see also Chapter 13, Larkum).

We can consider here the occurrence of at least four types of light-harvesting pigment-protein complexes (for a full description refer to Chapter 13, Larkum). These are the (1) phycobilin complexes, (2) chlorophyll *a*/*b* complexes, (3) fucoxanthin-chlorophyll complexes and (4) peridinin-chlorophyll complexes, although we note that each of these four types of complexes may have arisen more than once in the evolution of the algae. Phycobilin light-harvesting pigment-protein complexes occur to the exclusion of chlorophyll-based complexes in most Cyanobacteria and red algae. Other algae have chlorophyll-based complexes instead of, or as well as, the phycobilins (Rowan, 1989; Larkum and Howe, 1997; Raven, 1998, 1999; Raven et al., 2000; Table 2). The O_2-evolvers with only phycobilins as tetrapyrrole-based light-harvesting pigments other than chlorophyll *a* have higher ratios of PS I to PS II than do other O_2-evolvers (Falkowski and Raven, 1997; Larkum and Howe, 1997; Raven et al., 1999; Table 1).

The occurrence of structurally and spectrally different light-harvesting complexes in different higher taxa of algae led Engelmann (1883) to attempt to relate the zonation of higher taxa of intertidal and subtidal seaweeds on rocky shores to their pigmentation via the different light climates in which the various algal taxa grow. This insightful suggestion by Engelmann (1883) has been validly criticized on several grounds, e.g. the great variation in spectral transmission of coastal seawaters, the general absence of a 'green-brown-red' sequence of algal zones with increasing depth, and the high absorptance of many macroalgal thalli which via a large package effect, minimizes the differences in absorption as a function of wavelength among higher taxa of algae (Crossett et al., 1965; Larkum et al., 1967; Dring, 1981, 1982; Raven, 1984a,b; Kirk, 1994).

Engelmann's (1883) hypothesis could apply to optically thin algal structures, e.g. unicells and the young stages of macroalgae (Raven, 1984a,b, 1986, 1996, 1998, 1999). For the phytoplankton there is some relationship between habitat and genetically constrained pigmentation but there are several counter-examples (Raven, 1996, 1998, 1999). Vertical mixing (MacIntyre et al., 2000) over variable depths, which can be tens of meters, will clearly complicate any relationship of light climate and pigmentation. As for the young, relatively low absorptance, stages of macroalgae, Harvey (1836) was prescient in pointing out that coloration (as a taxonomic criterion) was best seen in reproductive stages (spores, gametes)

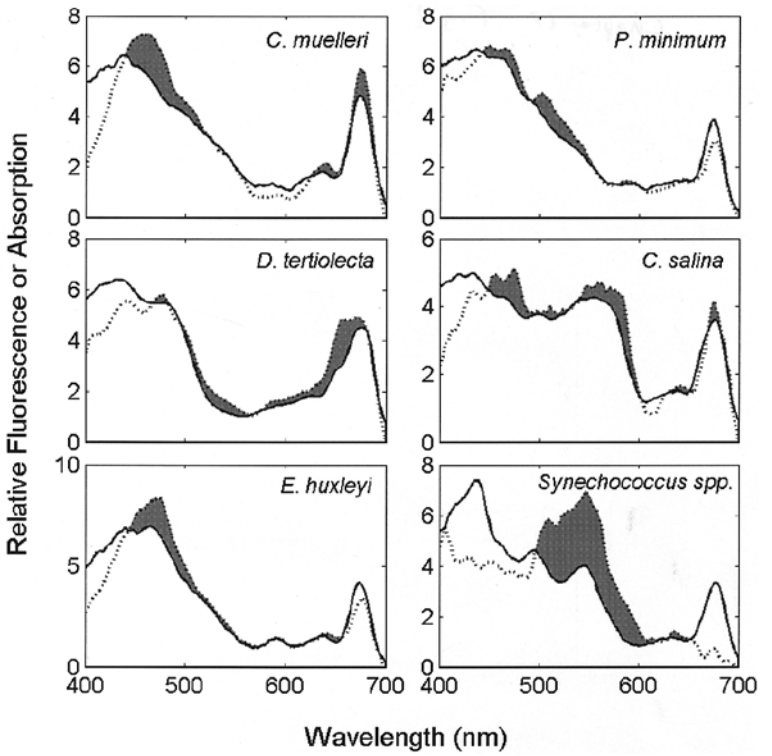

Fig. 1. Absorption (solid lines) and fluorescence excitation (dotted lines) spectra for the diatom *Chaetoceros muelleri*, the dinoflagellate *Prorocentrum minimum*, the chlorophyte *Dunaliella tertiolecta*, the cryptomonad *Chromonas salina*, the prymnesiophyte *Emiliania huxleyi* and the cyanobacterium *Synechococcus*. Spectra are normalized such that the areas under both the absorption and fluorescence spectra are equal. Fluorescence emission was measured at 730 nm, and arises primarily from Photosystem II. The filled area between the curves indicates the spectral region in which excitation energy is preferentially delivered to Photosystem II. In other regions of the spectrum, excitation energy is preferentially delivered to Photosystem I. The largest mismatch between absorption and fluorescence excitation spectra is for the cyanobacterium. Figure provided by Dr. David Suggett.

of seaweeds and freshwater macrophytes. However, attempts to interpret (à la Engelmann, 1883) the establishment of macroalgae in terms of their pigmentation and light quality, while not falling foul of high absorptance, still cannot explain fully the observed zonation patterns in terms of the (variable) spectral quality of incident radiation at a given depth in coastal seawater (Raven et al., 2000; Raven and Kübler, 2002).

The earliest members of the higher taxa of algae (Division, Class) were probably unicellular and of low absorptance. Thus, the spectral diversity of their pigments could have influenced photosynthetic performance at low incident irradiances, provided that the cells were dispersed rather than aggragated in a mat (Raven, 1996, 1999). Even so, it is not easy to relate the spectral differences in light-harvesting pigments among higher taxa of algae with the environment in which they presumably evolved, e.g. deep in the water column as a response to the higher UV-B output of the sun early in the Earth's history (Raven, 1996, 1999; Falkowski and Raven, 1997).

C. Photosynthetic Electron Transfer Chain

There are two locations in the photosynthetic electron transfer chain where markedly different catalysts perform similar functions. These are between the cytochrome b_6f complex and PS I where plastocyanin and cytochrome c_6 serve as electron carriers and downstream of PS I where flavodoxin can substitute for ferredoxin. Red algae and those derived by secondary endosymbiosis of red algal cells and euglenoids have only Cytochrome c_6. Other algae (Cyanobacteria, Chlorophyta) have only plastocyanin or both plastocyanin and cytochrome c_6 (a point

Table 2. Genotypic ('adaptive') variation in photosynthetic characteristics among algae

Characteristics	Cyanobacteria	Chlorophyta	Cryptophyta	Dinophyta	Euglenophyta	Haptophyta	Heterokontophyta	Rhodophyta
Photosynthetic light-harvesting pigments	Chlorophyll a + phycobilins[1] (or chlorophyll *a* + phycobilins + chlorophyll *b* or *d*[2], or chlorophyll *a* + chlorophyll *b*[3] (± chlorophyll *c*-like pigments)	Chlorophyll *a* + chlorophyll *b* ± chlorophyll *c*-like pigment)	Chlorophyll *a* + chlorophylls *c* + phycobilins (in thylakoid lumen, not phycobilisomes)	Chlorophyll *a* + chlorophyll c_2 + peridinin (or like almost every other algal Division as a result of secondary endosymbiosis)	Chlorophyll *a* + chlorophyll *b*	Chlorophyll *a* + chlorophylls *c* + fucoxanthin and derivatives	chlorophyll *a* ± chlorophylls *c* + fucoxanthin (b, c, p, x); chlorophyll *a* + violaxanthin (e)	chlorophyll *a* + phycobilin ± chlorophyll *d*
Redox catalyst for electron transfer from cytochrome b_6f to PS I	Plastocyanin and/or cytochrome c_6	Plastocyanin and/or cytochrome c_6	Cytochrome c_6 (?)	Cytochrome c_6	Cytochrome c_6	Cytochrome c_6 (?)	Cytochrome c_6	Cytochrome c_6
Occurrence of state transitions	Present	Present	Probably absent	Absent?	Present?	Absent?	Absent or low amplitude	Present
Occurrence of xanthophyll cycle[5]	Absent	Usually present (v-a-z or v-a)	Absent	Present (dd-dt, + v-a-z)	Present (dd-dt)	Present (dd-dt; + v-a-z)	Present (dd-dt + v-a-z in b, c; v-a-z in p, x)	Absent
Rubisco characteristics	Low CO_2 affinity, low CO_2/O_2 selectivity	Moderate-high CO_2 affinity, moderate-high CO_2/O_2 selectivity	Moderate CO_2 affinity, high to very high CO_2/O_2 selectivity	Low CO_2 affinity, low CO_2/O_2 selectivity	Moderate CO_2 affinity, moderate CO_2/O_2 selectivity	?	Moderate CO_2 affinity, high to very high CO_2/O_2 selectivity	Moderate to high CO_2 affinity; high to extremely high CO_2/O_2 selectivity
Glycolate metabolism (b)	Glycolate dehydrogenase; partial PCOC	Glycolate dehydrogenase (all except Charophyceae); glycolate oxidase (Charophyceae); PCOC	Glycolate dehydrogenase, then?	Glycolate dehydrogenase, then?	Glycolate dehydrogenase; modified PCOC	Glycolate dehydrogenase? then?	Glycolate oxidase; PCOC (p); glycolate oxidase; malate synthase (x); glycolate dehydrogenase, malate synthase (b); glycolate oxidase, then ? (e)	Glycolate oxidase; PCOC

[1]Cyanobacteria sensu stricto; [2]Cyanobacteria with chlorophyll *d* are assigned to the genus *Acaryochloris;* [3]Some cyanobacteria designated as having chlorophyll *b* actually use divinyl chlorophyll *b;* [4]Classes of Heterokontophyta are designated as follows: b = Bacillariophyceae; c = Chrysophyceae; e = Eustigmatophyceae; p = Phaeophyceae; x = Xanthophyceae; [5]Xanthophyll cycle components are designated as follows: a = antheraxanthin; dd = diadinoxanthin; dt = diatoxanthin; v = violaxanthin; z = zeaxanthin; [6]PCOC = Photorespiratory Carbon Oxidation Cycle; References: Badger et al. (1998); Falkowski and Raven (1997); Goss et al. (1998); Larkum and Howe (1997); Lohr and Wilhelm (1999); Raven (1987, 1988, 1997, 1999); Raven et al. (1989, 1999, 2000); Rowan (1989); and see text.

which will be considered later under acclimation) (Table 2) (see Raven et al., 1999, and Hope, 2000, for a discussion of kinetic comparisons of plastocyanin and cytochrome c_6).

There are considerable variations in the capacity for the Fe-containing ferredoxin to be replaced by the Fe-free flavodoxin among marine microalgae, and flavodoxin has been reported to be constitutive in some species (Geider and La Roche, 1994). There is a general correlation between the habitat of origin of a genotype and its capacity to produce flavodoxin under low-Fe culture conditions. La Roche et al. (1995) found that flavodoxin expression was induced by iron-limitation in all nine species of marine diatoms examined, including the oceanic isolate *Thalassiosira oceanica*. Erdner et al. (1999) examined 17 species from four classes of algae, and found no capacity to produce flavodoxin in one of three dinophytes and in the only cyanobacterium tested. Three of the five species which could not produce flavodoxin were from coastal (i.e. generally Fe-rich) habitats; one was of unknown origin, while the cyanobacterium *Synechococcus* sp. was from the Fe-poor Sargasso Sea and thus is a counter-example (Erdner et al., 1999). Another counter example is the ferredoxin-free (flavodoxin only) red macroalga *Chondrus* from coastal waters (Raven et al., 1999).

D. Photoprotection and Xanthophyll Cycle Pigments

Genetic differences among higher taxa of algae can also be seen in two traits related to photosynthetic light harvesting and photoprotection. These are state transitions and the occurrence of the xanthophyll cycle as a means of non-photochemical quenching of excitation energy (Lohr and Wilhelm, 1999; MacIntyre et al., 2000; Chapter 13, Larkum).

State transitions involve changes in the association of light-harvesting complexes with reaction centers as a function of the total irradiance, and the spectral distribution of photosynthetically active radiation (Chapter 13, Larkum). State transitions are known in the phycobilosome-containing Cyanobacteria and red algae, most green algae and in higher plants, but are apparently absent or small in chromophytes sensu lato (Raven et al., 1989; Fork et al., 1991; Larkum and Howe, 1997; Finazzi et al., 1999; MacIntyre et al., 2000). The variations in excitation energy transfer to the two sorts of reaction center can occur rapidly, i.e. over time intervals resembling those of variations in light-climate of the understory of a kelp forest, and may help to limit photodamage to D1 protein in PS II by limiting excitation energy transfer to PS II at high irradiances (Falkowski and Raven, 1997; Table 2). It is not easy to see ecological advantages in the qualitative or quantitative differences in state transitions among the various phyla and classes of algae. It is possible that the occurrence and extent of state transitions relates to the difference in absorption spectra between the pigments which supply excitation energy to PS II and those which service PS I. Thus, state transitions are well developed in Cyanobacteria (*sensu stricto*, with phycobilin) and Rhodophyta, and are absent or minimal in 'chromophytes' (e.g. Heterokontophyta): Table 2.

Xanthophyll cycles are involved in non-photochemical excitation energy quenching. The two characterized xanthophyll cycles involve de-epoxidation of violaxanthin to antheraxanthin and ultimately zeaxanthin (v-a-z) or de-epoxidation of diadinoxanthin to diatoxanthin (dd-dt). Zeaxanthin and diatoxanthin are the quenching, de-epoxidized forms of the xanthophylls, and violaxanthin and diadinoxanthin are the non-quenching forms. The v-a-z cycle occurs in members of the Chlorophyta (and Embryophyta), and the Eustigmatophyceae, Phaeophyceae and Chrysophyceae in the Heterokontophyta, although some green algae seem to lack a xanthophyll cycle (Rowan, 1989; Demmig-Adams and Adams, 1992; Franklin et al., 1996; Franklin and Larkum, 1997; Lohr and Wilhelm, 1999; Chapter 13, Larkum). In *Mantoniella* there is a reduced cycle where only the v-a interconversion occurs. The dd-dt cycle occurs in the Dinophyta, Euglenophyta, Haptophyta and in the Bacillariophyceae and Xanthophyceae in the Heterokontophyta, although in the cases examined there is a subsidiary v-a-z cycle in organisms with the dd-dt cycle (Rowan, 1989; Lohr and Williams, 1999). Despite the use of the v-a-z cycle in both green algae and embryophytes, the latter have more mechanistic similarities of their xanthophyll cycle with those algae predominantly using the dd-dt cycle (MacIntyre et al., 2000).

Notable absentees from the list above are the Cyanobacteria, the glaucocystophytes and the rhodophytes (Table 2). No data seem to be available for the glaucocystophytes. For Cyanobacteria and rhodophytes most data indicate the absence of a xanthophyll cycle (Table 2), although Ursi et al. (2003) present evidence consistent with the presence of a v-a-z cycle in the red alga *Gracilaria birdae*. Furthermore, Subramanian et al. (1999) found a reversible covalent change in photosynthetic

chromophores in the marine cyanobacterium *Trichodesmium* which, like the covalent changes in the xanthophyll cycles, reduced excitation energy transfer to PS II reaction centers at high incident photon flux densities. In *Trichodesmium* the covalent change involves the conversion of phycoerythrobilin (with efficient excitation energy transfer to PS II reaction centers) to phycourobilin (with substantial fluorescent loss of the light it absorbs) as light increases and vice versa as light decreases (Subramaniam et al., 1999). While it is not yet known if this phenomenon in *Trichodesmium* involves protein phosphorylation (as do state transitions) it is clear that it does involve covalent modification of photosynthetic chromophores (as do xanthophyll cycles).

MacIntyre et al. (2000) note that Cyanobacteria, which lack a xanthophyll cycle (but see above), have a very significant Mehler reaction activity (O_2 uptake by the reducing end of PS I) at light saturation (Kana, 1992). This can act as a sink for electrons when PS II activity exceeds net photosynthetic capacity. Diatoms may use non-assimilatory nitrate reduction as an electron sink (Lomas and Glibert, 1999), supplementing whatever Mehler reaction activity occurs (Bunt, 1965). Franklin and Badger (2001) have provided important data on the extent of Mehler reaction activity in marine macroalgae.

In view of the wide ecological range of the higher taxa of organisms with the light harvesting, redox catalyst, state transition and xanthophyll cycle characteristics and the great ecological overlap between taxa with differing traits described above, it is difficult to perceive the traits which characterize the various phyla and classes of algae as being adaptations to very specific habitats, even when the global environment at the time at which the trait evolved is considered (Raven 1984a; Falkowski and Raven, 1997; Raven et al., 1999, 2000). Further work on the mechanism of excitation energy quenching by de-epoxidized components of the xanthophyll cycle(s) may show whether the presence of phycobilisomes on the outer thylakoid membrane surface as major light-harvesters for PS II is incompatible with non-photochemical quenching in the xanthophyll cycle(s) at the inner side of the thylakoid membrane.

E. Ribulose Bisphosphate Carboxylase/ Oxygenase

There are very significant variations at the Class and Division levels in the kinetics of carboxylation and oxygenation by Rubisco (Table 2). One line of evolution of Rubisco starts with the cyanobacterial L_8S_8 (i.e. with eight large and eight small subunits) enzyme with a low CO_2 affinity and low CO_2/O_2 selectivity, and a high maximum specific reaction rate of carboxylation at CO_2 saturation. This Rubisco clade is retained in the plastids of green algae, euglenoids, chlorarachniophytes and higher plants, where the CO_2 affinity and CO_2/O_2 selectivity are higher, and the CO_2-saturated carboxylation rate is lower, than in the cyanobacterial enzyme (Table 2). These traits are especially pronounced in Rubiscos which function with diffusive supply of CO_2 (e.g. in the lichen alga *Coccomyxa*: Raven et al., 2000). They are less pronounced, or suppressed entirely, when CO_2 is supplied to Rubisco by a CO_2 concentrating mechanism (Raven et al., 2000).

A second line of Rubisco evolution occurs in prokaryotes as an L_8S_8 form in β-proteobacteria (i.e. organisms related to the ancestors of mitochondria). Lateral gene transfer has incorporated this variant of Rubisco into some Cyanobacteria, and into red algae and hence into all plastids derived from them by secondary endosymbiosis (Raven et al., 2000). In these Rubiscos the CO_2/O_2 selectivity is higher than the cyanobacterial line of Rubiscos, with very high values (three times the highest value in the cyanobacterial line) in some thermophilic and acidophilic red algae (Raven et al., 2000; Table 2). In all of these cases of lateral gene transfer the original (cyanobacterial) Rubisco has been displaced. The glaucocystophytes, which like red and green algae are the result of the primary endosymbiotic event which gave rise to plastids, resemble red algae in lacking chlorophyll *b* and having phycobilins, but resemble green algae in the form of L_8S_8 Rubisco that they contain (Badger et al., 2002).

Lateral gene transfer is also involved in the presence of L_2 (two large subunits) Rubisco in peridinin-containing dinoflagellates. This form of Rubisco originated in β-proteobacteria, and has a very low CO_2 affinity and CO_2/O_2 selectivity (Table 2; Raven et al., 2000).

The diversity of kinetics of Rubisco has significant impacts on the energy requirements for the net conversion of CO_2 to carbohydrate in photosynthesis (Raven, 2000; Raven et al., 2000) for the default condition of diffusion of CO_2 from an air-equilibrium solution as the means of supply of CO_2 to Rubisco, and with the photorespiratory carbon oxidation cycle

as the means of metabolizing phosphoglycolate (see below). Attempts to relate i) the genetic variation in Rubisco kinetics to the habitat occupied by the organisms today, ii) the occurrence of carbon concentrating mechanisms and iii) the inorganic C supply conditions when the various phyla and classes of algae evolved, have only met with partial success (Raven, 1997, 2000; Raven et al., 2000).

F. (Phospho)glycolate Metabolism

Significant phosphoglycolate production via Rubisco oxygenase activity occurs for photosynthetic organisms with diffusive supply of CO_2 from an air-equilibrium solution even when the Rubisco involved has the highest known CO_2/O_2 selectivity value (Raven et al., 2000; Table 2; Chapter 8, Beardall et al.). All algae, including those Cyanobacteria whose CO_2 concentrating mechanism maintains a very high CO_2 level around Rubisco, have a phosphoglycolate phosphatase and a glycolate oxidase and/or dehydrogenase, as well as some enzyme(s) metabolizing glyoxylate (Table 2; Raven et al., 2000). The diversity of pathways of glycolate metabolism in algae (Table 2) cannot readily be accounted as adaptive in terms of the present habitats of the organisms and the habitat at the time at which the algal taxon originated (Raven, 1997, 2000; Raven et al., 2000).

G. Enzymes Involved in Protection from Photooxidative Stress

The enzymes involved with the removal of active oxygen species generated (predominantly) by photosynthetic processes include superoxide dismutases, ascorbate and glutathione peroxidases, and catalase (Raven et al., 1999). These enzymes show significant taxonomic diversity within the algae, especially with respect to the different superoxide dismutases with their various metal ion requirements (Fe, Mn, Cu + Zn) and the capacity among eukaryotes to express an Se-containing glutathione peroxidase as well as an Fe-containing ascorbate peroxidase (Chadd et al., 1996; Raven et al., 1999).

Earlier, less complete data sets allowed some workers to make plausible correlations of the timing of the availability of metals and the origin of taxa with superoxide dismutases containing these metals, and especially the late origin of the green algal class Charophyceae when the free O_2 build-up made Cu available. However, later data, including the occurrence of Cu-Zn superoxide dismutase in a cyanobacterium and the early occurrence of other Cu-containing catalysts such as cytochrome oxidase and plastocyanin, make such historical adaptation arguments less plausible (Chadd et al., 1996; Raven et al., 1999), even allowing for the possibility of lateral gene transfer. Clearly the enzymes, together with the scavengers and quenchers, which remove active oxygen species evolved very early in the history of photosynthetic O_2 evolution, where, even with significant inorganic reductants available as O_2 sinks, there was local O_2 build-up (Canfield and Teske, 1996; Bjerrum and Canfield, 2002). These enzymes, scavengers and quenchers are the adaptive response to the qualitative presence of O_2 above the minimal (10^{-8} of the present atmospheric level) amount produced photochemically in the atmosphere. Clearly in an anoxygenic world, oxygen was a very dangerous chemical which had to be detoxified quickly. However, as with several of the enzyme systems discussed above, it is not easy to see adaptation in most of the current phylogenetic or environmental distribution of these enzymes. Perhaps these events took place much too early to have any phylogenetic significance in the modern world.

At lower taxonomic levels (genera, species, varieties) we might expect more recent environmental constraints to act as agents of natural selection than in the consideration of the characteristics of divisions (phyla) or classes.

An important point which should be considered in any analysis of possible selective advantages of putative adaptations is that a given trait may serve more than one selective end, and thus be acted on by several environmental factors. This may especially be the case for morphological traits. Thus, organism size among phytoplankton organisms impacts on efficiency (per pigment molecule) of the absorption of radiation, nutrient (including inorganic C) availability, sinking/flagellar motility, and susceptibility to grazing (Raven, 1998, 1999). In *Phaeocystis* spp. (Haptophyta) there is an alternation between unicellular zoospores (3 μm) and millimeter-size colonies (Moison and Mitchell, 1999). Despite a negative impact on the effectiveness of light and nutrient absorption, the colonial form is frequently favored, probably in relation to immunity from grazing by some herbivores (Moison and Mitchell, 1999; Ploug et al., 1999a,b).

