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ORIGINAL PAPER

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The gene family encoding the fucoxanthin chlorophyll proteins from the brown alga *Macrocystis pyrifera*

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Abstract Six members of a multigene family encoding polypeptide constituents of the fucoxanthin, chlorophyll *a*/*c* protein complex from female gametophytes of the brown alga *Macrocystis pyrifera* have been cloned and characterized. The deduced amino acid sequences are very similar to those of fucoxanthin chlorophyll binding proteins (Fcp) from the diatom *Phaeodactylum tricornutum* and exhibit limited homology to chlorophyll *a*/*b* binding (Cab) polypeptides from higher plants. The primary translation products from the *M. pyrifera fcp* genes are synthesized as higher molecular weight precursors that are processed prior to their assembly into the Fcp complex. The presumed N-terminal 40-amino acid presequence of the Fcp precursor polypeptide has features resembling that of a signal sequence. This presequence may be required for the protein to transverse the endoplasmic reticulum that surrounds the plastid in brown algae. A subsequent targeting step would be required for the protein to cross the double membrane of the plastid envelope. *M. pyrifera fcp* transcripts are of two sizes, 1.2 and 1.6 kb. The size difference is accounted for by the length of the 3' untranslated region, which can be up to 1000 bases. Transcript abundance's of members of the *fcp* gene family are dependent on light quantity, light quality, or both. Transcript levels of one gene increased approximately five- to tenfold in thalli grown in low intensity relative to high intensity white or blue light. Transcripts from this gene also significantly increase in red light relative to blue light at equivalent light intensities.

Key words Blue light · Chromophyta · Fucoxanthin *Macrocystis* · Phaeophyta · Signal sequence

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Introduction

A number of different light harvesting complexes (LHC) exist in photosynthetic organisms. These complexes capture radiant energy and transfer that energy to the photosynthetic reaction centers where it is transformed and used in the production of ATP and reducing power.

The light harvesting complex of terrestrial plants and green algae contains the integral membrane chlorophyll *a*/*b* binding (Cab) proteins (Green et al. 1991). There are a number of different classes of Cab polypeptides, all of which have three membrane spanning helices (Kühlbrant and Wang 1991; Kühlbrant et al. 1994). These polypeptides are encoded by a multigene family on the nuclear genome (Chitnis and Thornber 1988) and are synthesized in the cytoplasm of the cell as precursors with a presequence of 3–5 kDa. This presequence has been termed the "transit peptide" and is required for the transport of the protein across the double membrane of the chloroplast envelope (Schmidt and Mishkind 1986; Keegstra 1989; De Boer and Weisbeek 1991).

The major LHC in both cyanobacteria and red algae is the phycobilisome (Grossman et al. 1993). This macromolecular complex is peripheral to the photosynthetic membranes and is composed primarily of light absorbing molecules called phycobiliproteins. These proteins are pigmented by virtue of their covalent attachments to linear tetrapyrroles. In eukaryotic algae the phycobiliproteins are synthesized within the plastid while some of the phycobilisome linker polypeptides, which stabilize the interactions among the phycobiliproteins, are synthesized in the cytoplasm of the cell. Like the Cab polypeptides these linker polypeptides must traverse the chloroplast envelope. The mechanism by which they enter the plastid may be analogous to that used in higher plants (Apt et al. 1993).

Unique to the chromophytic algae, a group which includes brown algae and diatoms, is an LHC associated with photosystem II (PS II) that contains the pigments fucoxanthin and chlorophylls *a* and *c*. These pigments are noncovalently bound to fucoxanthin chlorophyll binding proteins (Fcp) that form an integral membrane complex that has been isolated from brown algae (Berkaloff et al. 1981; Duval et al. 1983; Peyriere et al. 1984; Caron et al 1988; Katoh et al. 1989; Berkaloff et al. 1990; Katoh et al. 1989; Mimuro et al. 1990; Passaquet et al. 1991), diatoms (summarized in Grossman et al. 1990) and other chromophytic algae (Grevby and Sundquist 1992). Purified complexes from brown algae typically contain fucoxanthin: chlorophyll *c*: chlorophyll *a* in a ratio of (0.6–1):(0.1–0.3):(1) (Passaquet et al. 1991). The complexes are composed of multiple Fcps with molecular masses ranging from 17–21 kDa. These proteins are immunologically related to polypeptides in the LHC complexes containing chlorophyll *b* (Fawley et al. 1987; Friedman and Alberte 1987; Hiller et al. 1988; Caron et al. 1988; Manodori and Grossman 1990; Passaquet et al. 1991; Grevby and Sundquist 1992; Plumley et al. 1993) and the PS I associated LHC of red algae (Wolfe et al. 1994). The partial amino acid sequence of a Fcp from the brown alga *Laminaria saccarhina* also indicates a low level of homology to other LHC polypeptides (Duoady et al. 1994).