V. Adaptation of Algal Photosynthesis to Environmental Extremes

A. Light

Dealing first with the availability of light, the classical suite of 'sun' and 'shade' adaptations found in studies of higher land plants by Björkman (1981)and others have significantly influenced views of algal adaptations to extremes of photosynthetically active radiation. In addition to newer constructions as to how the photosynthetic reactions at low photon flux densities can be seen as outcomes of mechanisms fostering the avoidance of photodamage as well as of means whereby the organism maximizes photon use at low photon flux densities (Osmond and Grace, 1995), there are a number of algal responses which are not entirely in accord with the higher plant 'sun/shade' paradigm. However, the general adaptive response in algae seems to involve larger photo-

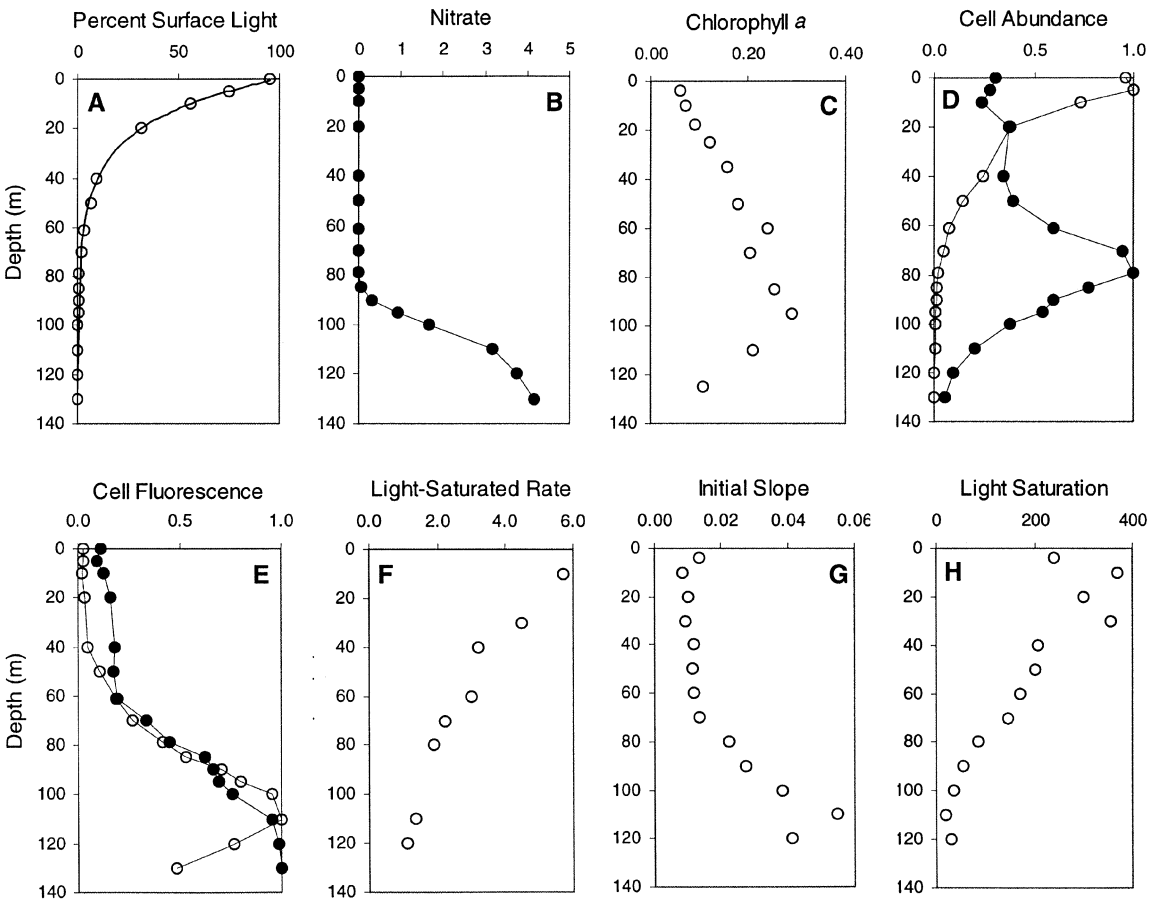

Fig. 2. Vertical profiles of environmental and biological variables for the oligotrophic North Atlantic Ocean. A) Attenuation of photosynthetically active radiation. B) Nitrate concentration (μmol l^{-1}) showing low values in surface waters and a pronounced nitricline below 90 m. C) Chlorophyll *a* concentration (μg l^{-1}). D) Relative abundance of *Synechococcus* spp. (open circles) and *Prochlorococcus* spp. (filled circles). Abundance is expressed as a percentage of the maximum value observed for each taxon. E) Relative cell-specific fluorescence arising from chlorophyll *a* for *Synechococcus* spp. (open circles) and *Prochlorococcus* spp. (filled circles). Fluorescence is expressed as a percentage of the maximum value observed for each taxon. F) The light-saturated photosynthesis rate (units of g C[g Chl *a*]$^{-1}$ h^{-1} of the phytoplankton assemblage. G) The light-limited initial slope of the photosynthesis-light response curve for the entire phytoplankton assemblage (units of g C m^2 s [g Chl *a* μmol photons h]$^{-1}$. H) The light saturation parameter for the entire phytoplankton assemblage (units of μmol photons m^{-2} s^{-1}). Observations of light, nitrate, cell abundance and cell-specific fluorescence were provided by Marcel Veldhuis (Netherlands Institute of Sea Research). The parameters of the photosynthesis-irradiance response curve were taken from Bouman et al. (2000).

synthetic units (total light harvesting pigment per PS I or PS II reaction center) in the shade-adapted plants.

Whether photoadaptation contributes significantly to the success of a species in nature has not been determined, although among the marine picophytoplankton, there is a tendency for the smaller divinyl chlorophyll *a* plus *b*-containing *Prochlorococcus* to live at greater depths than the larger chlorophyll *a* plus phycobilin-containing *Synechococcus* (Fig. 2). This may be related to the absorption maxima of the pigments of *Prochlorococcus* which are particularly appropriate for harvesting blue-green radiation deep in clear oceanic waters, and the smaller package effect in the smaller *Prochlorococcus* cells and hence the more rapid payback of light energy investment in pigments in a given light field in *Prochlorococcus* than in *Synechococcus* (Raven, 1984b, 1998, 1999; Bricaud et al., 1999; Ting et al., 2002). Furthermore, the depth distribution of *Prochlorococcus* clones is consistent with genetic adaptation to high- or low-irradiance environments (Moore and Chisholm, 1999; Hess et al., 2001). Specifically, *Prochlorococcus* clones with higher chlorophyll *b/a* ratios, higher light-limited growth rates and greater susceptibility to photoinhibition are found deeper in the water column. In the subsurface chlorophyll *a* maximum layer in the Sargasso Sea, *Prochlorococcus* sp. had growth rates that were twice those of the co-occurring phytoplankton (Goericke and Repeta, 1983; Goericke and Welschmeyer, 1993). However, it must be acknowledged that the deepest-growing benthic algae (Littler et al., 1985; Littler and Littler, 1994; Raven et al., 2000) are red crustose corallines with a large absorptance. In addition, these algae employ light-harvesting pigments with a high energy-cost in their production per unit light absorption rate in a given electromagnetic radiation environment (Raven, 1984a,b).

The depth distribution of *Synechococcus* and *Prochlorococcus* ecotypes may not be attributed exclusively to the marked light gradient. Vertical stratification is also evident in profiles, with nutrient-depleted waters of the surface mixed layer overlying the nutrient-replete waters below the thermocline. Given the nitrogen-limited condition of the waters in which *Synechococcus* is commonly found (Graziano et al., 1994) and the high nitrogen costs of phycobiliprotein synthesis, it is somewhat surprising to find *Synechococcus* as the most abundant photosynthetic organism in the oligotrophic North Atlantic. Parts of the ocean are Fe-deficient, and Fe deficiency induces the production of novel chlorophyll *a*-containing light-harvesting supercomplexes (IsiA) in *Synechococcus* (Boekema et al., 2001; Bibby et al., 2001a) which permit photosynthesis to occur with fewer Fe-rich PS I units with larger antenna complexes (Raven et al., 1999). The large PS I-IsiA units in Fe-deficient *Synechococcus* are evolutionarily related to such units in *Prochlorococcus* (Bibby et al., 2001b).

In this section we consider the absolute constraints on growth at the extremes of high and low photon flux density at which algae can grow. The upper limit of photon flux density for growth may be set by photoinhibitory damage (Osmond and Grace, 1995; Forster et al., 2001). Current perception is that damage to the D1 polypeptide is caused by one in every 10^6 to 10^7 of absorbed photons which arrives at photoreaction two reaction centers (Franklin and Larkum, 1997; Raven et al., 2000; Chapter 16, Franklin et al.). Restriction of this damage in high light environments involves a small photosynthetic unit size, sometimes accompanied by the ability to dissociate some light harvesting apparatus from the PS II reaction centers. It also involves mechanisms whereby non-photochemical quenching is increased, as well as regulatory and behavioral mechanisms which restrict photon absorption overall (see above). The extent to which these mechanisms occur is phylogenetically variable. Thus, Cyanobacteria and red algae cannot vary the extent of non-photochemical quenching via a xanthophyll cycle. Both of the avoidance mechanisms which restrict the extent of photodamage, albeit at the expense of the short-term photosynthetic potential, themselves are regulated, i.e. high irradiances lead to the dissociation of light harvesting complexes from reaction center two, and to the depoxidation of epoxycarotenoids to produce quenching carotenoids (see below). This must be distinguished from acclimation (considered below), in which the extent to which the underlying mechanisms are expressed alters in response to rather longer time scales, but still in response to environmental changes which are short-term relative to the time scale of several to many generations required for adaptation. Of course, the mechanisms that limit photodamage by varying the fraction of absorbed photons which reach photoreaction two centers are only necessary to the extent that photon flux density varies. Adaptation to continuous very high photon flux densities would only require a small photosyn-

thetic size to minimize the potential for photodamage while permitting maximal productive use of incident photons in photosynthesis at the high irradiance. Even with such a minimal photon harvesting capacity there might be constraints on effective photon use, with the possibility of spatial mis-match of the sites of primary photochemistry and of the catalysts of downstream reactions. However, there are precedents for photosynthesis without detectable photoinhibition at a very high incident photon flux density (Forster et al., 2001), even up to 3000 μmol photon m^{-2} s^{-1} in a dinoflagellate symbiont in a coral (Falkowski and Dubinsky, 1980; Long et al., 1994). Thus the potential for photodamage, presumably inherited from the earliest O_2-evolvers, can be dealt with in some algae and permit photosynthesis at one and a half times the maximal natural photon flux density of 2000 μmol photon m^{-2} s^{-1}.

At the other end of the scale we have algae growing at very low photon flux densities. Raven et al. (2000) consider the constraints on the lowest photon flux densities at which algal photosynthesis can occur. There are a number of constraints that restrict photosynthesis and growth at low photon flux density in the form of energy-consuming processes whose rates are invariant with photon flux density, and thus consume a large fraction of absorbed photons at lower photon flux densities. Among these processes are the redox backreactions of RCII, the leakage of H^+ through the thylakoid membrane, and the turnover of proteins in a manner independent of the rate of energy supply. The first two processes limit the rate of linear electron transport and of ADP phosphorylation respectively, while the latter consumes ATP. Raven et al. (2000) emphasize that these energy-consuming processes are sequential, so that their effects in constraining photosynthetic (and growth) rates are multiplicative. Using the data on the influence of these three processes on sun-adapted organisms (algae and higher plants) Raven et al. (2000) showed that it is difficult to explain the growth of algae at 0.5 μmol photon m^{-2} s^{-1}, let alone the growth of the crustose coralline red alga found at 274 m where the average incident photon flux density for 12 h per day not in excess of 0.02 μmol photons m^{-2} s^{-1}. Further investigation is needed of how the algae which can grow at less than 1 μmol photon m^{-2} s^{-1} cope with these energy-consuming reactions which use an increasing fraction of the energy input as the photon flux density decreases.

Turning to the shorter wavelengths (less than 400 nm), we find that UV-A (320–400 nm) can, in at least the short term, act in light harvesting for photosynthesis, and also can help (as does blue light) to offset the damage by UV-B. Corals have a host-derived fluorescent pigment that can be used to harvest UV-A under low light conditions (Salih et al., 2000). UV-A can also inhibit photosynthesis in its own right. UV-B (280–320 nm) either has no effect or, at higher flux densities, is always inhibitory of photosynthesis and growth. There are large genotypic differences in UV-B sensitivity among algae (Franklin and Forster, 1997). UV-B effects depend on the photon flux density of UV-A and of photosynthetically active radiation (Franklin and Forster, 1997; Chapter 16, Franklin et al.).

B. Inorganic Carbon Supply

Inorganic carbon supply variations in the past have presumably in part underlain the variations in inorganic carbon acquisition among the algae. The inorganic carbon acquisition mechanism varies among algae in terms of transport and the kinetic properties of Rubisco. Transport can be via diffusion of carbon dioxide, or pumping of CO_2, bicarbonate or H^+ at one or more membranes (Chapter 11, Raven and Beardall). A carbon concentrating mechanism can serve as a means for concentrating CO_2 at the site of Rubisco in steady-state photosynthesis to values above those in the bulk medium. Regarding the kinetic properties of Rubisco, a high affinity (low K_m) for CO_2, and a high selectivity factor for CO_2 relative to O_2, are generally correlated with a low specific reaction rate of the CO_2-saturated carboxylation reaction and vice versa (Badger et al., 1998; Raven, 1997). The perceived requirement for a means of concentrating CO_2 at the active site of Rubisco is greatest for organisms which normally grow at (i.e. have adapted to) low free CO_2 concentrations in the medium and/or have a Rubisco with a low CO_2 affinity and/or a low selectivity factor for CO_2 over O_2. Furthermore, a CO_2 concentrating mechanism allows the organism to fix more CO_2 per unit time per unit energy, carbon and nitrogen dedicated to Rubisco if it maintains saturating concentrations of CO_2 around Rubisco (see Chapters 8, Beardall et al. and 11, Raven and Beardall). This is especially the case if an organism has a Rubisco with a high specific reaction rate (and hence generally a low CO_2 affinity and CO_2/O_2 selectivity factor). A potential energetic disadvantage of this CO_2 concentrating mechanism

strategy comes from a consideration of photosynthesis and growth at low photon flux densities. The leakage of CO_2 from the high concentration maintained around Rubisco would constitute an energy cost that is essentially independent of the incident photon flux density. This would constitute a very significant energy loss at low light which operates in series with charge recombination in RCII and with H^+ leak through the thylakoid membrane (Raven et al., 2000). This problem is, apparently, dealt with adaptively by some algae which normally grow at low photon flux densities and which lack CO_2 concentrating mechanisms. Some of these are red algae with very high CO_2/O_2 selectivity factors for their Rubiscos, thus minimizing the energy cost associated with the phosphoglycolate synthesis which accompanies diffusive CO_2 entry from an air-equilibrium solution (Raven et al., 2000; Table 2). Of course, Rubisco with a high CO_2/O_2 selectivity, low CO_2-saturated specific reaction rate, and low K_m for CO_2, is not functioning at CO_2 saturation in today's air-equilibrium solution. To achieve a given rate of CO_2 fixation per unit biomass, an alga possessing this kinetic variant of Rubisco operating in today's atmosphere will require higher energy, carbon and nitrogen investments in Rubisco than would the same alga operating at CO_2 saturation. This is especially so for a Rubisco with a high CO_2 fixation rate per unit protein operating at CO_2 saturation.

The analysis in the previous paragraph implicitly relates to inorganic C entry from the medium into a small cell carrying out photosynthesis with the initial combination of CO_2 into covalent linkage in an organic C compound catalysed by Rubisco within a second or so of inorganic C entry. In secondarily aquatic embryophytic plants there are a wide range of mechanisms of inorganic C acquisition which involve a preliminary inorganic C assimilation into a C_4 dicarboxylic acid with a spatial (C_4-like) or temporal (CAM-like) separation of the $C_3 + C_1$ carboxylation and the subsequent Rubisco carboxylation, with or without uptake of inorganic C from sediment and transfer to photosynthetic structures (Raven 1984a, 1997). Such pathways are much less common in, or absent from, algae (Raven, 1984a, 1997).

C. pH

External pH impacts photosynthesis via the regulation of intracellular pH and the speciation of inorganic carbon species in the medium. Species of the single-celled green algal genus, *Dunaliella*, can grow over the pH range from < 1.0 to > 10.0 (Table 1; Raven, 1990). As well as implications for pH regulation and the energetics of plasmalemma cotransport processes, including any which involve inorganic carbon, the speciation of inorganic carbon is greatly influenced by pH (and is often a major determinant of pH), with a temperature and salinity-dependent switch from predominantly CO_2 below pH 5.9–6.5 to predominantly HCO_3^- from pH 5.9–6.5 to pH 9.2–10.5 and predominantly CO_3^{2-} at higher pH values. Apart from high intertidal rock pools, the pH range 0.5–7.5 and 8.5–11.0 in nature is essentially an inland waters phenomenon, and it is the algae of these habitats that have adapted to very low or very high pH with their extremes of inorganic carbon speciation. No incontrovertible proof of direct CO_3^{2-} uptake as inorganic carbon source for photosynthesis has yet been offered (Raven, 1997).

D. Oxygen Concentration

Extremes of O_2 concentration occur in nature with algae growing in hypoxic and hyperoxic environments (Raven et al., 1994; Chapters 8, Beardall et al., and 10, Raven and Beardall). Hypoxia is potentially a problem for respiration in the dark phase, with little evidence for an O_2 requirement for photosynthesis per se, but rather an inhibition by O_2 at low CO_2 concentrations if diffusive CO_2 entry to Rubisco occurs (Raven et al., 1994). Secondary deep chlorophyll fluorescence maxima of *Prochlorococcus marinus* populations occur in the upper regions (at depths of 80–140 m) of the oxygen minimum zones (<10 μM O_2) of the Arabian Sea and Eastern Tropical North Pacific Ocean (Goericke et al. 2000). Here, the abilities to use low light (<1 to 20 μmol photons m^{-2} s^{-1}), and withstand hypoxic conditions allows *P. marinus* to exploit a novel niche in the open ocean.

Hyperoxia up to several times the present air-equilibrium level can occur in high intertidal rock pools, in many bodies of freshwater, and in some organisms with CO_2 concentrating mechanisms even with external normoxia. Tolerance of hyperoxia may be improved by higher levels of enzymes that remove active oxygen species (Raven et al., 1994,1998). However, some hyperoxia-tolerant algae do not have very high levels of enzymes which remove active oxygen species (Raven et al., 1994, 1998). Most of the enzyme-catalyzed O_2 uptake reactions which

generate active oxygen species have a relatively high affinity for O_2, i.e. are at or near saturation at air-equilibrium concentrations of O_2 in solution. However, some active oxygen species may be generated by non enzyme-catalyzed reactions of O_2 with reduced redox intermediates, and the rates of such reactions are directly proportional to the concentration of O_2 (provided that the concentration of the reduced redox intermediate does not vary with O_2 concentration). Such reactions include the reduction of O_2 to $O_2^{\cdot -}$ by $PQ^{\cdot -}$ (in plastids) or $UQ^{\cdot -}$ (in mitochondria). Although this reaction is not thermodynamically favored, since the redox potential of the $O_2/O_2^{\cdot -}$ couple is more negative than that of the $PQ^{\cdot -}/PQ$ or $UQ^{\cdot -}/UQ$ couples, it does occur to some extent (Raven et al., 1998).

E. Temperature

Adaptation to different temperatures for growth involves changes in the degree of thylakoid membrane lipid saturation, the kinetics and quantity of enzymes such as Rubisco, and the ratio of light-harvesting pigments to downstream catalysts, in all cases compared to phylogenetically closely related organisms adapted to a different temperature regime. Adaptation to lower temperatures involves less-saturated thylakoid (and other membrane) lipids, and a lower ratio of antenna pigments to downstream catalysts (Geider, 1987; Raven and Geider, 1988). The very high sensitivity of zooxanthellate corals to small rises in summer temperatures, manifested in coral bleaching, is described in Chapter 19 (Yellowlees and Warner).

F. Pressure

The extent to which the pressure component of water potential impacts on photosynthesis is limited by the maximum depth to which sufficient photosynthetically active radiation penetrates, with the limit for benthic algae of 274 m corresponding to a pressure of 2.74 MPa in excess of atmospheric (0.1 MPa). Of course, even if photosynthesis in excess of respiration over 24 h cannot occur at depths below 274 m, planktonic algae can be circulated down to greater depths or sedimented to, and then resuspended from, greater depths, and retain their photosynthetic capacity. It is not clear if there are specific adaptations of algae to pressure tolerance. That phytoplankton can tolerate a pressure of 10 MPa in excess of atmospheric was shown by Platt et al. (1983) who found that the light-saturated photosynthesis rates of phytoplankton sampled from 10 and 1000 m were similar when incubated on deck. The light-limited initial slope and susceptibility to photoinhibition were higher in the sample collection from 1000 m, as expected if this population had acclimated to low light during transit to 1000 m. There are certainly adaptations to external osmolarity (the osmotic component of water potential) in the form of active water efflux from (functionally) wall-less cells growing at low osmolarities and the production of compatible solutes in the cells of organisms growing at higher external osmolarities.

VI. Acclimation of Algal Photosynthesis

Variations of chlorophyll *a*-specific photosynthesis rates and chlorophyll *a*-specific light absorption coefficients are evident in natural populations of marine phytoplankton (Kyewalyanga et al., 1998). These variations can arise from replacement of one population or assemblage of phytoplankton by another presumably better-adapted assemblage (succession or competition), or by physiological acclimation within a population or assemblage. Much of the variability in biomass-specific photosynthesis on seasonal time scales appears to be related to changes in species composition (Coté and Platt, 1983). However, photoacclimation is evident in vertical distributions of cell pigment content (Campbell et al., 1994), chlorophyll *a*-specific light-saturated and light-limited photosynthesis rates (Platt et al., 1982), and chlorophyll *a*-specific light absorption coefficients (Mitchell and Kiefer, 1988). Whether these vertical gradients of physiological responses are strictly due to acclimation, or are due to a combination of acclimation and adaptation (or competitive exclusion) is an area that deserves further research.

Photoacclimation involves co-ordinated changes in the composition and functioning of the photosynthetic apparatus in response to variations of photon flux density. Several strategies of photoacclimation in phytoplankton have been elucidated, based on changes in the photosynthetic unit size, photosynthetic unit number per cell, and Calvin-Benson cycle activities per cell (Richardson et al., 1983; MacIntyre et al., 2002). Although commonly described in terms of responses to the incident irradiance, the mechanisms underlying photoacclimation is likely

to involve the cell's perception of the rate of light absorption (Kana et al., 1997) rather than the irradiance per se. For example, the chlorophyll *a*:carbon ratio can be described as a function of the rate of light absorption in *Skeletonema costatum* even though chlorophyll *a*:carbon varies by over a factor of two as a consequence of variations in spectral quality at a given photon flux density (Nielsen and Sakshaug, 1993). Phytoplankton absorb a variable proportion of the photons incident on their surface (Bricaud and Morel, 1986). In addition, because the absorption spectrum of phytoplankton is not flat (Bricaud and Morel, 1986), the rate of light absorption depends not only on the irradiance, but also on spectral quality of the underwater light field and the absorption spectrum of the phytoplankton.

Photoacclimation involves changes in the composition and cellular abundance of light-harvesting pigment-protein complexes, in the cellular abundances and ratios of PS I:PS II reaction centers and of other catalysts within the electron transport chain and changes in the abundance of Calvin-Benson cycle enzymes, most notably Rubisco (Sukenik et al., 1987). Photoacclimation can also result in changes of photoprotective pigments (MacIntyre et al., 2002) and antioxidant defenses. Photoacclimation responses depend in part on whether the photon flux density of photosynthetically active radiation is light-limiting or light-saturating for growth. These two regions can be delimited by specifying the saturation irradiance (I_k) defined as the ratio of the light-saturated growth rate to the initial slope of the growth versus irradiance curve (Fig. 3A). Relative changes of cell pigment content, photosynthetic unit size and photosynthesis-irradiance response characteristics tend to scale with I/I_k (Fig. 3). Thus, knowledge of I/I_k provides considerable insight into the photoacclimatory response of nutrient-replete algae (MacIntyre et al., 2002). Marked increases of photosynthetic unit size (as defined by the ratio of light-harvesting pigments of PS II reaction centers) tend to accompany growth at irradiances less than I_k (Fig. 3C, D). The number of photosynthetic units per cell (or per unit cell biomass) also tends to be greater in low-light growth conditions.

The photoacclimation of many photosynthetic characteristics is typically a continuous function of photon flux density (Fig. 3). Therefore, it is incorrect to speak of high-light versus low-light-acclimated cells. Rather, cells acclimate along a continuum that can be defined by I/I_k (MacIntyre et al., 2002). As with adaptation to low photon flux densities, acclimation to different photon flux densities for growth often involves a larger photosynthetic unit size, with similar numbers of PS I and PS II reaction centers per cell (for unicells) or per unit thallus area (for macrophytes). In some cases the photosynthetic unit size is invariant with photon flux density (for unicells) or per unit thallus area (for macrophytes). In some algae the PS I:PS II ratio increases at low photon flux density (Raven et al., 1999).

Whether acclimation to lower photon flux densities involves larger photosynthetic unit sizes or more photosynthetic units, it always involves an increase in cellular pigment content leading to a higher optical thickness, and hence a greater package effect. This has implications for the time taken, in a given radiation environment, to absorb the photons needed to supply the energy required to synthesize the photosynthetic pigment-protein complexes (Raven, 1984a). Of course, the range of absorptances, and hence of package effects and the time taken to recoup construction costs, which occurs via acclimation in a given genotype is much less than the range found by adaptation in algae as a whole.

Recent evidence suggests that photoacclimation occurs in response to a signal transduction mechanism that involves the redox state of one or more components of the photosynthetic apparatus (Escoubas et al., 1995; Maxwell et al., 1995). Most attention has been focused on the ratio of reduced to oxidized plastoquinone (PQH_2/PQ) (Allen, 1993; Escoubas et al., 1995; Pfannschmidt et al., 1999). The ratio PQH_2/PQ appears to be an attractive signal because it provides a direct measure of balances or imbalances in the functioning of the photosynthetic electron transfer chain (Chapter 13, Larkum).