A number of genes encoding Fcps from the diatom *Phaeodactylum tricornutum* have been characterized. The amino acid sequences of diatom Fcps indicate that they are evolutionarily related to LHC polypeptides of terrestrial plants (Grossman et al. 1990; Bhaya and Grossman 1993). The *fcp* genes are in the nuclear genome and encode proteins that are synthesized in the cytoplasm of the cell as precursor polypeptides (Fawley and Grossman 1986). Targeting of the Fcp polypeptides to thylakoid membranes involves their transport across a set of endoplasmic reticulum (ER)-like membranes that surround the plastid (plastid ER) of the chromophytic algae (Gibbs 1981), followed by translocation across the double membrane of the plastid envelope. An amino terminal presequence present on primary translation products of the Fcps has a domain that is characteristic of an ER signal sequence and appears to function in transporting the proteins across the plastid ER (Bhaya and Grossman 1991).

To determine the similarity between light harvesting genes from diatoms and brown algae, explore protein targeting among groups of chromophytic algae and examine the effects of light on the expression of genes encoding light harvesting polypeptides in marine algae, multiple cDNAs encoding Fcps were isolated and characterized from the brown alga *Macrocystis pyrifera*.

Materials and methods

Culture conditions

M. pyrifera (L.) Ag. female gametophyte, strain MaI-1 was obtained from M. Neushul, University of California, Santa Barbara. This isolate was previously described as resembling *Macrocystis angustifolia* Bory (Neushul 1971). Maintenance cultures were grown in 50% modified PES medium (Magruder 1984) at 16°C in 2 l containers, bubbled with air and exposed to 35 μE m^2 per second continuous light.

Protein electrophoresis and immunoprecipitation

Protein samples were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (15% polyacrylamide) utilizing the Laemmli buffer system (Laemmli 1970). For immunoblots, proteins were electroblotted to nitrocellulose membranes (Schleicher & Schuell) and incubated sequentially with a polyclonal antibody against Fcp and protein A-peroxidase (United States Biochemical). In vitro translations were performed in rabbit reticulocyte lysates (Promega) and immunoprecipitated as described by Anderson and Blobel (1983).

Isolation of nucleic acids

Nucleic acids were extracted in 100 mM TRIS-HCl pH 8.0, 1.5 M NaCl, 20 mM EDTA, 20 mM DTT (dithiothreitol) and 2% CTAB (hexadecyltrimethyl ammonium bromide). Algal tissue was finely ground in liquid nitrogen and added frozen to the extraction buffer at a ratio of 1:10 (w/v). The solution was gently mixed at room temperature for 15 min, extracted with chloroform and centrifuged at 10000 g for 30 min. Polysaccharides were precipitated from the aqueous phase by the slow addition of 0.33 vol. ethanol followed by another extraction with chloroform. The aqueous phase was saved and used for either DNA or RNA isolation. DNA was precipitated from the aqueous phase with 1 vol. isopropanol for 4 h at room temperature. The precipitate was resuspended in 10 mM TRIS-HCl, pH 8.0, 1 mM EDTA (TE) at 0.1 vol. of the original extraction buffer. The solution was made to 1 g/ml CsCl and 600 μg/ml ethidium bromide prior to centrifugation in a Beckman Vti65 rotor for 6 h at 55000 rpm. The DNA band was removed from the gradient, the dye extracted with butanol, and the DNA precipitated with 2 vol. of ethanol and resuspended in TE to a final concentration of 1 mg/ml. For isolating RNA the aqueous phase was made to 3.0 M LiCl, 1% v/v β mercaptoethanol and placed at −20°C overnight. The precipitated RNA was collected by centrifugation at 10000 g for 30 min, solubilized in TE, extracted with an equal volume of phenol-chloroform (1:1), reprecipitated with 2 vol. ethanol, 0.3 M sodium acetate and resuspended in sterile H_2O to a final concentration of 1 mg/ml. Storage of the RNA was at −80°C. Poly(A)$^+$ RNA was isolated by oligo (dT)-cellulose chromotography (Maniatis et al. 1982).