Changes in expression of light-harvesting complexes, or light-harvesting complexes plus thylakoid redox components, or of PS II relative to PS I, can be related to the PQH_2/PQ ratio (Allen, 1993; Pfansschmidt et al., 1999). A high PQH_2/PQ ratio indicates a high excitation energy input to PS II relative to the capacity for CO_2 fixation, via PS I and the cytochrome b_6f complex. A high PQH_2/PQ ratio signals to the gene transcription system that the light-harvesting apparatus needs to be downregulated such that the excitation energy input to PS II is restricted. This is most readily achieved by decreasing the size of the photosynthetic unit for PS II rather than via fewer PS II with a similar, or smaller, complement of PS I. Conversely, a low PQH_2/PQ indicates a low excitation energy transfer to PS II

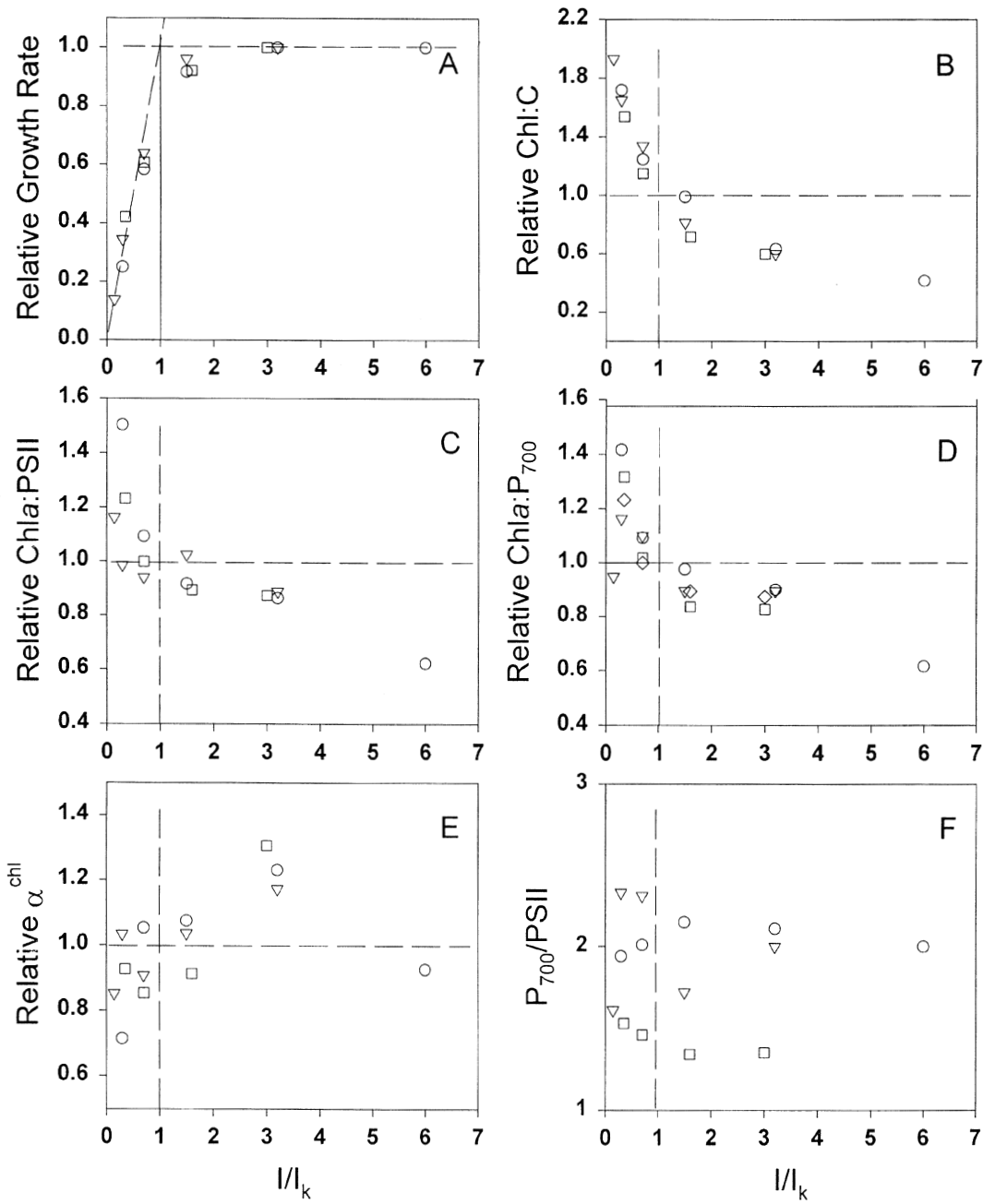

Fig. 3. Photoacclimation of the marine diatom *Thalassiosira weissfloggi* (inverted triangles), the haptophyte *Isochrysis galbana* (circles) and the dinoflagellate *Prorocentrum micans* (squares) to growth at irradiances of 30 to 600 μmol photons m^{-2} s^{-1}. Irradiance has been expressed relative to I_k. (A) Relative growth rate versus I/I_k. (B) Relative chlorophyll *a*:carbon versus I/I_k. (C) Relative Photosystem II unit size (chlorophyll *a* per O_2 evolved per saturating flash) versus I/I_k. (D) Relative Photosystem I unit size (chlorophyll *a* per P_{700} versus I/I_k. (E) Relative chlorophyll *a*-specific initial slope of the photosynthesis-irradiance response curve (α^{chl}) versus I/I_k. (F) Absolute PS II:PS I ratio versus I/I_k. Absolute values of these variable at an irradiance of 150 μmol photons m^{-2} s^{-1} are given in Table 3. Based on data from Falkowski et al. (1985) and Dubinsky et al. (1986). Although there have been a number of other studies where photosynthetic characteristics have been examined in cells acclimated to a range of growth irradiances, very few have all the data required to plot in the manner illustrated in this figure.

Table 3. Interspecific variability of photosynthetic characteristics of selected marine phytoplankton. Observations from Falkowski et al. (1985) and Dubinsky et al. (1986)

Species	Growth Irradiance[1]	I/I_k [2]	Growth Rate [3]	Chl*a*:C [4]	a^{Chl} [5]	α^{Chl} [6]	PSU_{O2} [7]	P_{700} [8]	PS II/PS I [9]
Thalassiosira weissflogii	150	0.75	1.15	0.035	4.2	0.37	2,340	1,350	1.7
Isochrysis galbana	150	1.5	1.1	0.014	11	1.2	1,554	835	2.1
Prorocentrum micans	150	.75	0.11	0.0046	12	0.78	2,350	855	1.5

Units are as follows: [1] μmol m^{-2} s^{-1}; [2] dimensionless; [3] d^{-1}; [4] g/g; [5] light absorption coefficient, m^2 g^{-1} Chl *a*; [6] chlorophyll *a*-specific initial slope; m^2 (g^{-1} chl*a*) mol O_2 (mol^{-1} photons); [7] mol chlorophyll *a* (mol O_2 in a saturating flash)$^{-1}$; [8] mol chlorophyll *a* (mol P_{700})$^{-1}$; [9] dimensionless

relative to the capacity for downstream processes, and signals the increased expression of light-harvesting antennae pigments, with or without an increase in the number of PS II units per cell or per unit thallus area.

It is not certain that PQH_2/PQ ratio is the sole regulation of the differential expression of genes for redox and related reactions of photosynthesis in response to variations in the incident photon flux density. It does, however, seem plausible that the PQH_2/PQ ratio should be the determinant of differential expression of genes associated with 'complementary chromatic acclimation' in those algae, especially members of the Cyanobacteria and the Rhodophyta, which have very different spectral absorptances of the pigments supplying energy principally to PS II (phycobilins with a minor component of chlorophyll *a*) relative to those supplying energy principally to PS I (predominantly chlorophyll *a*). Organisms acclimated to a predominantly green (phycobilin-absorbed) photon input for growth would have a low phycobilin to chlorophyll *a* ratio. Switching the photon input to red or blue wavelengths (i.e. mainly absorbed by chlorophyll *a*) decreases energization of PS II, lowers the PQH_2/PQ ratio, leading to increased expression of phycobilins. This brings absorption by phycobilins (supplying PS II) and chlorophyll *a* (mainly supplying PS I) closer to the 1:1 ratio required by linear electron transport.

Allen (1993) has suggested that the retention of some of the original endosymbiont genes by plastids (and mitochondria), rather than their transfer to the nucleus (or their complete loss), is related to the regulation of expression of organelle-encoded genes by organellar redox state as an indication of environmental conditions. Allen and Raven (1996) point out that the potential for mutation of organelle genomes as a result of active oxygen species produced in the organelle redox reactions might favor gene transfer to the nuclear genome. As the number of photosynthetic (or respiratory) genes retained in the organelle decreases, the marginal cost of the remaining bioenergetic genes is increased since the genes for replication, transcription and translation in the organelle must be retained for an ever-smaller number of bioenergetically active genes. While the suite of genes retained in plastids shows significant variation among higher taxa of algae, there is a core of genes in all of the plastids that have been investigated which code for one or more components of PS II, PS I, the cytochrome b_6f complex and Rubisco. The extent to which the presence of these genes in the plastid genome improves (e.g. speeds up, or makes more precise and accurate) the regulation of their expression in response to environmental cues needs further investigation, as does the significance of the phylogenetic variation in the suite of genes present in plastids. However, it is certainly possible that the variation in genes retained by the plastid genome can influence the rate or extent of acclimation of the photosynthetic apparatus.

The acclimatory processes described above have been modeled by Geider, McIntyre and Kana (1996) for phytoplankton cells. The key feature of the Geider et al. (1996) model is that the signal for acclimation of pigment content is assumed to be directly related to the quantum efficiency of photosynthesis. It is of interest that the observed changes in content of photosynthetic catalysts which Geider et al. (1996) model do not require increased rates of degradation

of catalysts whose content per cell decreases during acclimation in a growing cell population. This means that dilution during growth can account for the observed changes, and the enhancement (or initiation) of degradative reactions is not needed to explain the dynamics of these components whose content per cell is decreased during acclimation. It should be noted, however, that there are only two data sets that provide the necessary information for the Geider et al. (1996) formulation to be rigorously tested. Both of these data sets are for diatoms that can grow over a wide range of photon flux densities. Other organisms, in particular the Cyanobacteria where active degradation of phycobilisomes has been documented in response to nutrient limitation, may employ different mechanisms.

Whether a species employs dilution or degradation (turnover) to decrease pigment content following transfer to high irradiance may depend on the extent to which excitation energy transfer from the pigment bed to the reaction centers is subject to regulation. Diatoms can down-regulate excitation energy transfer to PS II when exposed to saturating photon flux densities. This is an important safety valve which allows the diatoms to retain pigments without placing undue excitation pressure on PS II. This, in turn, minimizes the potential for photoinhibition and photooxidative stress. In contrast, Cyanobacteria lack a xanthophyll cycle, and (with the exception noted above) appear to be unable to effectively quench excitation energy in the phycobilisomes. In addition, diatoms continue to synthesize diadinoxanthin following transfer to high light, whereas further processing of diadinoxanthin to fucoxanthin is inhibited. This allows diatoms to synthesize the precursor to a light-harvesting xanthophyll during exposure to bright light, and subsequently to rapidly transform the precursor (diadinoxanthin) into the light-harvesting fucoxanthin after return to low light. This may be particularly advantageous in a chronically low-light environment that is punctuated by brief exposure to bright light. Such an environment is experienced by cells cycling vertically in an optically deep mixed layer characteristic of the spring bloom conditions in temperate zones. If the diadinoxanthin also participates in the dd-dt cycle-related non-photochemical quenching, then there may be an added bonus of increased non-photochemical quenching during the bright-light exposure. If the mechanism of photoacclimation to bright light in Cyanobacteria involves active pigment degradation, than Cyanobacteria would be at a disadvantage in well mixed water columns.

Another acclimatory mechanism that is driven by variations in photon supply is the variation of the ratio of xanthophyll cycle carotenoids (violaxanthin/antheraxanthin/zeaxanthin and/or diatoxanthin/diadinoxanthin) to PS II reaction centers which operates in all higher algal taxa investigated except for the Cyanobacteria and most Rhodophyta (see Falkowski and Raven, 1997; Ursi et al., 2003). This mechanism operates by the altering the quantity of excitation energy reaching PS II relative to the capacity for downstream processing of electrons. Here it is possible that a high PQH_2/PQ ratio, signaling a potential excess of excitation energy reaching PS II to photochemical sinks for the energy, leads to the synthesis of more xanthophyll cycle carotenoids per PS II reaction center. However, the phenomenon of acclimation of xanthophyll cycle components has only been documented in a few algal species and its regulation is poorly documented (Falkowski and Raven, 1997).

Acclimation of the photosynthetic apparatus to variations in the supply of certain elements can, in various taxa, involve changes in the light harvesting antenna complexes of certain freshwater Cyanobacteria and in marine Cyanobacteria with the low Fe effect on isiA (Bibby et al., 2001a; Boekema et al., 2001). It can also involve the water-soluble redox agents involved in electron transfer from the cytochrome b_6f to P^+_{700} and from the reducing end of PS I to the ferredoxin-$NADP^+$ oxidoreductase (Raven et al., 1999). Growth of Cyanobacteria under S-deficient conditions can lead to the replacement of the usual phycobilins with phycobilins whose proteins contain less cysteine, i.e. less S (Mazel and Marliere, 1989).

The soluble redox protein in the thylakoid lumen which transfers electrons from the cytochrome b_6f complex with P^+_{700} is either the Fe-containing cytochrome c_6 (Rhodophyta, Heterokontophyta, Haptophyta(?), Euglenophyta), the Cu-containing plastocyanin (some Chlorophyta) or, in the cases of interest in terms of acclimation, either cytochrome c_6 or plastocyanin (Cyanobacteria, some Chlorophyta). The expression of cytochrome c_6 or plastocyanin in the organisms which produce either is regulated by the availability of Fe relative to Cu in the growth medium. A limited supply of Fe relative to Cu leads to the expression of cytochrome c_6 rather than plastocyanin, while a restricted supply of Cu relative

to Fe leads to the expression of plastocyanin rather than cytochrome c_6. The fraction of the total catalytic Fe in an algal cell which occurs in cytochrome is much smaller than the fraction of total catalytic Cu which occurs in plastocyanin. Thus, the expression of cytochrome c_6 rather than plastocyanin results in a greater proportional saving in catalytic Cu than the saving in catalytic Fe resulting from the expression of plastocyanin rather than cytochrome c_6 (Raven et al., 1999).

The final catalyst substitution concerns the replacement of the normal agent, the Fe-containing ferredoxin, with the metal-free flavodoxin, as the catalyst of electron transfer on the cytosol (Cyanobacteria) or stroma (eukaryotes) from the reducing end of PS I to the ferredoxin-$NADP^+$ oxidoreductase, and thence to $NADP^+$ (see Raven et al., 1999). In many, but by no means all, algae, Fe deficiency in the growth medium leads to the expression of flavodoxin rather than ferredoxin. Deficiency in this context does not necessarily mean that the availability of iron is so low as to restrict growth rate under otherwise optimal conditions (McKay et al., 1999; Davey and Geider, 2001). However, the detection of flavodoxin has been used as a biological marker of incipient and actual Fe deficiency in natural assemblages of marine phytoplankton (La Roche et al., 1996; Erdner and Anderson, 1999; Erdner et al., 1999). It is of interest that a red alga (*Chondrus crispus*) appears to constitutively express flavodoxin, with no capacity to produce ferredoxin; this needs to be followed up by molecular genetic approaches.

Having considered in at least semi-quantitative terms the benefits of the acclimatory substitutions among proteinaceous catalysts with different contents of S, Fe and Cu, it is appropriate to ask if there are costs of substituting a catalyst which has less, or none, or some, of that element. For the low-cysteine phycobilins, economizing in S, we do not know if the S-economical variant has a similar capacity to absorb photons and transfer excitation energy to that of the more S-demanding variant. In the case of cytochrome c_6 and plastocyanin it appears that these two catalysts have equal catalytic potential, regardless of whether they are tested in (where appropriate) homologous or heterologous thylakoid systems. For the ferredoxin/flavodoxin pair it appears that the Fe-containing ferredoxin has a greater catalytic potential (mol electron transferred per mol catalyst per second) for electron transfer from PS I to ferredoxin-$NADP^+$ oxidoreductase than does the metal-free flavodoxin.

Acclimation to variations in O_2 levels can involve changes in scavengers and quenchers of active oxygen species such as singlet-oxygen and the hydroxyl radical, and enzymes which remove the active oxygen species superoxide and hydrogen peroxide (Raven et al., 1994). However, variations in O_2 concentration do not always yield acclimatory changes in scavengers such as ascorbate or in the enzymes superoxide dismutase and ascorbate peroxidase (Raven et al., 1998).

Variations in inorganic C supply involves acclimation of the inorganic C acquisition mechanism except for those organisms which reply only on diffusive CO_2 entry and cannot express a CO_2 concentrating mechanism (Chapter 11, Raven and Beardall). The possibility that the morphology of benthic macroalgae using diffusive CO_2 entry may change as a function of inorganic C concentration (freshwater) or water flow regime (freshwater or the sea) in relation to diffusive CO_2 supply does not seem to have been investigated. Of course, algae with carbon concentrating mechanisms might also show morphological changes in response to variations in the diffusive supply of inorganic carbon to the thallus surface in addition to the cell-level acclimation discussed below.

The observed changes in inorganic carbon acquisition kinetics when algae which can express a CO_2 concentrating mechanism and which are growing with an unrestricted inorganic C supply are switched to a restricted inorganic C supply is an increase in the affinity for inorganic carbon. There is a wide range of mechanisms of CO_2 accumulation underlying the acclimation process in different organisms, and there is sometimes more than one mechanism of CO_2 accumulation involved in a single genotype. Qualitative or quantitative changes in the expression of catalytic mechanisms associated with CO_2 accumulation include periplasmic and intracellular (mitochondrial, stroma/cytosol, thylakoid) carbonic anhydrases, and of transporters at the plasmalemma and/or plastid envelope, involving ATP-driven CO_2 or HCO_3^- pumps, HCO_3^-/Cl^- or HCO_3^-/OH^- antiporters, or redox-associated inorganic C pumps (e.g. NADH dehydrogenase in Cyanobacteria which is involved in uptake of inorganic C from the medium and delivery of HCO_3^- to the cytosol) (Poole and Raven, 1997; Raven, 1997; Axelsson et al., 1999; Omata et al., 1999).

These data show widespread occurrence of acclimation of these inorganic carbon acquisition mechanisms to variations in inorganic carbon supply

across a wide taxonomic range and a diversity of mechanisms. It is not clear what signal transduction pathway converts the environmental signal to the changed algal performance in any system, so that it is premature to ask if similar signaling systems are involved in the diversity of CO_2 accumulation mechanisms in the range of organisms.

It is also important to ask what the ecological relevance of these acclimatory responses. In high intertidal rock pools, or freshwaters with relatively low inorganic C:N or inorganic C:P ratios, there are significant changes in inorganic C acquisition responses by algal genotype as a function of differences in concentration and speciation of inorganic C. The range of variation of marine phytoplankton C, N and P contents is reviewed by Geider and La Roche (2002). Even for organisms living in habitats with a more constant inorganic C supply there are acclimatory responses of the CO_2 concentrating mechanism. It is possible that these acclimatory responses relate to variation in other resource-supply, and other environmental, variables which interact with CO_2 concentrating mechanisms. It is known that CO_2 concentrating mechanisms in Cyanobacteria (Beardall, 1991) and eukaryotic macroalgae (Kübler and Raven, 1995) show inverse effects of reduced inorganic C supply and lower incident photon flux densities. Interactions of inorganic N, Fe and Zn supply with CO_2 concentrating mechanisms are predicted; interactions among these nutrients and have been found experimentally, but not always as predicted (Raven, 1991, 1997; Falkowski and Raven, 1997; Erica Young, personal communication).

Temperature acclimation by algae is considered by Anning et al. (2001) and Raven and Geider (1988).

VII. Regulation of Algal Photosynthesis

Regulation refers to the control of enzyme activity and energy dissipation pathways that occurs independently of net macromolecular synthesis or degradation. Regulation will be considered in terms of the (apparently) important physiological and ecophysiological functions that they perform and the catalysts which are regulated rather than categorizing them according to the means by which regulation is achieved, although the three processes we consider have different means of modifying the catalysts.

The first regulated process that we consider is the activity of Rubisco, i.e. the enzyme activity per unit of enzyme protein. MacIntyre et al. (2000) point out that short-term (tens of seconds-minutes) activation and deactivation of Rubisco can account for short-term variations in maximum photosynthetic rates in (for example) rapid changes in photon flux density during mixing in the surface mixed layer. Changes in the activation state of Rubisco may also underlie adjustment to steady-state photon flux density variations over time too short to permit acclimation (change in Rubisco protein content). Activity changes of Rubisco in algae appears to be dependent on carbamylation and decarbamylation and, perhaps, the level of tight-binding inhibitors such as carboxyanobinitol-1-phosphate. The role of Rubisco activase in regulating Rubisco activity in algae and Cyanobacteria is not clear; this enzyme is present in *Chlamydomonas* and, apparently some filamentous Cyanobacteria, but not the unicellular cyanobacterium *Synechocystis* PCC 6803 (Kaneko et al., 1996; Li, Zianni and Tabita, 1999). Since the rate of activation is much faster than the rate of deactivation, Rubisco may remain in an active state during short-term fluctuations in photon flux density (e.g. during rapid vertical movement in high-attenuation habitats, such as an estuary; MacIntyre et al., 2000).

A second regulated process which occurs over a time course of minutes is the state transition phenomenon. This changes the relative absorption by the PS I and PS II antennae by reversible dissociation of some pigment-protein complex from PS II (with or without reversible association with PS I), and (as we have already seen) state transitions occur in green algae, red algae and Cyanobacteria (Table 1) (Chapter 13, Larkum). Phosphorylation and dephosphorylation appear to underlie these responses. High irradiation, and darkness favor state I (preferential excitation of PS I), while excess excitation of PS I (e.g. by red-far red light) favors state II (preferential excitation of PS II). State transitions alter the photosynthetic effectiveness of organisms by excitation energy arrival at the limiting photoreaction centers (PS II or PS I), and thus altering efficiency of photosynthesis at low photon flux densities and the extent of diversion of excitation energy from PS II at high incident photon flux densities.

A third regulated process is the xanthophyll cycle in all of the algae investigated except Cyanobacteria and most of the red algae (Chapter 13, Larkum; Ursi et al., 2003), and the phycoerythrobilin-phycourobilin interconversion in the cyanobacterium *Tricho-*

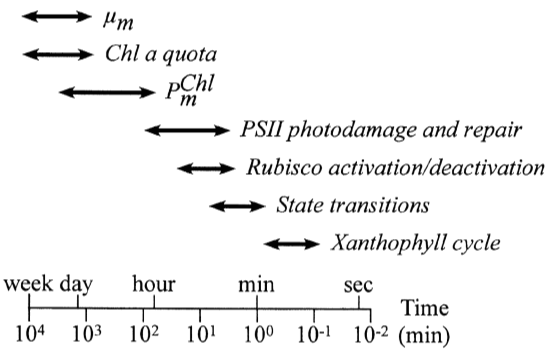

Fig. 4. First order time constants for acclimation of algal photosynthesis to step changes in photon flux density. Figure provided by Hugh MacIntyre (Horn Point Environmental Laboratory). Updated from MacIntyre et al. (2000).

desmium (see above). This process occurs over time-scales of minutes, and involves covalent modification to the chromophores which varies the capacity to quench excitation energy. The high-quenching forms of the pigments restrict excitation energy transfer to PS II, thus down-regulating PS II activity but also thereby limiting photodamage to PS II. This production of the pigment from which quencher excitation energy occurs when organisms are exposed to high photon flux densities, and involves activity of de-epoxidase enzymes in the xanthophyll cycle and phycoerythrobilin to phycourobilin conversion in *Trichodesmium*. De-epoxidation predominates under low irradiances.

VIII. Rates of Regulation and Acclimation

Many regulatory reactions occur so rapidly as to allow photosynthesis to track instantaneously the naturally occurring changes of environmental conditions (i.e. they have time constants of seconds or less) (Fig. 4). These include, for example the light-dark activation-deactivation of fructose-1,6-bisphosphate (and sedoheptulose-1,7 bisphosphate)-1-phosphatase; this takes place over seconds. Another example is activation-deactivation of the CF_0-CF_1, ATP synthetase. Other regulatory mechanisms, such as the three outlined in the previous section, occur with time constants of minutes, and as such may constrain rates of photosynthesis or photoprotection in rapidly changing light environments (MacIntyre et al., 2000).

Unlike regulation, which typically occurs rapidly and is reversible on short time scales (seconds to tens of minutes), acclimation involves the net synthesis and breakdown of macromolecules. As such, the achievable rate of acclimation to changing conditions is limited by the mechanistically achievable rates of macromolecule synthesis and/or degradation and what is selectively appropriate, granted the time course of environmental fluctuations.

Light is clearly the most rapidly varying environment factor, and one whose tracking by acclimation is likely to be costly in energetic (and C and N) terms. As such, rapid variations of light environment induce a number of regulatory responses. Over the longer term, phytoplankton appear to acclimate to the equivalent in terms of a steady photon flux density which is rather higher than the arithmetic mean of the varying photon flux densities which occur during the photoperiod (Gilstad et al., 1993; Kromkamp and Limbeek, 1993; Flameling and Kromkamp, 1997). The algae thus appear to be acclimated to variations in photon flux density in a manner which protects against photoinhibition in high photon flux densities rather than maximizing performance at the low photon flux density extreme of the variation. This procedure may limit restrictions on the photosynthesis during low photon flux density episodes due to photo-damage incurred in high photon flux density episodes; this damage (or photo-protective procedures) restricts photosynthesis at low photon flux densities to a relatively greater extent than at high photon flux densities.