Gel electrophoresis and hybridization conditions

DNA was resolved on agarose gels in TAE buffer (Maniatis et al. 1982). Total RNA (1–3 μg) was resolved on agarose gels containing formaldehyde (Rosen et al. 1990). Nucleic acids were transferred to Nytran filters (Schleicher and Schuell) which were then baked at 80°C in a vacuum oven for 2 h. Hybridizations were performed in a Bachofer rotary oven at 52° or 65°C overnight with 5 × SSPE, 1% SDS, 5 × Denhardts and 100 μg/ml DNA. Following hybridization the filters were washed twice for 30 min at the hybridization temperature in 10 or 50 mM phosphate buffer containing 0.1% SDS and exposed to X-ray film (Kodak XAR5) or analyzed on a PhosphorImager (Molecular Dynamics) to quantify the level of hybridization.

Cloning and sequencing

An *M. pyrifera* cDNA library was constructed in λ ZapII XR (Stratagene) following the manufacturer's protocol. Immunos-

creening of the library was conducted using a polyclonal antibody raised to Fcps from *P. tricornutum* (Fawley and Grossman 1986). Additional plaques with recombinant DNA containing *fcp* sequences were identified by plaque hybridization (Maniatis et al. 1982) using the insert to *M. pyrifera* Fcp cDNA clone pFcpF as a hybridization probe. Subclones were generated by digesting the insert DNA with appropriate restriction enzymes and ligating the fragments into Bluescript SK–. Sequencing was by the dideoxy chain termination method using Sequenase 2.0 modified T7 DNA polymerase (United States Biochemicals). Sequences were analyzed using the GCG Sequence Analysis Software Package (GCG-Inc).

Light and nutrient conditions

Cultures for RNA quantification were grown in 2 l containers with 25% PES medium at 14°C. Maintenance cultures were exposed to a 12 h light:12 h dark cycle, bubbled with air and illuminated at an intensity of 35 μE m^2 per second. Duplicate cultures were grown under the following light conditions: white, high (150 μE m^2 per second), medium (35 μE m^2 per second) and low (10 μE m^2 per second); blue, high (100 μE m^2 per second) and low (10 μE m^2 per second); red, high (100 μE m^2 per second) and low (10 μE m^2 per second). Light sources consisted of cool white (F20T12/CW, spectral distribution 410–720 nm, peak at 585 nm), blue (F20T12/B, spectral distribution 380–590 nm, peak at 485 nm) and red (F20T12/R, spectral distribution 610–700 nm, peak at 660 nm) fluorescent tubes (Philips). All cultures including those in which nutrients (nitrogen and phosphorus) were omitted from the medium were sampled after 14 days. Samples were taken at the middle of their respective light or dark periods (as indicated in the text).

Results

The Fcp and its precursor

Crude thylakoid membranes from *M. pyrifera* possess a prominent polypeptide of 20 kDa (Fig. 1, lane A). This protein band, which is probably composed of a number of different polypeptide species, cross reacts with a polyclonal antibody raised against the purified FCP complex from the diatom *P. tricornutum* (Fig. 1, lane B).

In vitro translation products synthesized in a reticulocyte lysate system primed with poly(A)$^+$ RNA isolated from *M. pyrifera* were 15–100 kDa (Fig. 1, lane C) in size. An abundantly labeled primary translation product (10%–15% of total incorporation) migrates with an apparent molecular mass of 23 kDa. Immunoprecipitation from total in vitro translation reactions with *P. tricornutum* Fcp antibodies identified the 23 kDa product (Fig. 1, lane D) as the Fcp precursor. The Fcp primary translation product is approximately 3 kDa larger than the mature protein. A second polypeptide that was immunoprecipitated had a molecular mass of 24 kDa and may represent a higher molecular mass Fcp or a different polypeptide that shares common epitopes with the Fcps.

Gene characterization

Approximately 10000 recombinant clones from the *M. pyrifera* λZapII cDNA library were screened with the *P.*

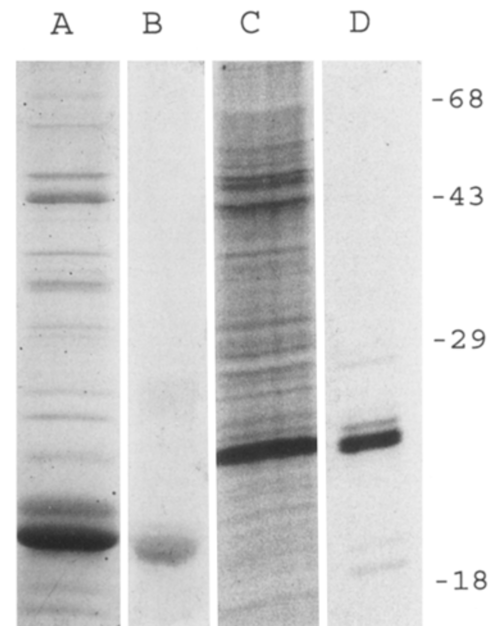

Fig. 1 SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of fucoxanthin chlorophyll binding protein (Fcp) polypeptides. Lane A, crude thylakoid membranes isolated from *Macrocystis pyrifera* plastids. Lane B, immunoblot of same sample as lane A, incubated with polyclonal antibodies to *Phaeodactylum tricornutum* Fcp (Grossman et al. 1990). Lane C, in vitro translation in rabbit reticulocyte lysate primed with poly(A)$^+$ RNA isolated from *M. pyrifera.* Lane D, immunoprecipitation of in vitro translation reaction with *P. tricornutum* Fcp antibodies. Size markers are in kilodaltons

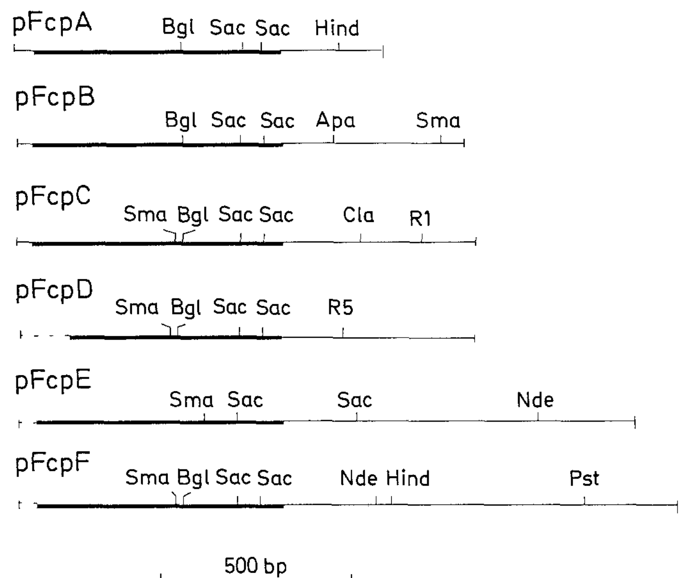

Fig. 2 Restriction maps of the different groups of cDNA clones (pFcpA-F) selected by DNA hybridization or immunologically screening a λZapII XR library of *M. pyrifera*, female gametophytes. Coding regions are indicated by *heavy bars.* Restriction sites for *Apa*I, *Bgl*II, *Cla*I, *Eco*RI, *Eco*RV, *Hind*III, *Nde*I, *Lac*I, and *Sma*I are indicated

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A   actcactcggaactcgtacatcttttctctccacctcctaaactcgaagATGAAGTCCGCTGTGATGGCTGTAGCTTGCGCCGCTGCGCCAGGCCTTCGTCGCCCCAGCGCCTTCAACGG
B   ------cc-caactc------------------------aaataccaagATGAAGTCCGCTGTGATGGCTGTCGCATGCGCCGCCGCGCCAGGCTTTCGTGGCCCCAGCGCCTTCAACGG
C   -----acctcaacttccaatcctttcacgt--cctacccaactcgaagATGAAGTCCGCTATCATGGCTGTTGCTTCCGCCGCC---CCAGGCCTTCGTGGCCCCAGCGCCTTCAACGG
D   ------------------------------------------------------------------------------------------------------------------------
E   ----------------------------------------------------------------ATGGCTATCGCTTGCGCCGCTGCGCCAGGCCTTCGAGGAGCCGAGCCGTTCAACGG
F   -----------------------------------------------------------------TGGCCATCGCATGCGCCGCCGCGCCAGGCCTTCGTGGCCCTAGCGCGTTCAACGG