Inorganic carbon concentrations can change such that free CO_2 concentrations can vary by an order of magnitude in an hour, and the resource cost of acclimation is relatively high if significant changes in Rubisco are involved. However, acclimation of transporters, carbonic anhydrases and photorespiratory cycle enzymes (or their surrogates in glycolate metabolism) to CO_2 availability have lower resource costs (Raven, 1991). The costs of acclimation to S deficiency by replacing major protein components (phycobilins of Cyanobacteria) with low-S homologs (Mazel and Marliere, 1999) can also be high, even if many of the amino acids used are scavenged from the high-S phycobilins which are degraded. Addressing N, P and Fe deficiency by increasing expression of transporters has a relatively low cost in energy, C and N, as has switching from ferredoxin to flavodoxin and cytochrome c_6 to plastocyanin as a means of economizing on Fe in catalysts. There are also changes in quantities of catalysts which contain

Fe and for which no non Fe-containing analogues occur. The concentrations of nutrients such as S, N, P and Fe usually vary rather slowly in the algal environment. However, rapid enrichment of the medium for phytoplankton results from mixing of deeper nutrient-rich water into the upper mixed layer/epilimnion and rapid deprivation and reinstatement of nutrients to intertidal marine algae occurs with the ebb and flow of tides.

What we know of time courses of acclimation to changes in resource availability is generally derived from the imposition of step changes in resource availability. For uptake and assimilation of chemical resources and replacement of catalysts with analogues which use less (or more) of a resource the half-time of acclimation at 20–25 °C is, even for the most rapidly growing algae, an hour or more. The time taken cannot be directly related to the quantity of material to be synthesized. For decreases in catalytic activity dilution during growth may occur with no further net synthesis until the new steady level has been achieved (Geider et al., 1996, 1998). For example, cell chlorophyll *a* content declines due to accelerated cell division without net breakdown following transfer from low to high light (Cullen and Lewis 1988; Anning et al, 2000; MacIntyre et al., 2002). The change of flavodoxin abundance following resupply of iron to Fe-limited cells appears to occur by dilution rather than by active breakdown (Davey and Geider, 2001). In some cases a turnover of catalysts can speed net decay if synthesis is decreased or abolished but breakdown is not (see above). The time taken for responses of catalysts to Fe deficiency is relatively slow, perhaps because large-scale changes in major photosynthetic catalysts is involved. These lengthy time courses of acclimation resemble those to variations in photon flux density where half-times of response of hours to step changes in photon flux density are common, even for rapidly-growing microalgae. The long half-time for acclimation to step changes in photon flux density contrasts with very rapid changes in photon flux density in the natural environment, e.g. cloud changes, wave frequency, and circulation in the upper mixed layer/epilimnion, with frequencies of seconds to tens of minutes (Gilstad et al., 1993; Kromkamp and Limbeek, 1993; Flameling and Kromkamp, 1997; Raven and Kübler, 2002.

An important development since the late 1970s has been the formulation of models that quantify the changes in photosynthesis and growth of algae as a function of variations in light, nutrients and temperature. These models can be used to relate cell-level phenomena to algal ecology (MacIntyre et al., 2000). Most of the earlier models (Shuter, 1979; Laws and Bannister, 1980; Kiefer and Mitchell, 1983) were developed for conditions of balanced growth, and typically invoke, optimally, criteria based on maximizing the efficiency of resource use in their formulations. However, some recent models (Kana et al., 1997; Geider et al., 1998) are dynamic in the sense that they are formulated for unbalanced growth in variable environments. They have been based on simple regulatory rules that do not necessarily maximize resource use (Geider et al., 1996, 1998; Kana et al., 1997) and/or simplified treatments of biochemical pathways (Flynn et al., 1997; Flynn and Hipkin, 1999). Validation of earlier models was limited to comparisons with balanced growth conditions in chemostat cultures. The recent models have been applied to batch cultures (Flynn et al., 1997; Geider et al., 1998) as well as to step-changes of photon flux density (Geider et al., 1998; Flynn et al., 2001). Such models must tread a fine path between undue simplification which may neglect important biochemical and biophysical phenomena and more complexity than is useable in analyzing laboratory and, especially, field data (Geider et al., 1998). The further development of such models is limited by gaps in our understanding of the mechanisms of regulation and acclimation as well as by the availability of suitable data sets for model validation.

IX. Conclusions

Our understanding of adaptation, acclimation and regulation in algal photosynthesis has advances significantly over the last decade. The advance in genome analysis of Cyanobacteria is greatly aiding our understanding of photosynthetic adaptation among Cyanobacteria, especially with respect to light use and inorganic carbon use. Genomics are also helping our understanding of acclimation in Cyanobacteria by indicating the range of genetic options for light harvesting and inorganic carbon acquisition and the conditions in which these options are exercised. Modeling is also increasingly significant in synthesizing data and in predicting the outcomes of environmental variations.

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Chapter 18

Photosynthesis in Marine Macroalgae

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Summary

Marine macrophytes live in a highly variable environment in respect to light, UV-radiation, temperature and also salinity. During evolution their metabolism has adapted to these strongly changing conditions, especially in the upper littoral zone. In the lower subtidal, the photosynthetic apparatus has adapted to low light conditions, to absorb maximally the incident photons and to utilize the absorbed energy with high efficiency. In the intertidal and upper sublittoral zone the photosynthetic apparatus is exposed to strong light stress when the absorbed amount of light energy is higher than the organism can use for its metabolism. This happens generally when plants are exposed to irradiances, which are above the mean level of irradiation occurring at their growth sites (e.g., light flecks or low tide around noon). Under light stress the amount of thermal energy dissipation increases and the energy flowing into photochemistry decreases also. Photoinhibition causes a decrease of the photosynthetic quantum yield (dynamic inhibition) and at much higher fluence rates or after a longer duration of high irradiation, also a decrease of the photosynthetic capacity (chronic inhibition). The absorption cross-section of the chromatophores can be diminished by chloroplast displacement or shrinking, especially in brown algae, which decreases the rate of photodamage to the light-absorbing apparatus. Irradiances, which exceed the protective capacities of these mechanisms, cause irreversible damage to the photosynthetic apparatus. Normally, the latter does not occur for long at the natural growth sites.

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In the daily cycle, dynamic photoinhibition of photosynthesis generally occurs in intertidal and upper subtidal algae, especially if low tide coincides with noon. Dynamic photoinhibition is a protective mechanism rather than photodamage. It enables the photosynthetic apparatus of macroalgae to cope with the temporary light stress in the upper littoral zones. Algae which are not able to decrease the energy pressure on photosynthesis by harmless thermal energy dissipation and/or a decrease of the absorption cross section are irreversibly damaged and, therefore, cannot grow in the upper littoral zones.

Kirk (1994) stated in his textbook that many macrophytes show photoinhibition of photosynthesis. However, he concluded misleadingly that 'ecologically this phenomenon is of less significance, since any given macrophyte species is generally to be found growing at a depth where the light intensity is one to which it is well adapted.' On the contrary, the studies performed in recent years show clearly that photoinhibition of photosynthesis is of great significance for the survival of macrophytes in their natural environment. In particular, algal zonation is thought to depend at least in part on the ability to withstand photoinhibition.

This chapter deals, therefore, with the special environmental conditions for marine macrophytes, the adaptation and acclimation of photosynthesis to these conditions, and it discusses fluorescence techniques recently developed especially for in situ (field) measurements.

I. Introduction

Solar radiation is the most important prerequisite for life on Earth. In the process of photosynthesis, photoautotrophic organisms convert light energy into chemically-bound energy. Macroalgal photosynthesis contributes substantially to the global primary production. About 5% of the total global oceanic production is based on marine macroalgae and seagrasses although their occurrence is restricted to only 0.6% of the area of the world's oceans (Smith, 1981). Therefore, macrophytes act also as an important sink for CO_2.

Macrophyte-derived carbon plays an important trophic role for many marine organisms, e. g. growth rates of benthic suspension feeders are greatly increased in the presence of detritus from benthic macrophytes. Stable carbon isotope analysis has confirmed that the nearshore food web is predominantly based on seaweed-derived carbon (Duggins et al., 1989). Moreover, macrophytes serve as shelter for marine animals, especially juvenile fishes. They stabilize sediments, buffer against large changes in nutrient concentrations in the water column (Duarte, 1995) and are important in terms of biodiversity (Norton et al., 1996).

A decline of macrophyte beds in Australia and North America may have contributed to a tremendous decrease in local fisheries (Edgar and Shaw, 1995a,b; Kirkman, 1997). If the productivity of coastal aquatic ecosystems is disturbed, this may have severe implications on the vitality of seabirds and land predators that feed on aquatic organisms. Additionally, macroalgae are used in industry for production of food and phycocolloids like carragenan, alginate and agar (Lüning, 1990; Guiry and Blunden, 1991).

Photosynthesis of many marine macroalgae is C_i (inorganic carbon) saturated under the present conditions (but see Chapter 11, Raven and Beardall), whereas seagrasses are CO_2 limited (Beer and Koch, 1996). As future global, and, hence, in the seawater dissolved CO_2 levels will increase (Houghton et al., 1990, 1992) it has been predicted that photosynthesis of seagrasses will be almost C_i saturated. In contrast,

Abbreviations: α – photosynthetic efficiency (maximum light utilization coefficient); Chl – chlorophyll; ΔF – variable fluorescence in light, $(F_m - F_t)$; $\Delta F/F_{m'}$ – effective quantum yield of PS II; DOM – dissolved organic matter; E_c – compensation point, when respiration equals photosynthetic rate; E_k – calculated light saturation index; ETR – electron transport rate; FCP – fucoxanthin-Chl *a/c* protein complex; F_m – maximal fluorescence yield of PS II after dark acclimation, all reaction centers are open; F_m' – maximal fluorescence yield of PS II in the light acclimated state, all reaction centers are closed; F_o – minimal fluorescence yield of PS II after dark acclimation, all reaction centers are open; FRR-fluorometer – fast repetition rate fluorometer; F_t – fluorescence yield of PS II due to actinic irradiation, reaction centers are partly closed; F_v – variable fluorescence yield in darkness, $(F_m - F_o)$; F_v/F_m – maximum quantum yield of PS II; FW – fresh weight; H_{sat} – period of light saturation of photosynthesis; h_v – light energy; I – irradiance; – I_o – impinging irradiance; K_d – vertical attenuation coefficients of downward irradiance; LHC – light harvesting pigment protein complex; PAM-fluorometer – pulse amplitude modulation fluorometer; PAR – photosynthetically active radiation; P-E curve – photosynthesis versus irradiance curve; PLSD – post hoc least significant difference test; P_{max} – maximal photosynthetic rate, photosynthetic capacity; PQ – apparent photosynthetic quotient (O_2:CO_2); PS – Photosystem; qE – energy quenching parameter; Rubisco – ribulose bisphosphate carboxylase/oxygenase; UVB – ultraviolet B radiation; UVR – ultraviolet radiation

no such change is expected for macroalgae, especially from the intertidal (Beardall et al., 1998). However, the important global function of seaweeds as a CO_2 sink may be reduced, if carbon assimilation of photosynthesis is inhibited due to increased UV-radiation as a consequence of stratospheric ozone depletion (Chapter 16, Franklin et al.). Therefore, biodiversity of macrophyte communities may change in future with anthropogenic climate effects.

Molecular oxygen is generated in photosynthesis, which is used in the respiratory metabolism of all heterotrophic organisms. Changes in irradiance and light quality can promote photosynthesis, but can also inhibit many biological processes if the radiation becomes excessive, or if short wavelength radiation with a high energy content is absorbed by biomolecules (Chapter 16, Franklin et al.). Consequently, damage to important components of plant metabolism does result in reduced photosynthetic and general metabolic activity and a decrease in biomass production. In particular during periods of strong sunlight, photosynthetic activity of marine macroalgae in the field is depressed (Ramus and Rosenberg, 1980; Coutinho and Zingmark, 1987). This depression, which is mainly caused by dynamic photoinhibition for protection (see Section V.B), follows a diurnal pattern, so that the lowest photosynthetic activity occurs mostly between noon and afternoon (Huppertz et al., 1990; Henley et al., 1991, 1992; Hanelt, 1992; Hanelt et al., 1993). The depression arises because energy supply by the photosynthetic apparatus exceeds the demand of the Calvin cycle and oxygen free radicals could be generated. It, therefore, depends on the irradiance of photosynthetically active radiation (PAR). It has recently been shown that ultraviolet (UV) radiation also has a strong impact on photosynthesis of aquatic organisms (Holm-Hansen et al., 1993a,b; Franklin and Forster, 1997; Häder and Figueroa, 1997; Chapter 16, Franklin et al.). Ever since the discovery of the Antarctic ozone hole in the 1970s (Farman et al., 1985), serious concerns have arisen about the impacts of increasing UVB radiation on the biosphere (Madronich et al., 1998; Björn et al., 1999). Moreover, recent research indicates that the stratospheric ozone layer is thinning not only over Antarctica but also over the polar regions of the Northern hemisphere (Jokela et al., 1993; Müller et al., 1997; Rex et al., 1997). As macroalgae and seagrasses are in general attached to the substrate, they have to cope with considerably different radiation regimes during their ontogenetic development. Low light adapted organisms from the Polar regions seem to react particularly sensitively to alterations in the solar spectrum (Kirst and Wiencke, 1995).

Increase of global temperature, arising from elevated atmospheric CO_2 levels (greenhouse effect), will lead to changes in the geographic distribution of marine macrophytes as during periods of lowered water temperatures in the ice ages (van den Hoek et al., 1990; Wiencke et al., 1994; Bischoff and Wiencke, 1995a,b), e. g., the southern distribution limit of the dominant canopy species *Laminaria hyperborea* will be shifted from Portugal to northern Denmark by a temperature increase of 2 °C, as blade initiation in this alga is inhibited by elevated temperatures (Breeman, 1990). As understorey species are shade-adapted, these species will loose their canopy shelter and will be exposed to damaging effects of full sunlight, e.g. experimental removal of adult plants of the canopy species *Ecklonia radiata* from algal forests in Australia resulted in tissue damage, photopigment destruction, reduced growth and low survivorship of the juvenile understorey plants of the same species (Wood, 1987). According to this study the UV component of radiation, rather than PAR, was responsible for the inhibition of growth and photodamage. Therefore, macrophytes exposed to increased levels of UV irradiation might be displaced to greater depths. This scenario points to interactive effects between changing temperature and light regimes, which have hitherto not been investigated.

II. Radiation Conditions in Coastal Waters

Photosynthetic activity of marine algae depends on the environmental light conditions, which can be quite different in the coastal area compared to the open ocean. Penetration of light is largely determined by scattering and absorption of biological and inorganic material, with higher concentration in coastal areas. Coastal waters show large temporal changes and regional differences in the concentration of dissolved and particulate matter influencing penetration of solar radiation into the water body. So Jerlov (1976) classified marine waters into nine types of coastal and five types of oceanic waters depending on the respective transmittance characteristics (Chapter 16, Franklin et al.).

Marine macrophytes can form communities (e.g. kelp forest) with vegetation layers comparable to

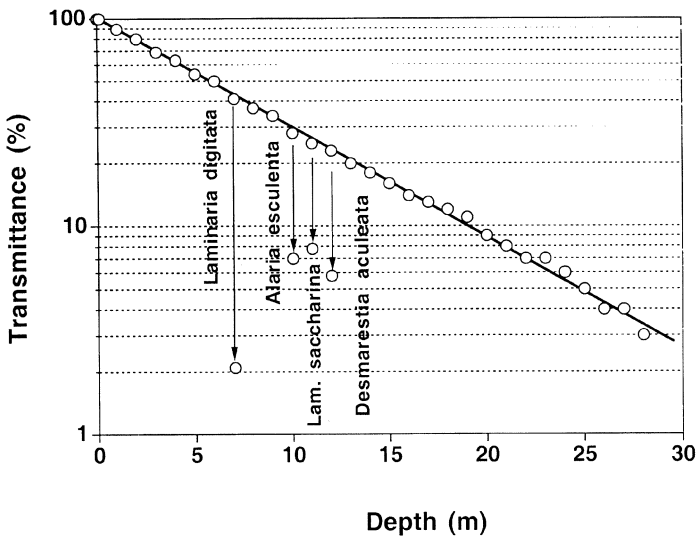

Fig. 1. Light transmittance within the water column and below the undergrowth of four brown algal canopy species from the Kongsfjord at Spitsbergen measured in early June (1996) in a transparent water body expressed as percentage of surface radiation (vertical attenuation coefficients of downward irradiance K_d(PAR) = 0.12)

terrestrial forests with canopy species overtopping the understorey species (Lüning, 1970, Dayton, 1985). This has effects on photosynthetic performance and adaptation of the photosynthetic apparatus of the different species within kelp forest ecosystems. Irradiance of photosynthetically active radiation (PAR) is strongly attenuated by the kelp canopy as shown in Fig. 1. for light measurements above and below a canopy of different large kelp species at the Kongsfjord in Spitsbergen. In addition, the change in the light field includes not only a decrease in the photon irradiance but also changes in the light quality. Salles et al. (1996) found that below the canopy the spectrum was enriched in green and in far red light, probably affecting photosynthesis as well as the photomorphogenetic development of the understorey.

Polar regions show particular strong seasonal changes of the radiation conditions. At 80° North the polar day lasts from mid April to the middle of August and the polar night from the middle of October to the middle of February. Furthermore sea ice cover, which still can persist during Arctic spring, confers a strong seasonal impact on exposure of marine organisms to solar radiation (Mehlum, 1991; Ito and Kudoh, 1997; Vincent et al., 1998). Consequently, as a result of the long polar night and prolonged sea ice and snow cover, Arctic macroalgae endure about six months of darkness. They are suddenly exposed to high solar radiation as soon as the ice cover breaks up in early summer (Bischof et al., 1999), when the sunlight penetrates deeply into the actual clear water and elevated UVB levels can occur due to ozone depletion.

Due to the wavelength dependent absorption in the water column and the presence of high concentrations of dissolved organic matter (DOM) (Björn, 1993), often characteristic for coastal waters, UVB radiation does not generally penetrate very deeply into the coastal water body (Jerlov, 1976; Kirk, 1994; Hanelt et al. 2000).

In a sheltered Arctic coastal water system a strong influence of melt water discharge on light transmittance was demonstrated by Hanelt et al. (2000). Within a fjord or most estuaries, a water system of a minor water exchange with the open ocean, the water is strongly stratified with a turbid fresh water layer above the relatively transparent salt water. This stratification significantly influences the fluences of PAR and UVR during summer time. However, eulittoral algae are fully exposed to high solar radiation at low tide and need to develop strategies to cope with the high radiation. Shade-adapted species either grow in the sublittoral zone (and are thus protected against high radiation by the water column above), or they grow as subcanopy species being shielded by other algae.

For annual calculations of biomass production, and for modeling the spectral light distribution in

relations to atmospheric studies, the underwater light climate should be determined during the course of the year to obtain a basic data base. For a better insight into the marine underwater ecosystem, the seasonal variation of the daily solar irradiance, light transmittance into the water body, including the UV-radiation range, should then be described to estimate possible implications on the primary productivity of benthic macroalgae under global change aspects.

III. Light Absorption by Macroalgae

The various classes of algae exhibit different absorption characteristics (Chapter 2, Larkum and Vesk). The color of the algae is mainly based on the accessory photosynthetic pigments of the light harvesting complex (LHC). Light absorption by the carotenoid fucoxanthin is mainly responsible for the brown color typical for the Phaeophyceae (Chapter 15, Mimuro and Akimoto), the phycobiliproteins for the red color of the Rhodophyceae (Chapter 14, Toole and Allnutt). If the LHC consists mainly of chlorophylls, the color is green, as is typical for the Chlorophyceae. The occurrence of the various types of pigments in the LHC and their arrangement in both photosystems is responsible for different photosynthetic efficiencies of different spectral wavebands which affects photosynthetic activity at different light qualities occurring within the water body. The action spectrum of photosynthesis of red algae shows a so-called blue and red drop, first described by Haxo and Blinks (1950). Green light causes the highest photosynthetic rates in these organisms because it is absorbed mainly by the phycobilins of the antenna of Photosystem II and transferred efficiently to both photosynthetic reaction centers. In contrast blue and red/far red light does not induce high electron transport rates because chlorophyll molecules act as the main antenna pigment in Photosystem I so that the reaction center of PS I is primarily activated (Butler, 1978). Blue and far red light induces principally charge separation in the reaction center of PS I and cyclic electron transport around PS I. As charge separation of PS II is not induced to an equal amount, linear electron transport rate is small and oxygen production rate is low (Haxo and Blinks, 1950; Hanelt et al., 1992). In contrast, energy absorbed by the LHC of Photosystem II (particularly the green wavebands) is also transferred to PS I by 'spillover' (Chapter 13, Larkum), and thus activates the linear electron transport so that oxygen production occurs with a high yield. Such monochromatic light conditions, however, rarely occur in natural systems so that these spectral effects on photosynthetic activity are rather artificial.

Engelmann (1883, 1884) pointed to the fact that most green algal species occur in the eulittoral and upper sublittoral, whereas brown algae grow often in deeper zones and many red algae can be characterized as deep water species. In deep water, where blue-green light prevails, the red pigments of the Rhodophyceae have an efficient absorption (Biebl, 1962). However, this is only partly valid because the absorption characteristic depends on several other factors, especially the thallus morphology. It also applies more to coastal waters than to oceanic waters (Larkum et al., 1967). A thick non-transparent algal thallus appears black and absorbs light over the whole spectral range. In addition accessory pigments do enhance light absorption in the blue-green range and examples of green algae growing in deep waters are not rare (Dring, 1981, 1982; Ramus, 1981). Anyhow, the deepest algae found are crustose red algae with a quite low light demand (Littler et al., 1986), which is not only due to their low growth rate and special morphology (one absorption layer) but also to their capability to use very efficiently the incident photons. Leukart and Lüning (1994) demonstrated in several red algal species that growth rate and photosynthesis depends on the light quality during culture and on the pigment content under these conditions. The light requirements were lowest in green light for all red algae investigated. The action spectra of growth followed the photosynthetic action spectra, with maximum efficiencies in the green wavebands. This points to the importance of light quality for survival at low photon fluence rates and corroborates the findings of Harder and Bederke (1957), Beer and Levy (1983) and Glover et al. (1986, 1987). The fading importance of phycobilins at increasing irradiances was demonstrated by Larkum and Weyrauch (1977). They investigated the photosynthetic action spectrum of the red alga *Griffithsia monilis* at different light levels and showed that at low irradiances the phycobilins are the major light-harvesting pigments, while at increasing irradiances chlorophyll and carotenoids contribute proportionally more as the absorption of phycobilins becomes light saturated. However, the phylogenetic development of pigments in deep-water algae adapted most probably to low light of a particular spectral

character in the sense of Engelmann (1883) is beyond question.

How is photosynthetic activity related to thallus structure? Many studies have shown (Ramus, 1978; Littler and Arnold, 1980; Weykam et al., 1996) that light absorption in species consisting only of photosynthetically active cells is higher compared to species consisting also of non-photosynthetic tissue in the medulla. Therefore, filamentous (e. g. *Urospora*, *Bangia*, *Ectocarpus*) or foliose (mono- or distromatic) thalli (e.g. *Monostroma*, *Ulva*, *Porphyra*) show higher photosynthetic capacities (P_{max}) determined on a fresh or dry weight basis than branched, corticated (e. g. *Plocamium*, *Desmarestia*) and leathery (e. g. *Laminaria*, *Iridaea*) species. These thallus forms have developed convergently in the various systematic groups.

Such morpho-functional relations can be demonstrated not only between different species, but also between different developmental and life-history stages within the same species. For example, net maximal photosynthetic rates (P_{max}) measured on a fresh weight basis are higher in two year-old than three year-old *Ascoseira mirabilis* from Antarctica (Gómez et al., 1996), reflecting the higher proportion of non-photosynthetic tissue in the older plants. A similar morpho-functional variation has been demonstrated in one to seven year old *Ascophyllum nodosum* (Stengel and Dring, 1998) and between gametophytes and sporophytes of Antarctic *Desmarestia menziesii* (Gómez and Wiencke, 1996). The gametophytes of this species are characterized by a higher photosynthetic capacity (P_{max}), dark respiration, photosynthetic efficiency at limiting irradiances (α) and hence a lower light saturation (E_k) for photosynthesis than adult sporophytes. These morpho-functional differences are regarded as a fundamental feature of the life strategy of this species. In general, growth of gametophytes and uncorticated sporophytes is favored at low fluence rates in winter in virtue of their filamentous organization, high assimilatory pigment content per biomass, high area/volume ratio and a low proportion of non-photosynthetic tissue. Thus, the 'shade adaptation' of gametophytes in Desmarestiales allows the alga to survive, reproduce and recruit under conditions unfavorable for large sporophytes, which require comparatively higher photon fluence rates for photosynthesis and growth (Gómez, 1997). Similarly, stress sensitivity of photosynthesis for excessive light is dependent on the thallus age and on the life history stage as shown for *Laminaria saccharina* (Hanelt et al., 1997b).

It should, however, be noted that morphology does not always reflect the potential photosynthetic activity. Dudgeon et al. (1995) indeed found differences between the heteromorphic generations of the red algae *Mastocarpus stellatus* and *Chondrus crispus* but these differences were as great or greater among morphologically comparable thalli as among morphologically distinct thalli. Similarly, species of the genus *Laminaria* are characterized by high photosynthetic rates on a dry weight basis and by a high carboxylating activity in the non-growing distal regions compared to the basal meristematic region (Küppers and Kremer, 1978). In contrast, in the macro-morphologically similar *Himantothallus grandifolius*, rates of photosynthesis are comparable in all parts of the blade (Drew and Hastings, 1992). On the other hand, in *Ascoseira mirabilis*, a species of the same macro-morphology, rates of light carbon fixation increase like in *Laminaria* from the basal to the distal parts of the blade (Gómez et al., 1995a). But opposite to the situation in *Laminaria* (Küppers and Kremer, 1978), light independent carbon fixation is highest in the distal thallus parts of *A. mirabilis* (Gómez et al., 1995a). These differences between species may reflect evolutionary divergence, specific morpho-functional relationships, different states of acclimation and/or adaptation to the specific external conditions and may also depend on seasonal changes in physiology related to the life-strategy of the individual species.

IV. Determination of Photosynthetic Rates

In the past, photosynthesis in macroalgae has mostly been measured by determination of oxygen evolution and less frequently by carbon assimilation. Few studies have been performed by measuring both oxygen evolution and carbon assimilation, and therefore, determinations of the apparent photosynthetic quotient (PQ = mol O_2:mol C) are rare. Hatcher (1977) measured PQs of *Laminaria longicruris* at between 0.7 and 1.5. Measurements made on five brown and two red algal species from Antarctica gave PQs between 1.1 and 5.3 (Thomas and Wiencke, 1991; Weykam et al., 1997). High values are typically obtained when nitrate is the dominant nitrogen source and low values when ammonium is mainly taken up due to the difference

in their reduction states (Williams et al., 1979). Enhanced production of lipids and proteins will also result in higher PQs than when polysaccharides are predominantly synthesized (Laws, 1991; Williams and Robertson, 1991).

The Kautsky effect of light-induced chlorophyll fluorescence has been used in a number of ways to measure photosynthetic parameters. The pulse amplitude modulation technique (PAM-method) was introduced by Schreiber and co-workers in 1986. This is now widely used to measure quantum yield and photosynthetic rate. In principle the fluorescence signal of PS II competes for the excitation energy with the photochemical energy conversion and heat dissipation (Fig. 2). During a strong pulse of white light, all electron acceptors of PS II become fully reduced for less than a second so that excitation energy is only dissipated by heat or fluorescence. Then, a lower fluorescence signal indicates a higher heat dissipation, which, as a competitor against the photochemical energy conversion, means a decrease of the quantum yield of the photosystem. At higher light intensities photoinactivation due to dynamic photoinhibition (Osmond, 1994; Chapter 16, Franklin et al.) causes a stronger thermal energy dissipation, which decreases the excessive energy input into the photochemical system. This optimizes the rate of photochemistry and diminishes the rate of photodamage. The increase of the non-radiative energy dissipation is variously suggested to be due to an increase of the zeaxanthin content in the Photosystem II antenna by Adams III and Demmig-Adams (1992), to an aggregation of the light harvesting complexes by Ruban et al. (1993) and/or to an increase of the amount of inactive Photosystem II centers (e.g. PS II_β centers), which may also be able to protect active photosynthetic centers (Guenther and Melis, 1990; Öquist and Chow, 1992; Critchley and Russell, 1994). However, the evidence for the first mechanism, the xanthophyll cycle, is gaining ground (Gilmore et al., 1998).

A low intensity modulated measuring light induces a modulated fluorescence signal from the plant, which can be detected by the instrument even against strong background light. Changes in the ratio of variable (F_v) to maximal fluorescence (F_m) of temporarily dark exposed plants are used as a measure of photoinhibition and recovery (Krause and Weis, 1991). $F_v = F_m - F_o$, in which F_o is the initial fluorescence, i.e. when all reaction centers of PS II are active or 'open,' and F_m is the maximal fluorescence

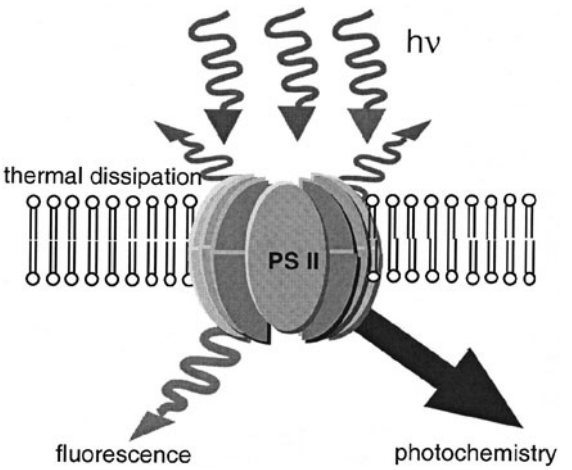

Fig. 2. Scheme of energy flow in Photosystem II. At normal light conditions the absorbed light energy ($h\nu$) is mainly used to drive photochemistry. A small amount of energy is always lost by fluorescence, which is used for calculation of the quantum yield of photosynthesis. A certain amount of energy is dissipated harmlessly by heat. Under excessive light conditions thermal dissipation increases strongly, and hence, decreases quantum yield of PS II (Hanelt and Nultsch, 2003).

determined under a strong saturating light pulse, i.e. when all PS II centers are 'closed'. By monitoring the dynamics of this signal, it is possible to measure the maximum quantum yield (F_v/F_m) in dark exposed plants and the effective quantum yield ($\Delta F/F_m'$) of Photosystem II (PS II) in light exposed plants without affecting the object being investigated. Earlier experiments showed that the results of fluorescence measurements are generally consistent with those of oxygen measurements and that F_v/F_m is a good measure for the photosynthetic efficiency in macroalgae (Hanelt, 1992; Hanelt et al., 1995, 1997c).

For determination of photosynthetic performance under natural light conditions field experiments are indispensable. The results of field and laboratory experiments on photosynthetic performance carried out under comparable conditions can be inconsistent (Hanelt et al., 1992, 1993; Hanelt and Nultsch, 1995). In laboratory experiments using cultured red algae a period of 48 h was required to attain full recovery of photosynthesis after photoinhibition, whereas in the field the same species had already fully recovered by the evening, hence much faster. A reason was that it was only possible to measure the optimal quantum yield (F_v/F_m) in algae after collection under water and temporarily dark-exposed in a special cuvette on the shore. Thus, short-term dynamic depression of

the quantum yield could not be assessed. With a newly developed underwater fluorometer (Diving-PAM; Schreiber et al., 1997), or a SCUBA-based fast repetition rate fluorometer (FRR-fluorometer) (Gorbunov et al. 2000) it is now possible to perform underwater measurements of the effective quantum yield in benthic organisms in situ. This gives information on how photosynthesis is regulated under water in relation to the impinging fluence rate.

The FRR-fluorometer measures, in contrast to the Diving-PAM, chlorophyll fluorescence transients using a controlled series of sub-saturating blue light flashes that cumulatively saturate PS II within about 100μs, i.e. within a single photochemical turnover. The optimal quantum yield of brown algae determined with this fluorometer seems to be somewhat lower than determined with a PAM-fluorometer (Gorbunov et al., 2000). As yet only measurements in corals have been published using this instrument (e.g. Lesser and Gorbunov, 2001; Lombardi et al., 2000). Investigations of corals, sponges and macrophytes using the Diving-PAM are already well established (Beer and Illan, 1998; Beer et al., 1998a,b, 2000; Schreiber et al., 1997) and many more will be surely done in future times. As experience of operating these new instruments is still limited, the following section gives more detailed information.

Use of submersible instruments opens a new field of ecophysiological in situ research. However, like all new techniques there are pitfalls for the inexperienced user. The main application consists in the determination of effective PS II quantum yield by the saturation pulse method (effective quantum yield of an irradiated sample = $\Delta F/F_m'$, Genty et al., 1989). Fluorescence yield of photosynthesis depends on the respective specimen and its physiological state. Generally, in situ, the yield is often unexpectedly low, so that e.g. with the Diving–PAM a high measuring light intensity (to induce modulated fluorescence) and high electronic signal gain of the instrument are required, which decrease the signal:noise ratio. A prerequisite for an accurate measurement is, however, that the measuring light does not affect electron transport and all reaction centers remain open, as high measuring light intensities induce electron charge separation and close reaction centers. Changes in the kinetics of fluorescence caused only by the measuring light indicate that some centers have already closed. The effective quantum yield ($\Delta F/F_m'$) of Photosystem II is defined as F_m'-F_t/F_m', where F_m' is the maximum fluorescence of light-acclimated photosynthesis and F_t the fluorescence level caused by the ambient light (PAR). The ambient light should be measured with the light sensor close to the specimen. Then, a relative electron transport rate is calculated as:

$$\text{rel. ETR} = \Delta F/F_{m'} * \text{PAR}.$$

However, as shading by the instrument will change the light environment, the fiber optics should be placed at an angle of about 45° and not directly on the thallus surface as by darkening of the thallus tissue the energy quenching parameter (qE) will quickly change. As a result, $\Delta F/F_m'$ of the light acclimated plant would not be accurately measured. As the variation in the fluorescence signal is often large, measurement of a large number of specimens in one location is necessary.

Halogen lamps used for actinic illumination cause major problems as the spectrum of these lamps changes when actinic light settings are varied by the filament current as e.g. in the Diving-PAM. (Fig. 3, Wien law of spectral shift by different filament temperature). Moreover, energy output of the lamp depends strongly on the battery voltage and is not stable and changes quickly.

The resulting shift in the ratio of blue to red light may have several effects on photosynthesis and could affect photosynthetic performance. For example, photosynthetic capacity of brown algae in red light is increased by an additional blue light pulse due to activation of carbon supply (Schmid and Dring, 1996). Whether the photosynthetic rate, and thus, the course of the P-E curve is affected by alterations in the blue:red light ratio in short term irradiation (by the Emerson enhancement effect) is not clear. However, one should consider that in Fig. 3 the lamp emission within the wavelength range of 400–550 nm changed by about 35%. This affected the quantum yields and quenching parameters of different macroalgae, in an unpredictable manner (data not shown).

Effective quantum yields determined in situ with underwater fluorometers are more realistic than values which were determined only with land based PAM fluorometers (Häder and Figueroa, 1997; Häder et al., 1996, 1997). As normal fluorometers are not watertight, the algal sample is generally removed from its growth site. Even if the period between collection of the alga and measurement is small (< 15 min; Häder and Figueroa, 1997), changes of the light field and temperature will rapidly affect effective

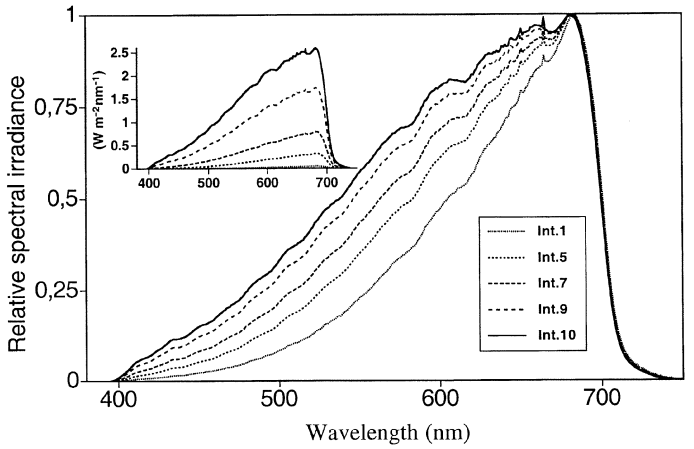

Fig. 3. Relative spectral irradiance of the internal PAM halogen lamp measured at different settings of the instrument. Blue light emission increases significantly with higher lamp current and instrumental setting (Int. 1 –10). These obvious changes of light quality are not visible if absolute irradiance is used as ordinate parameter as shown by the small graphical inset.

quantum yield, photochemical and non-photochemical quenching mechanisms. Therefore, the results obtained with underwater devices better reflect the real photosynthetic performance of algae at their growth site than any land based fluorometer can.

Figure 4 shows the relative electron transport rate (rel. ETR) for the brown alga *Dictyota dichotoma* calculated from in situ measurements in four different depth zones at noon. The curve of rel. ETR versus irradiance gives information similar to the normal photosynthesis versus irradiance curve (P-E curve) measured by oxygen production or CO_2 fixation. The relative electron transport rate increases at higher irradiance, and can never exceed values corresponding to a maximal yield of 0.8 (broken line). Deviation below this line reflects an actual depression of the respective photosynthetic efficiency due to individual light acclimation status. Nevertheless, the means of the measured effective quantum yields at different depths were statistically similar ($p > 0.05$, yield = 0.56). In the brown alga *Halopteris scoparia* growing in Spain on the Atlantic coast several centimeters below the water surface at low tide, the rel. ETR measured at different times depends only on the irradiance as the yield was nearly constant (Table 1). This indicates that photosynthesis of the high light acclimated species was not down regulated and never really saturated.

On Spitsbergen in the Arctic similar fluorescence measurements were made during low tide on sunny days on four brown macroalgae (Table 2). A clear relation between the photosynthetic performance of different thalli, blades or blade-regions and their distance from the water surface was found during low tide in several brown algae. The lowest mean effective quantum yield was measured in thalli or blade-regions close to the surface, and it increased with increasing depth. A blade of *Alaria esculenta* reaching from a depth of 30 cm up to the water surface showed a significant correlation between the quantum yield and its distance from the surface ($r = 0.47$, $n = 68$, $p < 0.0001$). This shows that photosynthesis acclimated to small changes in the ambient micro-light environment within a single organism

A further advantage of in situ fluorescence technique is that an appreciation of the variability of light-adapted status within a population can be measured. An example is shown for specimens of the red alga *Palmaria palmata* growing on Spitsbergen in about 1–2 m depth (Fig. 5). Plants grew in different micro-habitats with different micro-light conditions. Photosynthesis of the individuals was partly light-saturated as well as light-limited. The means show clearly that irradiances above 100 μmol $m^{-2}s^{-1}$ saturate photosynthesis. Under light limitation, the variance of the yield is rather small as photosynthesis is consistently performed almost with the highest efficiency or yield. At saturating irradiances, i.e. at the ETR_{max}-level, the effective quantum yield varies strongly, resulting in a wide variance in rel. ETR. This is again an effect of a different individual light acclimation status produced by fast down regulation of photosynthesis (qE, energy quenching mechanism) and dynamic photoinhibition of photosynthesis when

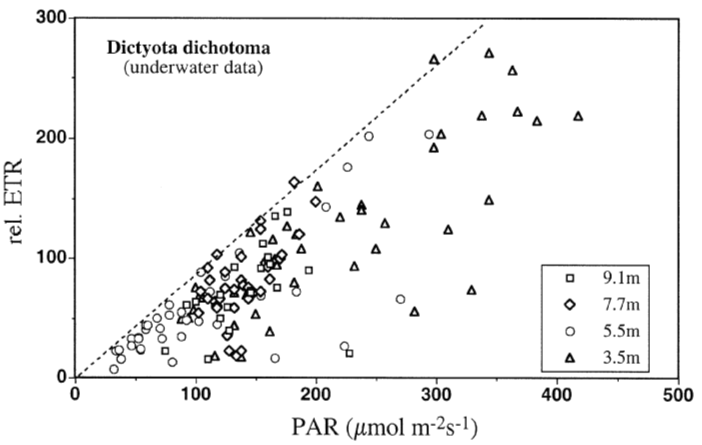

Fig. 4. Relative electron transport rate (ETR) of *Dictyota dichotoma* measured in different depths in the Mediterranean Sea. The broken line presents the maximal measured ETR in all depths and corresponds to an effective yield of 0.8 (after Hanelt and Nultsch, 2003).

Table 1. Differences in different photosynthetic parameters for *Halopteris scoparia* growing on the rocky shore of Punta Carnero (Atlantic coast). Mean values and standard deviations are shown, different letters denote significant differences of means for each parameter (Fishers PLSD).

Date Time	Counts (n)	Mean of yield ±SD (rel. units)	Mean of PAR ±SD (μmol m^{-2} s^{-1})	Mean of rETR ±SD (rel. units)
25.5.1996				
9:25	52	a 0.659 ± 0.08	a 95 ± 76.3	a 63.0 ± 52.9
12:31	53	a 0.651 ± 0.09	b 414 ± 316.3	b 266.6 ± 204.9
16:07	51	b 0.610 ± 0.09	a 54 ± 26.4	a 33.1 ± 17.2
26.5.1996				
12:45	56	a 0.677 ± 0.08	a 768 ± 349.2	a 522.2 ± 246.7
16:41	55	a 0.697 ± 0.06	b 206 ± 323.6	b 147.1 ± 236.8
19:32	57	b 0.627 ± 0.08	c 87 ± 97.5	c 53.8 ± 60.5

Table 2. Differences in different photosynthetic parameters measured in several brown algal species growing on the rocky shore of the Kongsfjord (Spitsbergen). Mean values and standard deviations are shown, different letters denote significant differences of means (Fisher PLSD). Date and time period of measurements are given.

Species / Depth Date / Time		Counts (n)	Mean of yield (rel. units)	Mean of PAR (μmol m^{-2}s^{-1})	Mean of rel. ETR (rel. units)
Alaria	surface	50	a 0.199 ± 0.127	a 205 ± 78.0	a 40.8 ± 29.7
esculenta	0.3 m	60	b 0.287 ± 0.111	b 251 ± 66.3	b 72.1 ± 33.5
15.5. (19–20:00)	0.6 m	54	c 0.449± 0.125	a 224 ± 149.0	c 98.4 ± 75.5
	0.6 m	88	d 0.538 ± 0.124	a , 194 ± 90.4	c 101.6 ± 51.6
Laminaria	0.2 m	27	a 0.430 ± 0.177	a 90 ± 27.9	a,b 36.7 ± 14.0
digitata	0.4 m	21	a 0.432 ± 0.215	a 112 ± 50.4	b 45.3 ± 30.0
11.5. (15–16:00)	1 m	30	b 0.550 ± 0.187	b 64 ± 54.7	a 30.8 ± 21.2
Chorda	0.4 m	88	a 0.289 ± 0.151	a 205 ± 117.9	a 53.2 ± 43.6
tomentosa	0.7 m	79	b 0.447 ± 0.216	b 134 ± 101.4	a 50.3 ± 41.5
26.5. (14–15:20)					
Acrosiphonia	0.3 m	53	a 0.209 ± 0.128	a 240 ± 152.3	a 43.5 ± 40.8
penicilliformis	0.7 m	46	b 0.303± 0.149	b 166 ± 51.7	a 49.5 ± 34.4
20.5. (10–11:30)	1 m	38	c 0.441 ± 0.172	c 59 ± 9.2	b 26 ± 13.5

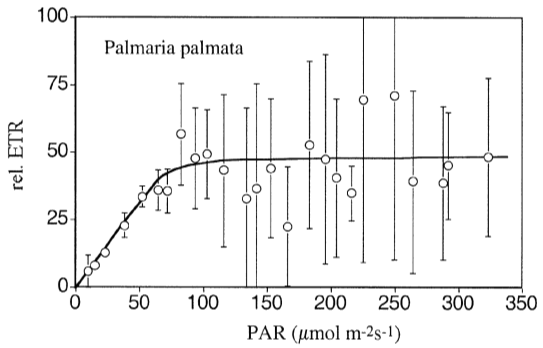

Fig. 5. Rel. ETR of the red alga *Palmaria palmata*. The curves show a non-linear curve fitted to the means of the different ETRs of individuals averaged for an irradiance interval of 10 μmol $m^{-2}s^{-1}$. Specimens were all measured at the same depths, but at different locations.

photosynthesis becomes saturated in situ. It provides strong evidence that the heterogeneity in the field irradiances results in a great variability of the photosynthetic parameters over short time periods.

V. Effects of Excessive Light on Photosynthesis

A. Photosynthesis and Chloroplast Arrangement

A prominent effect of exposure to excessive light is a change in the chloroplast distribution within the cell (Haupt and Scheuerlein, 1990). Chloroplast movement causes a change in the absorption cross section, according to the Lambert-Beer law, by the so called sieve effect (Furshansky, 1981). The higher plant *Tradescantia albiflora* has a greater tolerance of PS II against light stress than pea (*Pisum sativum*) due to its light-induced chloroplast rearrangement and chloroplast movement should be regarded as one of the photoprotective strategies used by this facultative shade plant (Park et al., 1996; Kasahera et al., 2002). Though chloroplast movement is also widespread among algae and was early described in the last century (Senn, 1919) the role of this physiological process is still not well investigated.

The model of Fig. 6 shows a cortex cell with its chloroplasts in the so-called low and in the high intensity arrangement. In the low intensity arrangement most chloroplasts are located adjacent to the periclinal walls, under high light conditions they show arrangement close to the anticlinal walls. These changes result in an increase of the light transmission through the thallus of about 12.5% and an increase in the self shading of the chloroplasts.

The photoreceptor responsible for the arrangement in the brown alga *Dictyota dichotoma* is a cryptochrome (Pfau et al., 1988; Hanelt and Nultsch, 1989). This pigment is adapted to the quality of the underwater light field, absorbing in the blue-green waveband to which the water body exhibits the highest transmittance. Red light, detected by phytochrome, is effective in the Conjugatophyceae *Mougeotia* and *Mesotaenium*, which grow close to the surface in fresh waters. Light induced changes in chloroplast

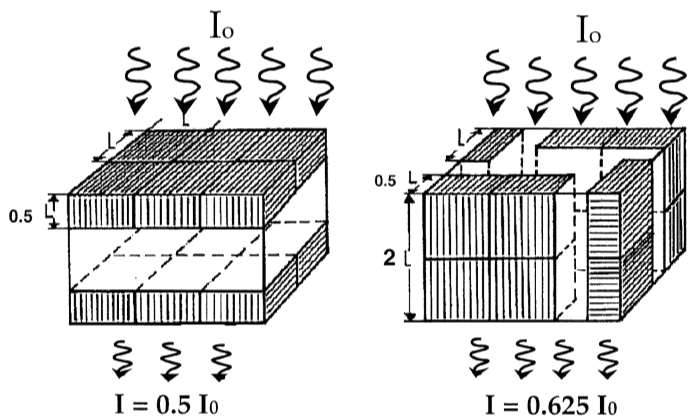

Fig. 6. Schematic drawing of a cortical cell with 6 chloroplasts in the low intensity (left) and in the high intensity arrangement (right). The irradiance (Io) impinging perpendicular onto the cell is attenuated by the chloroplasts with a theoretical size L. In this example the transmitted irradiance (I) increases about 12.5% due to the sieve effect.

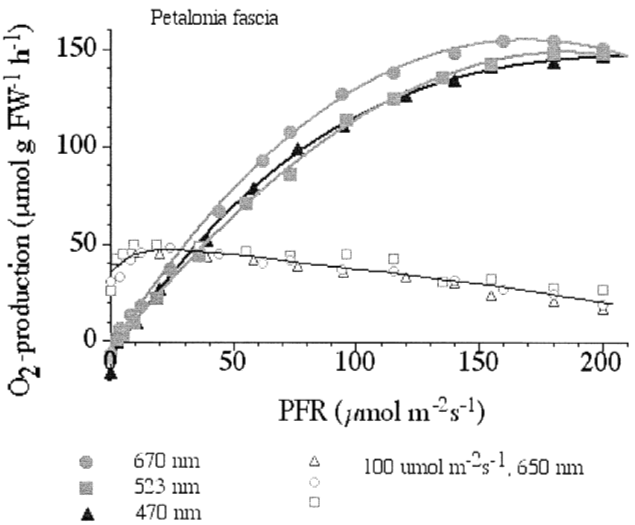

Fig. 7. Photosynthetic oxygen production versus irradiance curves of the brown alga *Petalonia fascia*. Oxygen production was measured at different wavelengths (full symbols). Only blue light can induce the chloroplast movement from the low into the high intensity arrangement and, therefore, is less absorbed in the high intensity arrangement. The open symbols show the oxygen production rate caused by a constant fluence rate measured subsequently after each irradiance applied to calculate the P-E curves. The decrease of this oxygen production rate is caused only by dynamic photoinhibition.

arrangements are widespread in brown marine algae (Nultsch and Pfau, 1979). In several green algae they are controlled mainly by circadian rhythms (Britz, 1979; Britz and Briggs, 1987).

In *Dictyota* daily changes in chloroplast arrangement have been demonstrated to occur under natural conditions in the field (Hanelt and Nultsch, 1990). On sunny days, in specimens exposed to the water surface, the chloroplasts occupy a position with lowest light absorption throughout the day. In depths lower than 4 m, the plastids show the low intensity arrangement even at midday and full sunshine, due to the low underwater irradiance. It has often been postulated that the chloroplast arrangement regulates photosynthetic activity (Zurzycki, 1975) though this is really questionable. Nultsch et al. (1981) did not observe a dependence of the photosynthetic activity on the chloroplast arrangement using polarized light to activate photosynthesis and chloroplast movement. Moreover, the kinetics of the chloroplast movement is relatively slow and photosynthetic activity is down regulated much earlier by dynamic photoinhibition (Hanelt and Nultsch, 1991) as demonstrated in the following example with the brown alga *Petalonia fascia* (Fig. 7).