A   TGCCGCCCTGACCACCTCGGCCAAGTCCTCTTCCGCCATGAAGATGTCGTTCGAGTCCGAGATCGGCGCCCAGGCCCCCCTCGGCTTCTGGGACCCGCTCGGCCTCCTGGCCGACGCCGA
B   TGCCGCCCTGACCACCTCGGCCAAGGCCTGTTCCGCCATGAAGATGTCGTTCGAGTCCGAGATCGGCGCCCAGGCCCCCCTCGGCTTCTGGGACCCGCTCGGCCTCTTGGCCGACGCCGA
C   TGCCGCCCTGACCACCTCGGCCAAGTCCTCTTCCGCGATGAAGATGTCGTTCGAGTCCGAGATCGGCGCACAGGCACCCCTCGGCTTCTGGGACCCGCTCGGCCTCTTGGAAGACGCCGA
D   ----------------------------------GCGATGAAGATGTCCTTCGAGCTTGAGATCGGCGCACAGGCACCCCTCGGCTTCTGGGACCCGCTCGGCCTCTTGGCCGACGCCGA
E   TGCCGCCCTCGCGACCTCCGCCAAGTCGTCCTCGGCCATGAAGATGAGCTTCGAGTCCGAGATCGGCGCACAGCCCCCGATCGGCTTCTGGGACCCGCTCGGGCTCGTGGCGGACGCGGA
F   CGCCGCCCTCTCGACCCCGGCGAAGTCCTCCTCCGCGATGAAGATGTCCTTCGAGTCCGAGATCGGCGCCCAGGCTCCCCTCGGCTTCTGGGACCCGCTTGGCCTCCTCGCCGACGCCGA

A   CCAGGACGGCTTCGAGCGCCTCCGCTACGTGGAGGTGAAGCACGGCCGCATTGCGATGCTCGCCATCGCCGGTCACTTGACCCAGCAGAACGCCCGCCTCCCCGGCATGCTCTCCAACTC
B   CCAGGAGCGCTTCGAGCGCCTCCGCTACGTGGAGGTGAAGCACGGCCGCATTGCGATGCTCGCCATCGCCGGTCACTTGACCCAGCAGAACACCCGCCTCCCCGGCATGCTCTCCAACTC
C   CCAGGACGCCTTCGAGCGCCTCCGGTACGTGGAGGTGAAGCTCGGCCGCATTGCGATGCTCGCGATCGCTGGCCACCTCACCCAGCAGAACGCCCGCCTGCCCGGCATGCTCTCCAACTC
D   CCAGGAGCGCTTCGAGCGCCTCCGGTACGTGGAGGTGAAGCACGGCCGCATTGCGATGCTCGCGATCGCTGGCCACCTCACCCAGCAGAACGCCCGCCTGCCCGGCATGCTCTCCAACTC
E   CCAGGAGCGGTTCGACCGCCTCCGCTACGTGGAGATCAAGCACGGGCGCATTGCTATGCTCGCCGTTGTGGGGCACATCACCCAGCAGAACACCCGCCTGCCCGGCATGCTTTCCTTCAA
F   CCAGGAGAGGTTCGAGCGCCTCCGGTACGTGGAGGTGAAGCACGGCCGCATTGCGATGCTCGCCATCGCGGGGCACCTGACCCAGCAGAACACCCGGCTGCCCGGCATGCTCTCGAACTC

A   GGCCAACCTCTCGTTCGCTGACATGCCCAACGGTGTGGCGGCTCTGTCCAAGATCCCCCCGGCGGGTCTCGCCCAGATCTTCGCGTTCATTGGCTTCCTGGAGCTGGCGGTGATGAAGAA
B   GGCCAACCTCTCGTTCGCTGACATGCCCAACGGTGTGGCGGCTCTGTCCAAGATCCCCCCGGCGGGTCTCGCCCAGATCTTCGCGTTCATTGGCTTCCTGGAGCTGGCGGTGATGAAGAA
C   CGCCAACCTCTCGTTCGCGGACATGCCCAACGGCGTGGCGGCTCTGTCCAAGATCCCCCCGGGCGGCCTCGCCCAGATCTTCGGGTTCATCGGCTTCCTTGAGCTGGCGGTGATGAAGAA
D   CGCCAACCTCTCGTTCGCGGACATGCCCAACGGCGTGGCGGCTCTGTCCAAGATCCCCCCGGGCGGCCTCGCCCAGATCTTCGGGTTCATCGGCTTCCTTGAGCTGGCGGTGATGAAGAA
E   GGAGAACCTCGCGTTCGCGGACTCGCCCAACGGCGTCGCCGCCTTCTCGAAGATCCCCCCCTGGGAACCCTCCAGATCATCCTGGCCATCGGATGCCACGAACTCTTCGTGGTGAAGCA
F   GGCGAACCTCTCGTTCGCTGACATGCCCAACGGCGTGGCGGCTCTTTCCAAGATCCCCCCGGGCGGCCTCGCCCAGATCTTCGGGTTCATCGGCTTCCTCGAGCTGGCGGTCATGAAGAA