Photosynthesis versus irradiance curves were recorded using blue (maximum at 470 nm), green (max. 523 nm) and red light (max. 670 nm). Blue light also induces the movement into the high intensity arrangement, whereas in green and red light the chloroplasts remain in the high absorbing low intensity arrangement. The shapes of the P-E curves show no significant differences for each waveband (Fig. 7) demonstrating that photosynthetic activity is independent of the respective chloroplast arrangement. Photosynthetic oxygen production of each sample, measured through the increasing PFR regimes, was also measured at a constant fluence rate (100 µmol m^{-2}s^{-1}, 650 nm) after each irradiance of the P-E curve. This oxygen production would be constant if photosynthetic activity was not regulated in the course of the experiment (see also Hanelt et al., 1992). However, this production rate decreased when the photon fluence rates of the blue, green and red light increased to higher values. The reason is that dynamic photoinhibition down-regulates photosynthetic efficiency even before chloroplast movement starts. Nevertheless, the displacement into the high intensity arrangement decreases the likelihood for a photodamage caused by excessive irradiances. Hanelt and Nultsch (1991) demonstrated that if the movement of the chloroplast is prevented the pigments are destroyed by high light to a greater extent than in a control where plastids are still able to move into the

high intensity arrangement. This effect was protective by the decrease of the absorption cross section. In contrast, pigment damage due to UV radiation is independent of the chloroplast arrangement (Hanelt and Nultsch, 1991), probably because UV is highly scattered within the cell so that a protective sieve effect of the high intensity arrangement is derogated. Therefore, chloroplast arrangements have evolved to prevent photodamage to the photosynthetic apparatus caused by excessive white light and not to regulate its activity.

As the fluorescence technique is more and more used in photosynthetic research it is important to determine if the chloroplast arrangement also changes the fluorescence signal due to the change of the amount of absorbed light. Brugnoli and Björkman (1992) argued that effects of high light induced chloroplast movements on fluorescence emission could be erroneously interpreted as quenching of F_o, F_m and F_v, and thus, would result in overestimation of the non-photochemical quenching coefficient. To verify this, we integrated the fiber optics of a PAM fluorometer into the optical pathway of a two beam microphotometer, developed to measure automatically the chloroplast arrangement (Hanelt and Nultsch, 1989). Blue light (120 μmol $m^{-2}s^{-1}$, 450 nm) was used to induce the chloroplast movement, determined by the microphotometer, and the fluorescence emission was detected by the PAM-fluorometer (Fig. 8).

The fluorescence parameter F_o does not significantly decrease in relation to the decrease of the absorption cross section. Though F_m decreases to a small extent, it is more probably an effect of the non-photochemical quenching parameter (qE). Most importantly, however, is that there was no significant change of the optimal quantum yield (F_v/F_m) (as also found by Brugnoli and Björkman, 1992). Not only was photosynthetic activity unaffected by the chloroplast movement, but also the quantum yields calculated by the fluorescence emission of the chlorophylls remained the same. This is an additional reason why PAM-fluorometry is well suited to measure photosynthetic performance of macroalgae.

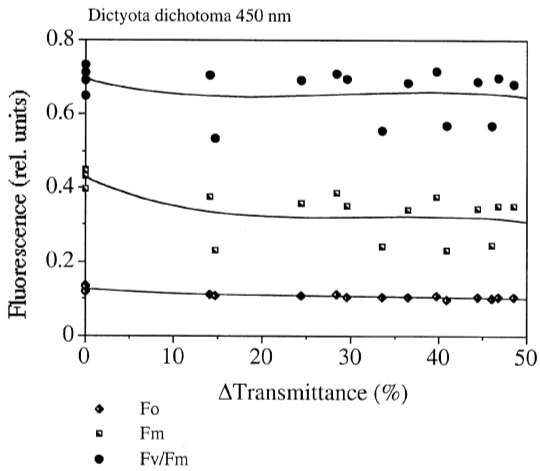

Fig. 8. In vivo PS II chlorophyll fluorescence in relation to the chloroplast arrangement of *Dictyota dichotoma*. The chloroplast arrangement was determined by the light transmittance through an algal thallus (abscissa) using a two beam microphotometer. The change of the absorption cross section did not induce significant effects on the fluorescence emission of this species (ordinate).

B. Changes of Photosynthetic Performance During the Day

Photosynthetic activity of marine macroalgae, growing in the intertidal and upper subtidal zone, is often depressed on sunny days in a typically diurnal pattern. Strongest depression, i.e. the highest degree of dynamic photoinhibition, occurs mostly in the early afternoon (Huppertz et al., 1990; Henley et al., 1991, 1992; Hanelt et al., 1993). Full recovery of photosynthesis is often reached at the latest in the evening in species capable of dynamic photoinhibition. Thus, photosynthetic oxygen production measured at non-saturating fluence rates and the optimal quantum yield (F_v/F_m) show an approximately inverse course in comparison to the fluence rate of daylight measured continuously during the day (Hanelt, 1992; Hanelt et al., 1993). A relation between the formation of zeaxanthin and a decrease in oxygen production and optimal quantum yield in macroalgae was also demonstrated (Franklin et al., 1992; Uhrmacher et al., 1995; Schofield et al., 1998). Most studies about photoinhibition have been performed so far with respect to photosynthetically active radiation (PAR). The early work of Larkum and Wood (1993) indicated that an increase of natural UV-radiation could cause effects similar to strong PAR. UV radiation depresses photosynthetic performance most probably by damaging the oxidizing site and the reaction center of Photosystem II (Holm-Hansen et al., 1993b; Chapter 16, Franklin et al.). It can be envisaged that an increase in the UV-component of the light impinging on cells will increase their susceptibility to photodamage, and, hence, affect the kinetics of the recovery processes

after photoinhibition in the daily cycle (Hanelt et al., 1997a; Bischof et al. 2000). Moreover, preliminary work also indicates an effect of UV radiation on N-uptake in macroalgae (Döhler et al., 1995), a result that suggests that further investigations are necessary.

The protection strategy of the plants under high light and UV stress mostly comprises dynamic photoinhibition and formation of screening pigments. Dynamic photoinhibition of photosynthesis is newly defined as a regulative protection mechanism against excessive radiation (Krause and Weis, 1991; Osmond, 1994). Excess energy, absorbed by Photosystem II, is dissipated by thermal radiation thereby avoiding photodamage. This process leads both to lowered photosynthetic capacity and quantum efficiency but is readily reversible, whereas photodamage (chronic photoinhibition) is not. In addition, it may be modulated by an increase in the zeaxanthin content of the PS II antenna (Adams III and Demmig-Adams, 1992) and/or by increasing the amount of inactive PS II centers, which are able to protect photosynthetically active centers (Öquist and Chow, 1992). All of these processes are partly based on enzymatic reactions, and are therefore also directly affected by the surrounding temperature.

C. Photoinhibition Induced by Photosynthetically Active Radiation (PAR) or UV-Radiation

Reduced photosynthetic capacity has been observed in plants exposed to high PAR, occurring as soon as light exposure exceeds the demands of photosynthesis; originally, this effect was termed photoinhibition (Powles, 1984; Chapters 13 (Larkum) and 16 (Franklin et al.)). In the field, high irradiances of PAR are generally accompanied by higher UV-radiation. Although the measurable effects of both wavebands, such as e.g. reduced photosynthetic efficiency, are similar, the mechanisms behind PAR- and UVR-induced inhibition of photosynthesis are very different, therefore both events should be carefully distinguished (Neale et al., 1993). Exposure to high irradiances of PAR can exceed the assimilatory capacity of the Calvin cycle (Ruban and Horton, 1994), as often occurs in macroalgae growing in the intertidal and upper subtidal zone at low tide during sunny days. Then, excessively absorbed energy may result in damage to the photosynthetic apparatus. However, dynamic photoinhibition (Chapter 16, Franklin et al.) enables the seaweeds to recover rapidly (within several minutes) after the offset of stressful conditions, e.g. in the afternoon or during rising tide (Hanelt et al., 1994). In contrast, during chronic photoinhibition, photosynthetic capacity is mainly affected by the impairment of the D_1 protein (Anderson et al., 1992). Due to the required *de novo* synthesis and the replacement of damaged D_1 protein in the thylakoid membrane, this effect is only reversible on a longer time scale (several hours) (Mattoo et al., 1984), observable by incomplete recovery before sun set. Chronic photoinhibition mainly occurs in seaweeds growing in the lower subtidal zone when exposed to high irradiances (Hanelt, 1998). These specimens have a lower ability to down regulate photosynthesis through the protective dynamic photoinhibition process.

In contrast to PAR, UVR cannot be regarded as being an 'excessive energy input' in a proper sense. The maximal irradiance is much smaller than of PAR (e.g. for the relations in an Arctic fjord see Hanelt et al., 2000) and the UV wavebands do not contribute substantially to energy supply for photosynthetic chemistry. As pointed out in Chapter 16 (Franklin et al.), UV exhibits adverse effects on photosynthesis in a more direct way, such as its absorption by aromatic and sulfhydryl-containing biomolecules, causing a direct molecular damage (Vass, 1997). Within the PAR range, the action spectrum of photoinhibition of macroalgae runs in parallel with the action spectrum of photosynthesis (Nultsch et al., 1987; Hanelt et al., 1995), and therefore, is directly related to photosynthetic pigment absorption. Thus UVB induced inhibition correlates much more to the absorption by DNA and proteins (Jones and Kok, 1966; Setlow, 1974).

Two aspects have received little attention so far. In many polar species growth rates are highest in Spring (Wiencke, 1990a,b), which are also affected by UVR, and not only photosynthesis of young thallus parts (Dring et al., 1996; Wood, 1987). Moreover, some species from the Arctic partly reproduce in spring. During this time, algal spores were found to be the most light-sensitive life history stage of the studied brown algae and are strongly affected by increased UVB radiation, both in respect to their photosynthetic performance and their susceptibility to DNA damage (Wiencke et al., 2000). As has been widely publicized, increased UVB due to ozone depletion occurs mainly in the polar spring due to atmospherical and geographical reasons. Therefore, polar species will be most exposed to the anthropogenic UVB increase.

VI. Algal Photosynthesis Under Low Light Conditions

Whereas photosynthesis of algae growing in the eulittoral must be adapted to strong light conditions, especially during low tide on sunny days, sublittoral species growing in deep water must be adapted to chronic low light. For planktonic algae, generally a lower depth limit of 1% of the surface light is regarded as defining the euphotic zone (Steemann Nielsen, 1975). Brown kelps reach their lower growth limit at about 0.6–1.2% of surface light, whereas for the deep-grown red macroalgae a minimum of 0.001–0.05% was determined (Lüning, 1981, 1985, 1990). Deepest crustose macroalgae seem to survive at an absolute light minimum of about 0.01 μmol $m^{-2}s^{-1}$ (Littler et al., 1986). Low light adapted species are typically characterized by high photosynthetic efficiencies and very low light compensation (E_c) and saturation points (E_k), ranging between 1 and 15 and between 14 and 52 μmol photons m^{-2} s^{-1}, respectively (Dunton and Jodwalis, 1988; Wiencke et al., 1993; Weykam et al., 1996; Brouwer, 1996; Eggert and Wiencke 2000).

Low light requirements of photosynthesis allow sufficient production during a long time of the day. Using data on daily changes of in situ irradiance and P-E derived parameters such as P_{max}, dark respiration, E_c and E_k, it is possible to calculate the daily periods over which C assimilation exceeds C losses due to respiratory activity at different water depths. This metabolic C balance is thought to determine the lower distribution limit of algae. Due to low E_k values, species from King George Island (Antarctica) are exposed during spring-summer for periods ≥12 h per day at ≤20 m depths to irradiances above saturation (period of saturation H_{sat}). Under these conditions, species such as *Palmaria decipiens* or *Desmarestia anceps* achieve positive C balances close to 3 mg C g^{-1} FW d^{-1}. At 30 m depth daily light availability decreases to values close to 7 or 9 h, but C balance is still positive in four of five studied species (Gómez et al., 1997). Only in the case of *Desmarestia anceps* from 30 m, a negative C balance was determined, indicating that at this depth the alga is at its lower distribution limit. In contrast, *P. decipiens, Gigartina skottsbergii* and *Kallymenia antarctica* and especially *Himantothallus grandifolius* are metabolically able to grow in even deeper waters.

The stable carbon isotope composition of macroalgae is an ideal marker for their depth distribution. Rapid carbon assimilation under high photon fluence rates leads to ^{13}C enrichments, probably due to extracellular and/or intracellular isotopic disequilibria resulting in a trend towards more positive carbon isotope values with increasing photon fluence rates (Wiencke and Fischer, 1990). Isotopic analyses of sediment trap samples from the 2000 m deep King George Basin revealed a strong contribution of seaweeds to the total organic carbon pool of the deeper basin waters in spring and summer (Fischer and Wiencke, 1992).

In conclusion, distribution of marine macrophytes depends not only on the lower light limit which accommodates biomass production to the minimal energy input (Kirst and Wiencke, 1995) but also on the discussed tolerance to the high light conditions close to the water surface (Hanelt, 1998). Changes in the water transparency can shift both light limits so that the algal distribution pattern is affected by seasonal changes of the light transmittance.

VII. Seasonal Photosynthetic Performance of Macroalgae

The seasonal photosynthetic performance of macroalgae is very strongly related to the life strategy of the individual species. In species with an opportunistic life strategy, the so-called season responders (Kain, 1989), photosynthesis is more directly related to the actual combination of abiotic factors, especially light conditions, temperature and nutrient concentrations. In *Ulva lactuca* for example, photosynthetic oxygen production is highest in summer during the period of optimum growth (Fillit, 1995). Results become difficult to interpret, when the photosynthetic measurements are performed at the same experimental temperature throughout the year although the temperature in the habitat is varying seasonally (Israel et al., 1995; Vergara et al., 1997). In the Antarctic, which is characterized by almost constant low temperatures and high nutrient concentrations (Drew and Hastings, 1992), photosynthesis of season responders depends on the light conditions only. The red alga *Iridaea cordata*, for example, shows a relatively constant photosynthetic activity throughout the year, even when the alga is cultivated for 6 months in darkness and is exposed to light only during photosynthetic experiments (Weykam et al, 1997). This species is therefore able to survive long dark periods in regions with

unpredictable light conditions such as in the Ross Sea. In this area, the sea is covered by ice for about ten months each year, but occasionally light can penetrate the ice through cracks and polynyas.

In the so-called season anticipators, which grow and reproduce in a strategic annual rhythm in response to a trigger, e. g. daylength (Kain, 1989), seasonal optima of photosynthetic capacities are mostly recorded in late winter to early summer. A baseline study is the work by Drew (1977a) on the brown algae *Laminaria digitata*, *L. hyperborea* and *L. saccharina* from Scotland. He measured seasonal photosynthetic performance in the laboratory at 10 °C and found a peak of photosynthetic capacity in spring in all three species. After adjustment of the metabolic rates to habitat temperatures the seasonal maximum is shifted to summer. As the spring peak coincides with nutrient regeneration and its subsequent depletion a relation is obvious. However, such spring peaks also occur in season anticipators from the Antarctic region, where nutrient depletion has never been determined in coastal regions and temperature is almost constant. In situ photosynthetic rates of the endemic Antarctic brown alga *Himantothallus grandifolius* increase, like growth rates, before sea ice breaks up and show their annual optimum soon after that (Drew and Hastings, 1992). The same results have been obtained in the brown algae *Ascoseira mirabilis* and *Desmarestia menziesii* from Antarctica (Gómez et al., 1995b; Gómez and Wiencke, 1997). In both species growth in late winter is presumably supported by the storage carbohydrate laminaran (Gómez et al, 1998a,b). The low molecular weight compound, mannitol, is presumably used as substrate for respiration during the period of active growth. In the distal region in *A. mirabilis* it may serve as substrate for light independent carbon fixation or is stored as laminaran, which attains it highest content here (Gómez and Wiencke, 1998).

In the season anticipator *Palmaria decipiens*, one of the most common Antarctic red algae, P_{max} and α exhibit strong seasonal optima under spring conditions (Weykam and Wiencke, 1996; Lüder et al., 2001a). Concomitantly, concentrations of Chl *a*, allophycocyanin, phycoerythrin and phycocyanin are highest. When thalli of this species are kept for six months over the winter in darkness (Weykam et al., 1997; Lüder et al. 2001b) the alga looses its ability to photosynthesize, primarily due to a decrease in the content of all three phycobilins. The content of floridean starch decreases gradually in the dark and drops suddenly when new blades are formed in August (Weykam et al., 1997). After reexposure to light, photosynthetic activity and phycobiliprotein levels increased within two weeks to normal values. The strategy of *P. decipiens* is therefore strongly synchronized to the periodic changes of the light conditions and differs considerably from that of the season responder *Iridaea cordata* (see above).

VIII. Adaptation and Acclimation of Photosynthesis and Respiration to Temperature and Salinity

A. Photosynthesis

Like most metabolic processes, photosynthesis is dependent on temperature. Macroalgae are able to adapt and to acclimate to different temperatures to a different degree. Temperature adaptation is the result of evolutionary processes over long time periods, short-term acclimation is achieved within days or weeks (Berry and Björkman, 1980; Davison, 1991; Chapter 17, Raven and Geider).

Comparing the photosynthetic activities of species from the various phytogeographic regions a considerable adaptation to the different temperature regimes is obvious. This applies especially to warm temperate and tropical species, which exhibit maximum rates of photosynthetic capacity between 25 and 35 °C (Lüning, 1990). These optima are in the same range as temperature optima for growth of species from these regions (Bischoff-Bäsmann et al., 1997; Pakker and Breeman, 1996; Pakker et al., 1995, 1996) reflecting the long exposure times to high temperatures since Mesozoic times. In comparison, photosynthesis of species from the Polar regions is less strongly adapted to the ambient temperature regime. In species from the Arctic, where water temperature rarely exceed 5 °C, optima for photosynthetic capacity are located at around 20 °C (Healey, 1972) and highest growth rates are achieved at temperatures between 10 and 15 °C (Wiencke et al., 1994). In stenothermal species from the Antarctic, the temperature optima for photosynthesis range between 5 and 15 °C (Drew, 1977b; Wiencke et al., 1993; Eggert and Wiencke 2000), values well above the temperature optima for growth which are located at temperatures below 5 or 10 °C (Wiencke et al., 1994; Bischoff-Bäsmann and Wiencke, 1996). These differences in temperature requirements may reflect

the different exposure times of Arctic and Antarctic species to low temperatures in the geological past, 2 million years in the Arctic and 14 million years in the Antarctic (Wiencke et al., 1994). On the other hand these species seem to be well enough equipped to live at the low temperatures as P_{max} values of endemic Antarctic species measured at 0 °C are similar to data from temperate algae measured at higher temperatures (Drew, 1977b; Thomas and Wiencke, 1991; Wiencke et al., 1993; Dunton and Jodwalis, 1988, Weykam et al, 1996; Eggert and Wiencke 2000).

Short-term temperature acclimation of photosynthesis serves to optimize photosynthesis in species exposed in their habitat to temperature fluctuations. Acclimation can involve a shift in temperature optima as has been demonstrated in *Laminaria saccharina* (Davison, 1987). However, this is not the case in four red algal species from the Antarctic (Eggert and Wiencke 2000). For only two species an acclimation potential to low temperatures was demonstrated. *Ballia callitricha* acclimates to 0 °C since individuals grown at 0 °C overall attain higher photosynthetic capacities compared to individuals grown at 5 °C. Similarly, *Gymnogongrus antarcticus* is able to acclimate in the same way to 0 and 5 °C.

Low temperature acclimation of photosynthesis requires a compensation for the lowered activity of the photosynthetic enzyme systems. This may be achieved either by an increase in the concentration of enzymes or by production of isoenzymes with modified properties. Elevated concentrations of ribulose bisphospate carboxylase/oxygenase (Rubisco) were determined in the brown alga *Laminaria saccharina* at a low compared to a high temperature (Machalek et al., 1996). In the same species an increase in the activity of this and other enzymes of the Calvin-Benson cycle has been demonstrated at low temperature (Davison, 1987; Davison and Davison, 1987). Moreover, individuals grown at lower temperatures may exhibit lower Q_{10} values resulting in a reduced sensitivity to temperature changes (Davison, 1987).

Photosynthetic efficiencies (α-value) in *Laminaria saccharina* are higher in individuals grown at high temperatures. This correlates with increased light absorption due to increased pigment content, PS II reaction center densities and the content of fucoxanthin-Chl a/c protein complex (FCP; Davison et al., 1991; Machalek et al., 1996). Similarly, in the red alga *Chondrus crispus* α-values and concentrations of Chl *a* and total phycobilins are positively correlated with temperature (Kübler and Davison, 1995). So it is obvious that there is a general similarity between acclimation of photosynthesis to high temperatures and to low light (Gerard, 1988; Greene and Gerard, 1990), a phenomenon found also in microalgae (Maxwell et al., 1994).

B. Respiration

Like photosynthesis, respiration can acclimate to temperature. Dark respiration rates are lower at all experimental temperatures in individuals grown at a higher temperature compared to individuals grown at a lower temperature (Davison et al., 1991; Eggert and Wiencke 2000). Overall, photosynthetic capacity, respiration, light compensation point (E_c), light saturation point (E_k) increase in saturating light with increasing experimental temperatures irrespective of the growth temperature. Individuals acclimated to a lower temperature attain higher values of photosynthetic capacity, respiration, E_c and E_k compared to individuals acclimated to a higher temperature. In the best case, the same values are attained at the respective growth temperatures. For a deeper insight in the area the review by Davison (1991) is pertinent.

C. Salinity

Supra- and eulittoral species are not only exposed to different temperatures and light conditions, but also frequently to air and low or high salinities due to the tidal regime and the weather conditions. In closed rock pools during low tide or in uncovered algae, drying up, the salinity increases, whereas during rainfall and, especially, in polar regions by melting of snow and ice the salinity can even decrease (Hanelt et al., 2000). Species from the higher shore generally exhibit an increased ability to tolerate immersed conditions and changing salinities. Some species take even advantage of e.g. emersion and exhibit higher photosynthetic rates during exposure to air compared to submerged conditions. A large number of investigations have been published in this field and for more information the interested reader is referred to the reviews by Kirst (1990), Kirst and Wiencke (1995) and Davison and Pearson (1996).

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Chapter 19

Photosynthesis in Symbiotic Algae

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Summary

Symbiosis is an evolutionary strategy that often confers an ecological advantage on the partners. Algae exist in symbiosis with a number of hosts and in a range of different anatomical relationships including exosymbiosis (lichens) and endosymbiosis (corals). Each of these imposes on the alga a chemical environment significantly different from that of free-living algae and this impacts on algal photosynthesis. This chapter examines how symbiosis influences the acquisition of inorganic carbon by the alga, its subsequent photosynthetic fixation and translocation back to the host. Inorganic carbon acquisition in symbiotic associations is invariably more complex than in free-living algae as it often is acquired through the host tissue which is different in nutrient composition and concentration, as well as pH, from the surrounding aquatic environment. In many instances the algae posses carbon-concentrating mechanisms to ensure the CO_2 concentration is adequate for productive carbon fixation by Rubisco. For the majority of algal symbionts photosynthetic carbon fixation is the major contribution algae bring to symbiosis. The translocated photosynthate provides much of the carbohydrate

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required for respiratory energy generation in the host. Evidence suggests that the host influences carbon fixation in the symbiotic algae to that effect. In symbioses between dinoflagellates and invertebrates, the secretion of photosynthate form the symbiotic algae to the host is believed to be under the control of a 'host factor.' However, the chemical nature of this factor has not yet been established. This chapter also examines the possible link between thermal/light stresses on the algal photosynthetic apparatus in the dinoflagellate-coral symbioses. There is compelling evidence that this initiates coral bleaching; the breakdown of the symbiotic association resulting in the expulsion of the algae from the symbiosis. The mechanism by which thermal/light stress triggers this process is thought to occur is either through the inactivation of a key component in the carbon fixation process (e.g. Rubisco or the carbon-concentrating mechanism, or by damage to the Photosystem II reaction center, probably the D1 protein.

I. Introduction

Symbiosis is the term coined by de Bary in the 19th century to describe associations where two (or more) organisms live together. This is interpreted to embrace those associations where the relationship is mutualistic, but not parasitic, indicating that both partners derive apparent benefit from the association. Douglas (1994) suggested a more restricted definition in which the main determinant was that (at least) one of the partners acquired a new metabolic capacity. There is a large spectrum of symbioses, many of which involve algal symbionts, and some of which are significant components of major ecosystems. Two such examples are tundra and coral reefs. Lichens constitute the dominant vegetation in large parts of the tundra. These associations comprise ascomycete or basidiomycete fungi and algae or, to a lesser extent, Cyanobacteria. Coral reefs thrive on photosynthetic carbon fixation by symbiotic dinoflagellates living within corals and other invertebrates such as clams, foraminifera and anemones. These provide the framework for the generation and maintenance of coral reefs. Indeed carbon fixation by symbiotic dinoflagellates on the coral reefs of the Red Sea is estimated to be an order of magnitude greater than free-living phytoplankton (Muscatine, 1980). The majority of symbioses are however cryptic and poorly documented, particularly their photosynthetic properties.