A   CGTTGAGGGCTCCTTCCCCGGAGACTTCACGCTCGGCGGCAACCCCTTCGGTGCGTCGTGGGACGCCATGTCTGAGGAGACCCAGGCCTCCAAGCGCGCGATCGAGCTCAACAACGGCCG
B   CGTTGAGGGCTCCTTCCCCGGAGACTTCACGCTCGGCGGCAACCCCTTCGGTGCGTCGTGGGACGCCATGTCCGAGGAGACCCAGGCCTCCAAGCGCGCGATCGAGCTCAACAACGGCCG
C   CGTGGAGGGCTCCTTCCCCGGAGACTTCACGCTCGGCGGCAACCCCTTCGCTTCGTCGTGGGATGCCATGTCCGCGGAGACCCAGGCCTCCAAGCGCGCGATCGAGCTCAACAACGGCCG
D   CGTGGAGGGCTCCTTCCCCGGAGACTTCACGCTCGGCGGCAACCCCTTCGCTTCGTCGTGGGATGCCATGTCCGAGGAGACCCAGGAGTCCAAGCGCGCGATCGAGCTCAACAACGGCCG
E   GGTGGAGGGCTCCTTCCCCGGGGACTGCACGACCGGTGGCAACATCTTCCAGTCGGCATGGGACAACATGTCCGAGGAGACCCAGGCCTCCAAGCGCGCGATCGAGCTCAACAACGGACG
F   CGTCGAGGGCTCCTTCCCCGGAGACTTCATTATCGGCGGCAACCCCTTCGCTTCCTCGTGGGACTCGATGTCCTCGGAGACGCAGGCCTCCAAGCGCGCGATCGAGCTCAACAACGGCCG

A   CGCCGCGCAGATGGGCATCCTCGCGCTCATGGTGCACGAGGAGCTCAACAACAAGCCCTACGTCATCAATGACCTCGTCGGCGCCTCGTACACCTTCAACTGAgcgtaattttaaatccg
B   CGCCGCGCAGATGGGCATCCTCGCGCTCATGGTGCACGAGGAGCTCAACAACAAGCCCTACGTCATCAACGACCTCGTCGGCGCCTCGTACACCTTCAACTGAagccccttgttagcccc
C   CGCCGCGCAGATGGGCATCCTCGCCCTCATGGTACACGAGGAGCTCAACAACAAGCCCTACGTCATCAACGACCTCCTCGGCGCCTCGTACAACTTCAACTAAagccttcctttagcttt
D   CGCCGCGCAGATGGGCATCCTCGCCCTCATGGTACACGAGGAGCTCAACAACAAGCCCTACGTCATCAACGACCTCCTCGGCGCCTCGTACAACTTCAACTAAaacttaaaaccttgcac
E   CGCCGCGCAAATGGGCATCCTCGCCATGATGGTGCACGAGCAGCTGTCCAACCAGCCCTACATCATCAACGACCTCGCCGGCGCCGCGTACCAGTTCAACTGAgcgttacgattatagcg
F   CGCCGCGCAGATGGGTATCCTGGGAATGATGGTGCACGAGGAGCTCTCCAACCAGCCCTACATCACTAACGACCTCCTCGGCGCGTCGTACACCTTTAACTAAagccctgcttttttta

A   ccccccccccctcccccccggcagaggtggcggatcggagggaaataatttgtcggatagcctctgtcgaactctcgtctcgtgacctctcggacccccagtttttaaggtcacgaagc
B   atagggtagtttttcggtgtgaacagaaaaaaggcacacggccacgaagaattttgtttttcggccggaagcgggcccccgcttcttgttttcccgcgtacatttccttctcctttcat
C   tgtgactttaggttgttgtgtggtgtggcttgaagaaagctgttgttaatagacggaaactttttttcaagcgacgtgtggccggcgcctgtcttgtttcttggggtggttggcgttt
D   gtgtgcgcacgcgtggttgtttttgctttgtgggggtgggggcgtgttttgcctttcgcagcatagctaccgtcttccggcaacgatataaataaaacgcatttcacgaagcggactc
E   tgccgcggtttgagctggcgaatacgagagacgggggagtggtggggggtgtttgcgtcccttctcctgccttggatacttttgcgtttctttgggggggaactctaaacccagatgaaga
F   gcgctgcacggggagtgacgggggggggggggatgttgaaagatattgtagcagcgtccaccccaccgcgcatcgctaccgattgtcggggtattatcaggggagaaaaattgttatttcc

A   ttaagccggttctggtgatgtcaagagggtctttggcggctagttttgctgacatgccactcaacaatttaacgcgcaacgtttgtaagcaagAAAAA...
B   tagatccggcacccaacgcgtgtaaaattatatggggatctcccgtgtttttttcttgtcaaaagcacacacgggggggggggttgcacgggaaggaggcagttgtccgggagaggggaa
C   taactcgtgtgcagcaccgacagcaacgtttgtttttttgttgcccaaggctgctcagtcgatcgatagggggtggttggctttttttggctcgtttttttcgcaaggggaggggggtgaccc
D   attttgatatcggggggcggcggaagcgccgatttcaggagccgtggtcgtgcaaaatttaatttccttggatttgtagttacagaagtagtcgggagctagctttgatttgtctggt
E   aaccgttgttgggggggtatttttagacggaagcaatcacaagagagagctctagggttgcctccgggagttcaagcgcagatgaggcagttttgatggggtctgccgttcttcttctga
F   tctcttgtttttcctggctgtgttgaccttgtgtgtgggagtgtttgatgatgttacatatttttccgtcacgtgcacgtgcttttgttttctttttgggcgcatatgatcgcggtg

B   ggaggggcttgcgggacgtaaaattgctcaactcatcatggcgccatttttactaggtcggcaacaacatttcggaagggaggattttagggtctcatcggggagaggagggaggctttt
C   cccccgccgtcatgtttcgtctgcgtcccttttttaagctgttcttttttttccgttgggtgcatcgaatacctaaaaacaggataagattctgcacgatttttgaattccctttggtatcgc
D   tggtttttttggacttttacacttcctttttgacgtcgttggtttttgctcgtttttgcggtcggcttattgccacagcaaaataggtgagccgttcgctgacatgacttgaccctggtatt
E   tgctggtcgtcggggcgcattgttcctcgccgaagaggcggtccctacgacagagagataaaagggcggtgcctcccttgttaggaggccttttttgtatgccgttttgtttttttcgaggc
F   ttttaatttttacgtgtttgagcagcaagcttgggggcgtagatgacctttttcgtttcttgcgtcgtcccccctcctcccccccccccacacacaacgcctcccttgcaatgtctaacc

B   attgacacgccggggggtctagcccggggtcagagatggcgtagagacaatttacactcctttatttctcaAAAAA...
C   acgcgtgtacaagagaaatcagcattttaattactagggtggacatataatgaatcgaacgaacaaacaaacaaaaatcgaagcggtccttgcAAAAA...
D   tttttgggtcgaggtgtcggtcggggcggggggggggggctgcttcatcggacgggtttaacgaccactattaataatcaattcctggaacttgaaacAAAAA...
E   gacgcacatacactttttaagttcgcgcgtcggtcgcgccctgctgtgatacgatgaattttaacacgagacgcgcaatttgcaatgtcgctgtcccgtgcgtacgtactcgtggtaagta
F   tggccagagtaggggtgcgtctggggtgtaggagagcggggggtcggggcagcaatcgaatacgaggggttatccttttttcacgcggtagatgttaagagggggggtttgcgccgttcct

E   caattcgagagaatgagggcggttcaagattattccgcaggatgcaaagctgtacacttatagcagtctgggtccgtttgtagaagacgttccttcccttcgtttttgttgtgccttgaa
F   ggtcgcaacattctttcggttgtagttagttttttttccttggtagttactgtggggcgtatggggcggcactcggagcgtttcgacttttggcagattgatcgatcgatgatagagtaga

E   aaggcaccgcgggcaatgttaaaaaataacaaaaaaataccatatgatgtggtgtgcaggttttgtgagtatcatacatgcagagttctctttctgggttgtggttgacttgaaataccg
F   ggggacgggggggtggaatgcaacgcgtcgcgaatgctgattggttggttggttatctgttagatttttttgatgttgcgtttcgtgggggggttttccttgcgtgccccgcgtgtgttgag

E   ggggtgtcccaagctgaggagagagaacactgctgcgggcgttcgttcctcgttcctcgtatcagctactcgtatacatacgtattaatagcaaccgggcaagaacgatgaacacgagaa
F   catatacttttgtgatgtgaggctttccgtcttgttcgagtctgcagacccggccggtgctgttgtcaggagggagatggatggaacggtgtcgtgtctgtttttttcttggatgaggggg