Little is known of the physiological and molecular relationships that exist between host and algal symbiont in the majority of these associations. At present detailed understanding of algal symbioses at the molecular level is restricted to just a few, primarily lichen, dinoflagellate-invertebrate and *Chlorella*-hydra associations. The former is a terrestrial exosymbiosis while the others are both endosymbioses where the alga lives within the body mass of the host.

The formation and maintenance of symbioses is invariably considered in terms of what the symbiont can deliver to the host. In most of these associations it is the increased availability of nutrients that is paramount; *Rhizobium* fixes nitrogen, mycorrhiza provide phosphate and in algal associations it is the photosynthetic fixation of inorganic carbon and the resultant export of photosynthate to the host. However the advantage to the symbiont is more problematic and in algal symbioses is usually explained in terms of a stable and defined environment for the symbiont.

Previous chapters have described in detail the physiology, biochemistry and molecular biology of algal photosynthesis. This chapter is restricted to those aspects of algal photosynthesis and its related processes that are specific to algal symbiotic associations. The primary emphasis will be put on the dinoflagellate-invertebrate symbioses, which are currently of major biological, environmental and political interest because of the impact of global warming on coral reefs (Hoegh-Guldberg, 1999).

A number of organisms harbor dinoflagellate symbionts but the archetype is the hermatypic coral. These and other marine invertebrates, most of which occur in tropical waters, harbor dinoflagellates and many of these associations provide the biological

Abbreviations: ACP – Chl *a*-Chl c_2-peridinin-protein; CA – carbonic anhydrase; CCM – CO_2-concentrating mechanism; C_i – inorganic carbon; [C_i] – inorganic carbon concentration; D1 – 32 kDa Photosystem II reaction center protein; dPAR – photosynthetically active radiation; FRR – fast repetition rate fluorometry; LHC – light harvesting complex; PAM – pulse-amplitude modulation fluorometry; PCP – peridinin-Chl *a* protein; PS II – Photosystem II; PSU – photosynthetic unit; Rubisco – ribulose bisphosphate carboxylase/oxygenase; S_{rel} – relative specificity factor for Rubisco pertaining to the carboxylase to oxygenase reaction kinetics $(V_c*K_o)/(K_c*V_o)$; UVR – ultraviolet radiation; Z-tubules – zooxanthellae tubules in which zooxanthellae reside

driving force for coral reefs. These centers of biodiversity are in decline because of the ravages of pollution, over-fishing and global warming. The latter is directly correlated to the increased incidence of 'coral bleaching' (Hoegh-Guldberg, 1999) and has been a driving force for fundamental research into these organisms in an effort to better understand the relationship and the role that photosynthesis plays in the symbiosis.

II. Algal Symbiotic Associations

The impetus for symbiotic associations comes from the ability of one partner to provide a metabolic capacity that the other lacks thus making the association more productive. In the case of algal symbiotic associations, photosynthesis adds the capability for autotrophic fixation of inorganic carbon to the association and the subsequent export of photosynthate provides a major nutritional source for the host. Algal assimilation of ammonium and nitrate and in some instances nitrogen fixation can also add autotrophic capacity to the association.

There is a large diversity of algal symbiotic associations (Reisser, 1992). Many of these symbioses are between unicellular algae and invertebrates although other host phyla such as fungi (lichens) do form symbioses. In the majority of these associations little has been documented apart from a description of the anatomical relationship between host and alga. While with many associations the governing principles of the relationship are similar, each is unique and comparisons between associations at a more detailed level should be avoided. For instance dinoflagellates participate in a large number of symbiotic associations with marine invertebrates. While most, but by no means all of these algal symbionts, are dinoflagellates of the genus *Symbiodinium* it is clear that these algae are taxonomically diverse. There appears to be no correlation between *Symbiodinium* strain and host taxa (Trench, 1993). Phylogenetically distantly related hosts (e.g. corals and clams) can contain strains of *Symbiodinium* that are closely related, if not identical, while closely related hosts may harbor distantly related *Symbiodinium* strains. At the same time some hosts may contain several genotypically distinct *Symbiodinium* (Carlos et al., 2000, van Oppen et al., 2001) and juvenile tridacnids have the capacity to modify the resident population of strains within their tissue (Belda-Baillie et al., 1999). This phylogenetic diversity in host and symbiont undoubtedly confers differences in the metabolic relationship between the partners.

In all algal symbioses two questions come to the forefront: (1) what do the algae provide to the host and (2) what do they get in return? Both directly relate to algal photosynthesis and revolve around carbon acquisition, photosynthetic carbon fixation and the secretion of photosynthate to the host. However before dealing with these it is necessary to consider the metabolic relationship in terms of the host algal interface.

III. The Host-Algal Interface

The interface between host and alga is critical to the metabolic relationship between symbiotic partners. Yet this is probably the least understood aspect of algal symbioses. With lichens the algae are exosymbionts and the host fungi do not envelope the symbiont. However in all endosymbiotic associations the alga is completely enclosed either within host cells or within host tissues.

In the majority of invertebrate-algal symbioses the association originates from ingestion of the alga by the host or, in some instances, the maternal inheritance of the algal symbiont (Trench, 1987). Algal symbionts in these associations are therefore associated with the digestive system of their host and preexisting transport pathways within the host membrane may facilitate metabolite transport in both directions. This impacts on the acquisition of inorganic carbon for photosynthesis and the translocation of the resulting products. In the case of intracellular symbionts such as *Chlorella* in hydra and *Symbiodinium* in corals and sea anemones, the alga is enclosed within the perialgal vacuole (Fig. 1), which is of host origin. All nutrients that are required by the alga have to be transported across the perialgal vacuole membrane, as do all algal photosynthetic products provided to the host. Neither the transport systems nor the chemical environment within the vacuole have been characterized to any degree. In tridacnids *Symbiodinium* is located within tubes diverging from a diverticulum of the host stomach (Norton et al., 1992).

It has been suggested that the vacuolar pH within the perialgal vacuole housing *Chlorella* symbionts is acidic (Mews, 1980). *Chlorella* is known to

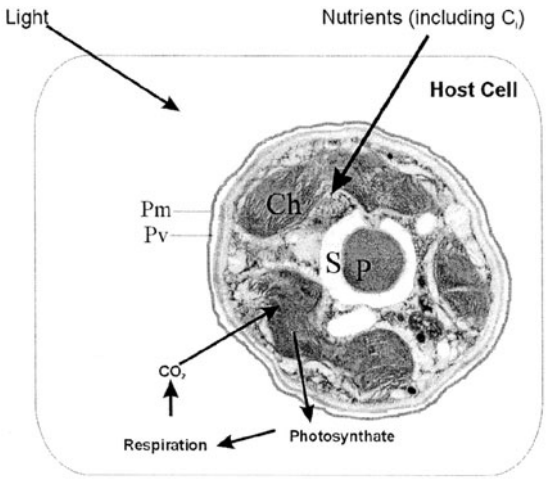

Fig. 1. Anthozoan endoderm cell with intracellular dinoflagellate symbiont: *Symbiodinium* cell within the perialgal vacuole (Pv) which is bounded by the perialgal membrane (Pm). Carbon fixed within the chloroplast (Ch) is exported to the host cell and the CO_2 generated by respiration is recycled to the algal symbiont. Nutrients required by the symbiont must pass through the host cell before acquisition by the dinoflagellate symbiont. A pyrenoid (P) surrounded by a starch sheath (S) are found within *Symbiodinium* cells

translocate carbohydrate to its host (*Hydra viridis* and *Paramecium bursaria* among others) and the only factor known to stimulate release is low pH (Cernichiari et al., 1969). Indeed in the case of the *Spongilla fluviatis* and *Acanthocystis turfaceae* symbioses, translocation is postulated to be through a pH dependent active transport against a concentration gradient (Fisher et al., 1989; Matzke et al., 1990). Experimental evidence supporting this is, however, ambiguous. Immunocytochemical analysis following exposure of the hydra to a weak base was not consistent with a low pH inside the vacuole (Rands et al., 1992) while results obtained with the carboxylic ionophore monensin are consistent with a proton pump in the vacuolar membrane (Schüßler and Schnepf, 1992). Further analysis using pH sensitive fluorochromes is required to resolve this issue as the proposed low pH not only impacts on carbohydrate translocation but will also have a major influence on the species of inorganic carbon available for photosynthesis.

The *Symbiodinium* perialgal vacuole has not been examined to the same extent. In *Anemonia viridis*, enzyme cytochemistry shows that the vacuolar membrane possesses both phosphatase and ATPase activity but their function is not clear (Rands et al., 1993). Also, this organism does not appear to possess an acidic vacuolar space.

IV. Carbon Acquisition, Fixation and Secretion

In the majority of symbioses, translocation of photosynthetically fixed carbon is the primary benefit the algal symbiont brings to a host. Indeed in some instances (e.g. hermatypic corals) the algal symbionts translocate more than 90% of their fixed carbon to the host, contributing significantly to the respiratory carbohydrate required by its partner (Falkowski et al., 1984, Muscatine et al., 1981). These measurements are based on both O_2 flux data and the use of ^{14}C-labeled bicarbonate in intact symbioses.

Given that most algal symbionts are either endosymbiotic or at least enclosed by the host it might be concluded that photosynthetic productivity is reduced compared to their free-living counterparts. This question was addressed by Raven (1992) who examined the net photosynthetic rates of symbiotic assemblages and equivalent free-living algae on a substrate area basis. He concluded that lichens, despite their low growth rate, were equally as productive as mats of terrestrial microalgae, whereas corals exhibited substantially greater net photosynthesis than both mat-forming algae and erect macroalgae and seagrasses. One can conclude from this that both the physical conditions and biological environment combine to ensure high levels of productivity by the algal symbionts.

Some symbiotic algae do however exhibit low photosynthetic rates. For instance, many hosts (in particular plants e.g. *Azolla*, *Gunnera* and cycads) living in symbiosis with cyanobacterial symbionts (often *Nostoc*) that fix nitrogen have low photosynthetic rates indeed sometimes undetectable. This has been taken to indicate that their main contribution is nitrogen fixation and that they may be dependent on the host for organic carbon and energy requirements. The nitrogen is invariably released as ammonium and the algal symbiont has low capacity for ammonium assimilation.

There is nothing unique about the mechanism of photosynthetic carbon fixation in algal symbionts but there are important physiological constraints imposed on the alga by the host. While exosymbiosis allows the alga to directly exchange nutrients with the environment, endosymbiosis confers on the host

effective control of the environment surrounding the alga and therefore the supply of essential nutrients that can influence photosynthesis. Endosymbiosis also offers significant potential for intervention by the host in algal metabolism. In this section we discuss the supply of inorganic carbon (C_i) to the alga, carbon fixation and the influence of nitrogen supply on photosynthetic outcomes, and the translocation of photosynthate to the host and its control.

Many of these experiments have been conducted with radioactive tracers, particularly ^{14}carbon-labeled compounds. Their use has enabled the uptake of inorganic carbon, its photosynthetic fixation and secretion to be followed in cultured symbiotic algal populations, algae freshly isolated from the host or in the intact symbiosis. All three experimental designs have their disadvantages. Cultured algae (if available) and those freshly isolated from the host do not equate to the natural symbiotic environment. On the other hand, experiments using the intact symbiosis are complex and identification of the source (host or symbiont) of labeled product and determination whether the host or symbiont is responsible for synthesis can be difficult to establish.

A. Inorganic Carbon Acquisition

Endosymbiotic associations reliant on photosynthesis derive their inorganic carbon from two sources, respired CO_2 from the host and inorganic carbon from the surrounding marine or freshwater environment. Phototrophic assimilation of respired CO_2 can not provide net growth to the host as it merely recycles carbon. However acquisition of inorganic carbon external to the host and its subsequent photosynthetic fixation result in net CO_2 fixation by the symbiotic association. This requires transport of inorganic carbon into the algal cell and implies the host tacitly or actively transports inorganic carbon to the endosymbiont. In some symbioses this strategy appears diametrically opposed to that of non-symbiotic relatives. Most animals including invertebrates have mechanisms to remove CO_2 from their tissues rather than acquiring it. However the flux in algal symbiotic associations results in the same outcome except that the alga is the sink for C_i and the source is the host and its external environment.

With free-living algae the concentration of the external pool of inorganic carbon and the influence of pH on its molecular form (CO_2, HCO_3^- or CO_3^{2-}) are important factors in the uptake of inorganic carbon. In aquatic algal symbioses the process is more complex since the external pool of C_i has first to pass through the host and consequently the host [C_i] and pH are significant factors in the supply of inorganic carbon to algal symbionts. Chapter 11 (Raven and Beardall) has dealt with C_i acquisition in free-living algae. There is no evidence that C_i acquisition in algal symbionts is any different; however, it is clear that in some cases the host may also play an active role in the supply of C_i to the symbiont.

Most studies on C_i acquisition have been conducted on *Symbiodinium* symbioses, in particular anthozoans and tridacnids. Both are endosymbioses but differ markedly in that the algal symbionts in corals and anemones are intracellular while in clams they are extracellular (Norton et al., 1992). In addition the dinoflagellate symbionts in anthozoan polyps are found in the endodermal layer that is in direct contact with the small pool of seawater in the coelenteron (Fig. 2), while the clam symbionts are enclosed within tubules buried in the mantle tissue. The boundary layers between seawater and symbiont are therefore different.

Results support different in vivo mechanisms for the acquisition of C_i by dinoflagellate symbionts in anthozoans and tridacnids. Photosynthetic rates measured as a function of external pH (6.5 to 8.5) in freshly isolated *Symbiodinium* from coral parallels the concentration of bicarbonate despite the increased CO_2 concentration at low pH (Allemand et al., 1998). In contrast, *Symbiodinium* from tridacnids demonstrate a strong pH dependence with highest rates occurring at low pH values indicating a preference for CO_2 as the C_i source (Leggat et al., 2000). Interestingly, however, the *Symbiodinium* population from tridacnids adapt following isolation, and when maintained in seawater for two days bicarbonate becomes the main source of C_i for photosynthetic carbon fixation (Leggat et al., 1999).

Isotope fractionation indicates that corals themselves acquire C_i from the bicarbonate pool (Land et al., 1975). These conclusions are consistent with more recent studies which indicate that the rate of photosynthesis cannot be supported by the spontaneous conversion of bicarbonate to CO_2 in the water column (Benazét-Tambutté et al., 1996). While the endodermal cells that line the coelenteron of the polyp have direct access to the seawater pool, in anemones at least this pool is not sufficient to account

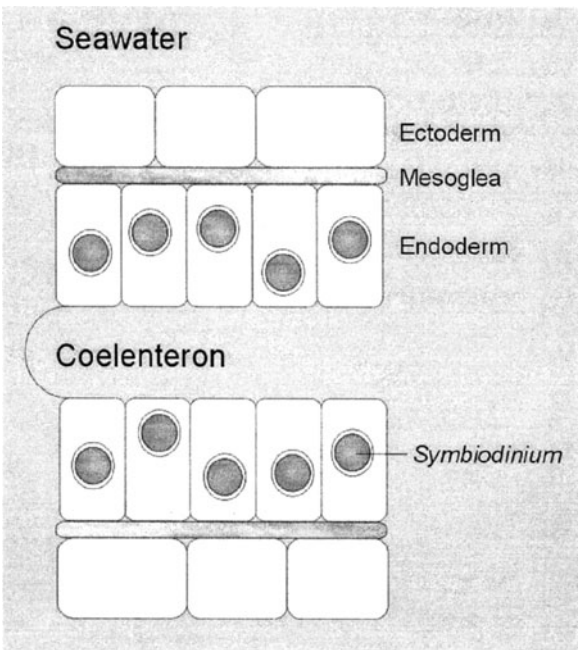

Fig. 2. Anthozoan polyps: Arrangement of tissue layers in anthozoan polyps showing the presence of the dinoflagellate symbionts, *Symbiodinium*, within the endodermal cells

for the observed rate of photosynthetic carbon fixation (Furla et al., 1998a,b). Consequently it has been argued that the major supply of C_i comes through transepithelial transport across the ectodermal and mesogleal layers to the symbionts in the endoderm (Allemand et al., 1998). Active transport is believed to be an important element in this process.

There is also strong circumstantial evidence that carbonic anhydrase participates in facilitating the transport of C_i since its presence in both corals and sea anemone polyps is directly correlated with the presence and abundance of the symbiotic dinoflagellates in the symbiotic association (Weis et al., 1989; Weis, 1991, 1993). In addition photosynthesis is significantly inhibited in the presence of the carbonic anhydrase inhibitor, diamox (Weis et al., 1989). More recent experiments have shown that carbonic anhydrase expression and synthesis are enhanced in the sea anemone, *Anthopleura elegantissima*, in the presence of dinoflagellate symbionts (Weis and Reynolds, 1999). This supports the earlier observation that a 31 kDa protein with the characteristics of carbonic anhydrase is present in *Anthopleura elegantissima* tissue only in the presence of the symbionts (Weis and Levine, 1996). It is also interesting to note that in the coral, *Pocillopora damicornis*, there is an inverse relationship between carbonic anhydrase activity and water flow indicating that carbonic anhydrase is implicated in the acquisition of inorganic carbon (Lesser et al., 1994).

It is clear therefore that further detailed investigation is required before we can understand the mechanism(s) whereby C_i acquisition occurs in corals and sea anemones.

In contrast to anthozoans, the symbionts isolated from tridacnids acquire C_i primarily as CO_2 *in hospite* (Leggat et al., 1999, 2000). This is supported by the demonstration that the mantle tissues where the algae reside contain significant amounts of host expressed carbonic anhydrase and during bleaching the 32 kDa CA is depleted (Yellowlees et al., 1993, Baillie and Yellowlees, 1998). This isoform is involved in the supply of CO_2 to the algae in the Z-tubules (Leggat, 2001).

The 32 kDa carbonic anhydrase is one of two isoforms found in the mantle, the other, a 70 kDa isoform, being located in the mantle epithelium. Both these isoforms are also located in the gills where they facilitate uptake of C_i into the haemolymph. The 70 kDa is an unusual carbonic anhydrase

in that it is membrane localized and contains two catalytic domains (Leggat, 2001).

The need for a carbon concentrating mechanism (CCM) in symbiotic dinoflagellates to meet their photosynthetic carbon requirements was predicted (Raven, 1992). The argument became more compelling with the discovery of a Form II Rubisco in *Symbiodinium* (Whitney et al., 1995, Rowan et al., 1996). Subsequently measurements of intracellular C_i up to seven times greater than expected from passive diffusion were shown (Leggat et al., 1999). However this is significantly less than the 20- to 80-fold, relative to ambient C_i, shown in the freshwater dinoflagellate, *Peridinium gatunense*, which acquires a CCM under C_i limiting conditions (Berman-Frank and Erez, 1996).

Lichens are dependent on water for metabolic activity of the symbiosis. Paradoxically this causes swelling of the fungal hyphae thus decreasing CO_2 diffusion to the algal symbiont. To obviate this, most algal symbionts in lichens have been shown to possess a CCM (Badger et al., 1993; Palmqvist et al., 1994). Direct and indirect evidence has been obtained for Cyanobacteria and a wide number, but not all, microalgae found in symbiosis with lichens (Palmqvist, 1995). The cyanobacterial symbionts possess carboxysomes while the microalgal symbionts with CCMs contain pyrenoids. However, some microalgal symbionts, including *Coccomyxa*, do not possess a CCM and are also devoid of pyrenoids. Consequently they utilize low external CO_2 concentrations less efficiently than those with CCMs (Palmqvist et al., 1997). Evidence has also been produced which indicates that, in trebouxioid lichens at least, the CCM can range in activity and that this is dependent on environmental factors (Smith and Griffiths, 1998).

B. Carbon Fixation

In all algal symbionts studied Rubisco is recognized as the primary CO_2 fixation enzyme. This has been clearly demonstrated in *Symbiodinium* where 3-phosphoglycerate is the first product of CO_2 assimilation (Streamer et al., 1993). However chromatographic analysis of extracts from *Symbiodinium* soon after exposure to ^{14}C-labeled bicarbonate reveals that dicarboxylic acids including malate, succinate and glutamate are major ^{14}C-labeled products (Trench, 1971). This is probably due to the presence of significant levels of β-carboxylating enzymes, particularly phosphoenolpyruvate carboxylase, in these dinoflagellates (Ting, 1976; Tytler and Trench, 1986). However there is no evidence that Rubisco is other than the major enzyme in CO_2 fixation.

Rubisco has been isolated from a number of symbiotic algae including *Prochloron*, the cyanelles of *Cyanophora paradoxa* and *Symbiodinium* (Andrews et al., 1984; Burnap and Trench, 1984; Whitney and Yellowlees, 1995). The first two express a Form I Rubisco. Both large and small subunits of the cyanelle Rubisco are encoded on the cyanelle genome and are co-transcribed as a single transcript (Starnes et al., 1985). Both amino acid sequence and gene arrangement are consistent with the cyanelle having evolved from an endosymbiotic cyanobacterium.

Symbiodinium expresses a Form II enzyme previously found only in anaerobic prokaryotes (Whitney et al., 1995). This has been demonstrated in other dinoflagellates (Morse et al., 1995) with the exception of some *Peridinium* species that express a Form I Rubisco encoded by the rbcLS operon in their diatom endosymbiont (Chesnick et al., 1996; Chesnick et al., 1997).

The presence of a Form II Rubisco poses the question of how an oxygenic phototroph can fix CO_2 productively using this isoform. All Form II Rubiscos show poor discrimination between CO_2 and O_2 and Whitney and Andrews (1998) have shown dinoflagellates are no exception. However calculations based on a S_{rel} of 37 indicate that a three- to four-fold concentration of external CO_2 within the cell is sufficient to account for the photosynthetic rates exhibited by symbiotic dinoflagellates. This is within the capability of the CCM present in *Symbiodinium* (Leggat et al., 1999).

The Form II 'from *Symbiodinium* along with that from other dinoflagellates is very unstable and has not been purified in an active form (Bush and Sweeney, 1972; Whitney and Yellowlees, 1995). The reason for its instability is unknown even though a wide range of techniques has been applied in attempts to purify a stable enzyme (Whitney and Yellowlees, unpublished). The purification of an active Form II from this eukaryote may shed light on how this unique enzyme functions.

The instability of the *Symbiodinium* Rubisco (or possibly another Calvin-Benson Cycle enzyme) has been suggested as a possible cause of bleaching in corals (see Section VI). If elevated temperatures result in the loss of Rubisco activity then over-reduction of the electron transport chain at high

photosynthetic irradiances and rates may result in damage to PSII and bleaching (Warner et al., 1996, Jones et al., 1998).

The presence of a Form II Rubisco in dinoflagellates in an oxygenic environment indicates the potential for photorespiration, as does the presence of the enzymes required for a functional photorespiratory pathway (Muscatine, 1990). However all reports indicate photorespiration is very low (Crossland and Barnes, 1977; Trench and Fisher, 1983): a fact backed up by the low concentrations of both glycolate and phosphoglycolate found in the symbionts (Trench, 1971b; Streamer et al., 1993). This may be due to the *Symbiodinium* Rubisco being found exclusively within the pyrenoid (Marendy, Ludwig and Yellowlees, unpublished) thus limiting its exposure to O_2.

Experiments with *Chlamydomonas* indicate that, during acclimation to low CO_2, Rubisco localizes increasingly to the pyrenoid (Morita et al., 1997; Kaplan and Reinhold, 1999). One can only speculate whether this analogy is true for *Symbiodinium* but the free-living dinoflagellate *Amphidinium carterae* contained Rubisco in both the chloroplast stroma and pyrenoid (Jenks and Gibbs, 2000). The pyrenoid localization of Rubisco correlated with increased light levels. More recent experiments have shown that in the dinoflagellate *Gonyaulax*, the presence of pyrenoids correlates with the maximum rate of carbon fixation (Nassoury et al., 2001). At these times Rubisco is sequestered within the pyrenoid while at other times it is present in the stroma. While pyrenoid formation is out of phase with maximum O_2 evolution it is consistent with the hypothesis that Rubisco localization in the pyrenoid is required for productive carbon fixation and that a CCM is associated with this organelle in dinoflagellates.

The pyrenoid in *Symbiodinium* chloroplasts is almost completely surrounded by a starch sheath (Fig. 1). Under normal *in hospite* growth conditions the starch sheath is thick. However when tridacnids are exposed to elevated nitrogen concentrations, the starch sheath in *Symbiodinium* decreases significantly in size (Ambariyanto and Hoegh-Guldberg, 1996). This is indicative of nitrogen limitation under normal growth conditions. It is widely accepted that the host, through limitation of nitrogen resources, controls *Symbiodinium* numbers. However it is probably more appropriate to view it from the perspective that algal photosynthesis is directed towards carbohydrate production (and its export) and not the mobilization of reserves for cell proliferation. In this way the host can 'milk' the algae to meet its energy demands. The host is therefore manipulating algal photosynthetic carbon fixation ensuring that the host's metabolic demands are met rather than fostering an increase in population of symbionts.

A similar metabolic situation exists in the *Chlorella-Paramecium bursaria* symbiosis (Dorling et al., 1997). Here it has been proposed that translocation of photosynthate may inhibit cell growth by inhibiting assimilation of nitrogen without reducing photosynthetic capacity of the algal cells. A similar argument is invoked with the *Chlorella*-hydra symbiosis where low rates of algal growth are attributed to carbon translocation with a consequent lack of fixed carbon to support nitrogen assimilation (McAuley, 1992, McAuley et al., 1996). This regulation of carbohydrate metabolism by nitrogen availability is well characterized in higher plants (Stitt and Krapp, 1999).

C. Translocation

While most algal symbionts can store carbohydrate as starch, much of the photosynthetically reduced carbon is exported to the host. This occurs primarily as carbohydrate with the molecular form dependent on the species of algal symbiont. Lichens, for instance, transport only a single sugar to the fungal host and this differs with algal species. Cyanobacterial symbionts secrete glucose while eukaryotic algal symbionts transport one of the polyols, erythritol, ribitol and sorbitol (Fahselt, 1994). The polyol transported is dependent on the genus. All these sugars are derived directly from intermediates of the Calvin-Benson Cycle.

In most instances symbiotic *Chlorella* translocates maltose to its host (e.g. *Hydra viridis* and *Paramecium bursaria*) (Cernichiari et al., 1969; Ziesenisz et al., 1981), although glucose is translocated in the *Spongilla fluviatilis-Chlorella* symbiosis (Fischer et al., 1989). Maltose constitutes in excess of 90% of the photosynthate released from the symbiont although alanine and glycolate are also known to be translocated (Mews, 1980). As indicated previously, there is evidence to suggest the release of maltose is pH dependent.

Like many other symbionts cyanelles translocate glucose to their host (*Cyanophora paradoxa*) although

the proportion of translocated fixed carbon (5–15%) may be significantly less than in other symbioses (Trench et al., 1978; Schlichting et al., 1990).

The most studied translocation process is that of the dinoflagellate *Symbiodinium*. The original experiments were conducted on a *Symbiodinium* isolate from the giant clam *Tridacna crocea* (Muscatine, 1967). The algae, freshly isolated from their host, were incubated in the presence of ^{14}C-bicarbonate and the major labeled product secreted by the symbiont was glycerol. Many subsequent reports from both in vivo and in vitro experiments are consistent, with glycerol as the major compound released from *Symbiodinium* in both corals and other symbiotic coelenterates. However glucose, lipids, a number of amino acids, particularly alanine, and dicarboxylic acids have also been detected (Trench, 1971; Muscatine et al., 1972; Patton and Burris, 1983). It appears likely that the molecular nature of the photosynthate translocated may be dependent on a number of factors including the species of both alga and host and the nutritional state of the association.

In giant clams, in vivo experiments clearly show that glucose is the major photosynthetically derived carbohydrate within the haemolymph (Streamer et al., 1988) despite the fact that glycerol is released from *Symbiodinium* in vitro (Muscatine, 1967). The haemolymph glucose concentration fluctuates in a diel manner, directly proportional to irradiance (Rees et al., 1993), while the concentration of glycerol was extremely low. If glycerol is translocated to any significant extent in this symbiosis it must be absorbed rapidly by the mantle tissue from the tubule system housing the symbiotic dinoflagellates.

The initial experiments of Muscatine (1967) also demonstrated an increase in the excretion of carbohydrate by *Symbiodinium* occurs in the presence of host extracts. This has been repeatedly confirmed and, depending on the symbiotic association, the increase is between two- and 20-fold. This has lead to the hypothesis that a 'host factor' can stimulate the release of photosynthetically derived carbohydrate from the algal symbiont to the host. Much of the evidence as to the nature of the 'host factor' is contradictory and there is no consensus as to the chemical identity of this compound. Results indicate that it is both a low molecular weight compound and a large molecule (Sutton and Hoegh-Guldberg, 1990; Grant et al., 1998); and that it is heat stable and heat labile (Sutton and Hoegh-Guldberg, 1990). However the weight of evidence supports the presence of a heat stable, low molecular weight molecule as the 'host factor.'

Current studies on 'host factors' focus on the ability of amino acids to stimulate translocation. A synthetic mixture of amino acids based on that found in a host tissue extract from *Pocillopora damicornis* emulated the 'host factor' properties of the original extract (Gates et al., 1995; Gates et al., 1999). Taurine, a non-protein amino acid, has also been shown to stimulate not only the release of photosynthate from *Symbiodinium* (host = *Aiptasia pulchella*) but also the photosynthetic rate of the alga. This was not due to a metabolite of this sulfur containing amino acid (Wang and Douglas, 1997).

There is circumstantial evidence that 'host factor' from different hosts may be similar in structure as it stimulates secretion of photosynthate from most dinoflagellates. The original experiments of Muscatine (1967) showed that tissue homogenate from either the giant clam *Tridacna crocea* or the coral *Pocillopora damicornis* caused secretion of photosynthate from their respective symbiont population. More recently host tissue homogenates from *Tridacna derasa* were shown to stimulate release of photosynthate from free-living dinoflagellates but not from unicellular algae of other families (Masuda et al., 1994). This, however, is not consistent with experiments on the sea anemone *Anthopleura elegantissima* where photosynthate is translocated from both dinoflagellate and zoochlorellae symbionts (Engebretson and Muller-Parker, 1999).

While the chemical identity of 'host factor' is still uncertain, its mechanism of action has not received any attention. Whatever the mechanism, 'host factor' can also affect a number of other metabolic events including increasing the rate of photosynthesis by the symbiont (Trench, 1971b; Gates et al., 1999). In addition it is also clear that 'host factor' only induces the secretion of newly synthesized photosynthate (Trench, 1971b). Pre-incubation of symbionts with $NaH^{14}CO_3$ and then chased with unlabeled bicarbonate in the presence of 'host factor' did not result in release of labeled glycerol. This supports the hypothesis that 'host factor' diverts photosynthetic carbon fixation from intracellular metabolism to secretion and that preformed algal metabolic pools cannot be readily accessed by the host. One could speculate therefore that 'host factor' may operate by influencing signal transduction pathways within the symbiont. Signal transduction cascades invariably

control multiple functions within the cell and while knowledge of these pathways in unicellular algae is scant their involvement is worth investigation. For instance some isoforms of the channel protein aquaporin are known to transport glycerol and can be regulated by pH variations, phosphorylation and binding of auxiliary proteins (Engel et al., 2000). This could be a good candidate for the secretion of glycerol and its control by host factor.

D. Chloroplasts and Cyanelles

The cyanelles of *Cyanophora paradoxa* and the algal chloroplasts found in certain ascoglossan molluscs are extremely unusual symbioses. The symbiont after all does not contain a full genetic complement and in each case the symbiont is free in the cytosol with no host membrane surrounding the symbiont. Both however are photosynthetically active and translocate fixed carbon to the host.

The cyanelle is ostensibly a plastid found in the cytosol of a glaucocystophyte protist (Chapter 2, Larkum and Vesk). Its recognition as a symbiosis is primarily because it has retained a prokaryotic cell wall between the two envelope membranes. However its genome is more akin to that of a chloroplast, coding for a restricted number of the cyanelle protein complement. The remaining genes are nuclear encoded and the expressed proteins possess transit peptides that target them to the cyanelle. Post-translational protein translocation has now been demonstrated in vitro for a number of cyanelle nuclear encoded proteins (Schwartzbach et al., 1998).

The symbiosis between chloroplasts and ascoglossan molluscs is ephemeral. The chloroplasts derived from a number of both chlorophyte and chromophyte algae are selectively retained by these herbivorous saltwater molluscs. Other cellular contents are digested. These chloroplasts are capable of light and CO_2-dependent oxygen evolution in the host and can survive for up to nine months in the mollusc. Expression of chloroplast encoded proteins including D1 and Rubisco LS can continue for eight months (Pierce et al., 1996; Mujer et al., 1996); however this also implies that the nuclear-encoded chloroplast proteins from the alga must be extremely stable. It has been shown that the life-span of chromophyte-derived chloroplasts (*Vaucheria litorea*) is longer than that from chlorophytes (*Codium fragile*). Whether the increased coding capacity of the chromophyte chloroplast contributes to their longevity is unclear.

V. Photoacclimation and Photoadaptation

In order for photosynthetic organisms to optimize light absorption during exposure to different light regimes, certain physiological adjustments must take place within the chloroplast. Photosynthetic pigments and the associated pigment-binding proteins that form the light harvesting complexes (LHCs) are responsible for the light absorption properties of algae. The regulation of these complexes is closely linked to the dynamic nature of the light field found in aquatic environments (Falkowski and LaRoche, 1991). Given the importance of the translocated carbon fixed by the symbiotic algae to the total energy budget of reef-building corals and other organisms (Muscatine, 1990), many studies have focused on better understanding how the symbionts optimize light absorption in corals via photoacclimation.

Symbiotic dinoflagellates utilize similar strategies of photoacclimation found in other species of algae and terrestrial plants, yet their unique location *in hospite* (i.e. within the respective animal host) introduces a slightly different scenario for light harvesting capacity relative to free-living microalgae. The depth distribution of organisms containing symbionts spans a wide range of photon flux densities (from very low light to > 2500 μmol quanta $m^{-2}s^{-1}$ in shallow water habitats). Likewise, these algae are largely contained within benthic organisms with little to no mobility. Such physical conditions have provided symbiotic dinoflagellates the time and location to evolve specific adaptations to their particular photic environment (Iglesias-Prieto and Trench, 1994). In addition to the loss of light with increasing depth, there is a considerable amount of shading due to packaging of the algal cells within the endoderm. In reef corals and other cnidarians, densities of symbiotic dinoflagellates tend to decrease with increasing depth (McCloskey and Muscatine, 1984; Battey and Porter, 1988; Masuda et al., 1993). The physiological reason for this pattern is not entirely clear, although two predominant hypotheses have been put forward: one, that a lower algal density may reduce self shading, and two, that hosts in deep water contain less tissue biomass per unit surface area, and therefore provide less space for the algae to occupy. Meanwhile, when

photon flux density is reduced at the same depth (e.g. during natural or experimental shading) dinoflagellate densities remain constant while pigment content tends to increase. Recent work suggests animal proteins similar to green fluorescent proteins may influence light absorption and, in some cases, energy transfer of light to the periphery LHCs of *Symbiodinium* (Dove et al., 2000). Likewise, animal behavior can also affect light absorption, as polyp contraction will further attenuate incident irradiance.

Photoacclimation in symbiotic algae is typically documented as an increase in photosynthetic pigment content and alterations in photosynthetic productivity (Falkowski and Dubinsky, 1981; Chang et al., 1983; McCloskey and Muscatine, 1984; Porter et al., 1984; Leletkin et al., 1996). Photoacclimation involves structural and physiological changes in the photosynthetic unit (PSU) of the algal cell, with the PSU comprising the LHCs and the reaction centers of the photosystems. The rise in pigment content may be due to an increase in the size of the photosynthetic unit (an enlargement of the light harvesting antennae), the number of the PSUs (an increase in both LHCs and reaction centers), or a combination of both pathways (Chang et al., 1983; Wyman et al., 1987). When different species of symbiotic dinoflagellates are cultured under identical conditions they exhibit significant differences in their photosynthetic capacity, thus displaying true signs of photoadaptation. Further, changes in the number and size of the PSUs in cultured dinoflagellates can correlate to the respective ecological distribution of the hosts from which they were originally isolated (Iglesias-Prieto and Trench, 1994). While changes in light harvesting at the biochemical level are known for only a few species of symbiotic dinoflagellates, current data indicate that protein and pigment composition can vary significantly between species.

At least two different primary light harvesting complexes have been identified within some free living and symbiotic photoautotrophic dinoflagellates, a water soluble peridinin-Chl *a* protein (PCP) complex and a membrane bound Chl *a*-Chl c_2-peridinin-protein (acpPC or ACP) complex (refer to Chapter 4, Durnford and Chapter 13, Larkum). Depending on the algal species, the PCP complex may be composed of a monomeric apoprotein of 35 kDa, a dimeric apoprotein with subunits of 15 kDa each, or a combination of the two proteins may be found in tandem (Iglesias-Prieto et al., 1991). The membrane bound ACP complex consists of at least two apoproteins 19–20 kDa in size (Hiller et al., 1993; Iglesias-Prieto et al., 1993) that have some homology to other LHCs found in chromophytes (Hiller et al., 1995). Cellular concentrations of these chlorophyll protein complexes increase in different species of symbiotic dinoflagellates to varying degrees during exposure to reduced light levels (Iglesias-Prieto and Trench, 1997).

The use of photosynthesis vs. irradiance curves to infer information about the status of the relative size and content of the PSU is a common feature of many older photoacclimation studies (Prezelin, 1987). However, more recent work suggests that using such extrapolations can be erroneous and are not a substitute for direct PSU measurements (Iglesias-Prieto and Trench, 1994). A further confounding factor is comparing cultured algae to symbionts within the host, as algal nutrient status in these two locations may be quite different. Algal cultures are typically maintained in nutrient replete media in log phase growth. Given the potential for nutrient limitation within the host (Muller-Parker et al., 1988), a decrease in nitrogen availability could lead to the preferential pathway of increasing the PSU size relative to the more metabolically costly process of synthesizing new PSUs. This situation has yet to be empirically tested in *Symbiodinium.*

VI. Coral Bleaching and Photoinhibition

A. Causative Agents and the Implications for Reef Communities

'Bleaching' in algal symbioses is a generic term that has been used to describe any phenomena where reef building corals and other organisms lose their symbiotic dinoflagellates and/or algal pigments and become visibly pale. Many forms of environmental perturbation can elicit a bleaching response including sedimentation (Dollar and Grigg, 1981), fluxes in salinity (Coles and Jokiel, 1992), lowered temperature (Steen and Muscatine, 1997), elevated temperature (Jokiel and Coles, 1990), ultraviolet radiation (Lesser, 1990), and high solar radiation during sub-aerial exposure (Brown et al., 1994). While many of these factors are responsible for small-scale bleaching events, recent large scale bleaching events that have occurred globally are correlated with prolonged exposure to sea water temperatures that are at or slightly above the annual recorded maximum for the

specific sites of bleaching (typically in the range of 30–32 °C) (Coles and Jokiel, 1977; Glynn, 1990; Hoegh-Guldberg and Salvat, 1995). While many experimental bleaching studies have documented the loss of algal density and pigments (Hoegh-Guldberg and Smith, 1989; Warner et al., 1996) most of the data collected from naturally bleached corals have indicated only a loss in algal number from the host (Jones, 1997).

Mass bleaching events can have several negative impacts on reef building corals as well as the surrounding reef community as a whole. The most severe result of bleaching is coral death. Post bleaching mortality rates have been noted to reach near 100% in shallow reefs of Indonesia (Brown and Suharsono, 1990). Elevated temperatures such as those recorded during the 1982–83 El Niño-Southern Oscillation event lead to mortality rates that have driven some species of hydrocoral close to the point of extinction (Glynn and Feingold, 1992). High mortality rates were also documented during bleaching in 1998 at some sites on the Great Barrier Reef (Hoegh-Guldberg, 1999). Bleaching may lead to reduced rates of calcification and reproduction in reef building corals. Calcification and colony growth are significantly higher in corals that contain dinoflagellates, due in part to the increased rates of photosynthesis when symbiotic dinoflagellates are present (Gattuso, 1999). Bleached colonies of the major Caribbean reef-building coral *Montastraea annularis* were unable to complete gametogenesis during the reproductive season after a bleaching event in Florida in 1987 (Szmant and Gassman, 1990), and numerous Pacific species have shown similar losses in fecundity after the 1998 bleaching in Australia (Hoegh-Guldberg, 1999).

While some species of coral may show signs of recovery in density of dinoflagellates and pigmentation in less than a year, the animal biomass and energy stores lost due to bleaching can take significantly longer to reach levels found in unbleached colonies (Fitt et al., 1993). Given the above examples, there is little doubt that substantial community shifts can take place if the impacts of coral bleaching are severe. Coral mortality along with the reduction in growth rate and reproduction will directly lead to a competitive loss for space with other invertebrates as well as macroalgae. Given the current evidence for sea surface temperature warming in the tropics, and that recent global bleaching events are considered to be the most severe on record, many have stressed the importance of understanding how these symbioses are negatively impacted at the cellular level. The need for understanding the potential biochemical pathways that may lead to acclimatization and resistance to bleaching in some host species and symbionts is equally compelling.

B. Photoinhibition

Many studies have shown that exposure to elevated temperature as well as a combination of elevated temperature and light has a negative impact on the photosynthetic physiology of symbiotic dinoflagellates (Coles and Jokiel, 1977; Hoegh-Guldberg and Smith, 1989; Fitt and Warner, 1995; Lesser, 1997; Jones et al., 1998; Warner et al., 1999). Evidence also suggests that the coral host plays a role in the overall colony response to thermal perturbation (Edmunds, 1990; Sharp et al., 1997; Downs et al., 2000). Few studies have focused on delineating which partner in the symbioses is effected first, yet the algae may be more susceptible to thermal damage. Stress to photosynthetic pathways in symbiotic algae can be placed in the context of what is currently known about processes of photoinhibition (Osmond, 1994; Chapter 16, Franklin et al.)

1. Effects of Light and Temperature in Chronic Photoinhibition

Any stress or perturbation that effectively reduces the amount of excitation energy that is used for photochemical energy production can be deemed as a source of photoinhibition. Many large-scale thermal bleaching events occurred during periods of doldrums thereby allowing for high levels of photosynthetically active radiation (PAR) and ultraviolet radiation (UVR) to penetrate deeper into the relatively clear water column. Elevated temperature may predispose the photosynthetic apparatus to further damage by excess light, thus leading to a state of chronic photoinhibition. Chronic photoinhibition is classified as damage to the photosynthetic apparatus of the alga that is non-reversible and requires de novo repair of specific photosynthetic proteins. Many components of the photosynthetic pathway are susceptible to thermal stress including the oxygen evolving complex (Havaux, 1993) and reaction centers of PS II, as well as enzymes involved in Calvin-Benson cycle activity such as Rubisco activase (Crafts-Brandner and Salvucci, 2000). An indirect way to measure

disruption at the PS II reaction center is by analysis of chlorophyll fluorescence kinetics. Several fluorescence techniques are now being utilized to study photoinhibition in symbiotic dinoflagellates including pulse-amplitude modulation fluorometry (PAM), and rapid induction analysis by fast repetition rate fluorometry (FRR). Such techniques have shown that dinoflagellates within different species of corals can have different thermal tolerance levels (Warner et al., 1996).

Many areas within the chloroplast are susceptible to thermal perturbation. A primary target of photoinhibitory damage at the PS II reaction center is the D1 protein (Ohad et al., 1994). Under normal homeostasis, the D1 has a high rate of turnover that involves a light-dependent inactivation and degradation coupled with de novo synthesis and insertion into the thylakoid membrane (Matto et al., 1984). During chronic photoinhibition the rate of D1 loss does not keep up with the rate of repair. This is indeed one such pathway of PS II disruption in dinoflagellates within naturally bleached corals (Warner et al., 1999). However, the biochemical and molecular pathways that lead to PS II disruption in the symbionts are still not known. Other work has hypothesized that the initial point of damage is beyond the light reactions and closer to the dark reactions at some point in the Calvin-Benson cycle (Jones et al., 1998). Chloroplast membranes contain a high proportion of galactolipids with highly unsaturated fatty acids, and are therefore susceptible to damage via active forms of oxygen during periods of high light and/or temperature exposure (Tardy and Havaux, 1997; Asada, 1999). Elevated levels of oxygen free radical scavengers such as superoxide dismutase, catalase, and ascorbate peroxidase are found in heat stressed cultured *Symbiodinium* (Lesser, 1996), in intact symbioses, and in non-symbiotic sea anemones (Lesser et al., 1990; Nii and Muscatine, 1997). Such oxidative stress can lead to an increase in lipid peroxidation in corals, leading to a loss in the structural integrity of the thylakoid membrane. The process by which the algae are removed from the host during natural bleaching is still an open question. It is believed that since the loss in photosynthetic capacity would result in a decline in the translocation of photosynthate to the host, that this may act as a signal for the removal of the algae perhaps by exocytosis (but see Ralph et al., 2001).

2. Photoprotection in Symbiotic Dinoflagellates and the Potential for Bleaching Resistance

As previously mentioned, some species of *Symbiodinium* are more tolerant to thermal/light stress than others (Warner et al., 1999). A common feature of many species of plants and algae is the capacity to down-regulate light energy capture and utilization in order to protect the photosynthetic apparatus from photoinhibitory damage (Chapter 13, Larkum). These processes fall into the category of dynamic photoinhibition, as they are largely reversible (typically on the scale of minutes to a few hours) and do not necessarily require any de novo repair processes to take place. Chlorophyll fluorescence has proven useful for quantifying photoprotection as the degree to which light energy is dissipated away from the reaction centers of PS II via non-photochemical pathways. When exposed to temperatures up to 36 °C, dinoflagellates within the coral *Siderastrea radians,* a species that does not bleach frequently, showed a higher capacity for energy dissipation compared to dinoflagellates within *Montastraea annularis*, a species with a long record of bleaching (Warner et al., 1996). Dynamic photoinhibition is notable in several species of shallow reef corals as a mid-day decline in the photosynthetic efficiency of PS II (as measured by the effective quantum yield $\Delta F/Fm'$) that subsequently recovers in the late afternoon and early evening (Brown et al., 1999; Hoegh-Guldberg and Jones, 1999). Further work on this phenomenon in shallow reef corals has shown that this mid-day drop in PS II efficiency correlates quite strongly with the rapid conversion of the xanthophyll pigment diadinoxanthin to diatoxanthin. The dissipation of excitation energy by the cycling of xanthophyll pigments is widely recognized to occur in other species of algae and plants in response to high irradiance (Demmig-Adams and Adams, 1992; Olaizola et al., 1994; Franklin et al., 1996). However, it is important to note that published xanthophyll analyses have only been performed on corals taken from extremely shallow water habitats (Brown et al., 1999), and it remains to be seen if this pathway is equally important in corals from deeper locations. Other mechanisms of photoprotection include enhanced rates of protein turnover (e.g. D1) as well as selective inactivation of PS II reaction centers. The former mechanism has been proposed to occur in thermally tolerant species of *Symbiodinium* in culture (Warner et al., 1999), while the latter

mechanism has been shown to occur in other classes of algae and terrestrial plants (Neale and Melis, 1990; Schnettger et al., 1994) and may very well be an important component of photoprotection for symbiotic dinoflagellates in situ.

A key focus in *Symbiodinium* research is to understand the genetic diversity of the algae and how it may relate to the physiological responses during periods of homeostasis and environmental perturbation. Previous work has shown that symbiotic dinoflagellates can differ considerably in their ultrastructure, biochemistry and specificity to different hosts they may inhabit (Schoenberg and Trench, 1980a,b,c; Blank and Trench, 1985). Use of restriction fragment length polymorphisms (RFLP) of the 18S and 28S rRNA genes (Rowan and Powers, 1992; Baker and Rowan, 1996), as well as analysis of the internal transcribed spacer region 2 (ITS 2) (LaJeunesse and Trench 2000), has shown that there is high genetic diversity within several lineages of symbiotic dinoflagellates. Molecular work has also facilitated the identification of more than one taxon of alga within some species of reef-building corals (Rowan and Knowlton, 1995; Baker and Rowan, 1996). This has led to the hypotheses that the distribution of multiple taxa within a single host may be regulated, in part, by physiological differences of the resident algae. Rowan et al (1997) compared natural bleaching patterns of two Caribbean corals in Panama, *Montastraea annularis* and *Montastrea faveolata,* and observed marked differences in bleaching with shallow water colonies having greater bleaching on the sides of the colonies and deep colonies showing greater bleaching on the tops of the colonies. This suggests that such patterns may be due to the differential tolerances to light and elevated temperature in dinoflagellates residing in particular portions of the corals. While this finding is quite important, current evidence indicates that not all species of *Symbiodinium* within a lineage or clade will react to environmental perturbation in the same way.

3. The Influence of Seasonal Patterns of Host and Symbiont Physiology

There are significant seasonal changes in the coral tissue biomass and density of symbiotic dinoflagellates (Stimson, 1997; Fagoonee et al., 1999; Fitt et al., 2000). Coral biomass and symbiont densities are typically the highest during the winter and early spring, when water temperature and irradiance are low, while the lowest animal biomass and symbiont densities occur at the end of the summer, when temperature and light are high (Fitt et al. 2000). While this correlation seems quite simple, it places the phenomenon of coral bleaching into a new context that has previously been overlooked. Due to limitations of space and other physiological constraints, it is understood that a coral colony can maintain a finite population of algal symbionts. Seasonal patterns of tissue biomass and algal density may be due to the combined effects of light and sea water temperature impacting the metabolic rates of both partners in the symbioses. Likewise, the photosynthetic efficiency of the symbionts follows the same seasonal pattern, with highest PS II quantum yields occurring in the mid-winter to early spring, and the lowest yields being noted at the end of the summer (Warner, personal observation). In light of this strong seasonal cycling, it appears that coral bleaching is best viewed as the end point of a physiological continuum in which corals and their symbionts may be 'stressed' long before any visible signs of algal loss are noted. Therefore, the combination of elevated (or extended) summer high temperatures and light can provide an extension of this pattern that leads to the collapse of the symbioses.

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