E   aaaaatcgtgaatgcaacctgaagaaaaaagAAAAA...
F   aggaggaggaagcctagttttttttttcttgtgtgtgctccacgaaatcgagaggggagagagtgaggtgcaggcttactttgttgttgttggtgttttgttgacgatcttttggtcgaag

F   agagtgccaagcaacgaaataatgagaaacccgttttcccagAAAAA...
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Fig. 3 Complete nucleotide sequences and alignment of *M. pyrifera fcp* cDNA clones. Bases in *uppercase letters* correspond to the coding region. The nucleotide sequences presented here have been deposited in the DDBJ, EMBL and GENBANK data bases under the accession numbers U10064(pFcpB), U10065(pFcpA), U10066(pFcpD), U10067(pFcpC), U10068(pFcpF), and U10069(pFcpE)

A

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          .         .         .         .         .         .         .         .         .         .         .
MFcpA     MKSAVMAVACAAAPGLRRPSAFNGAALTTSAKSSSAMKMSFESEIGAQAPLGFWDPLGLLADADQDGFERLRYVEVKHGRIAMLAIAGHLTQQN-ARLPGMLSNSANLSFA
MFcpB     M              F G              AC                              ER                           -T
MFcpC     M   I    S  -    G                                          E    A                           -
MFcpD     ...................................       L                     ER                           -
MFcpE     .....  I         GAEP      A                    P I        V    ER D       I         VV  I   -T        FKE  A
MFcpF     ...... I         G         S P                                  ER                           -T
LAMFcp    ......................................................<         EX F                         -T           >...
DIAFcp3   M F  F SLL S AAPA---  -----QQ  RT V TN A  N L   P    F     V  G EK D       I    S   V  Y V E GI    DIDY GT-  E
AMPLHC    ......................................<X  N R V D V  F   FT  GSVEN KK AQT  I   V    TM YI  EITGK   Y  P TGVKYD
NANOLHC   ......................................<   N A VTG  EY    FS >.................................................
           ------ Pre -------                   ⇑                                       -------- I --------

          .         .         .         .         .         .         .         .         .         .         .
MFcpA     DMPNGVAALSKIPPAGLAQIFAFIGFLELAVMKNVEGSFPGDFTLGGNPFGASWDAMSEETQASKRAIELNNGRAAQMGILALMVHEELNNKPYVINDLVGASYTFN>
MFcpB                                                                                                                >
MFcpC                   G      G                           AS       A                                       L    N   >
MFcpD                   G      G                           AS           E                                   L    N   >
MFcpE      S      F     L TL  ILA  CH  F V Q        C T   I QSA  N                         M    Q S Q  I    A  A Q   >
MFcpF                   G      G                     II    ASS   S  S                      GM     S Q  IT   L        >
LAMFcp    .....................................................<     R E  -             >............................
DIAFcp3    SI   F   TT SG  I   V            DIT GEFVG RNDFID G-- SFD    KMQ       Q              Q GSLIPN>
AMPLHC     DI   LG I  V A  WG MI YAA S  >
PEPFRAG   ................................................................<                     GG  S                >
                ------------ II ----------                                ------------ III --------
```

B

```
                  .  *.     *    . ..
MFcpB    I      RLRYVEVKHGRIAMLAIAGHLTQQ
         III    SKRAIELNNGRAAQMGILALMVHE
DFcp3    I      RLRYVEIKHGRISMLAVAGYLVQE
         III    QKRAIELNQGRAAQMGILALMVHE
TCab3C   I      KNRELEVIHCRWAMLGALGCVFPE
         III    ELKVKEIKNGRLAMFSMFGFFVQA
TCab6A   I      RYKESELIHCRWAMLAVPGIIVPE
         III    ELKVKEIKNGRLALLAFVGFCVQQ
```

Fig. 4A, B Amino acid sequence alignments. **A** Homology between *M. pyrifera* Fcps (MFcpA-F), *Laminaria saccharina* Fcp peptides (LAMFcp; Douady et al. 1994), *P. tricornutum* Fcp3 (DIAFcp3; Grossman et al. 1990), and N-terminal sequences from *Amphidium carterae* LHC (AMPLHC; Hiller et al. 1993) and *Nannochloropsis* sp. LHC (NANOLHC; Sukenik et al. 1992) and the Lys-C peptidase fragment from *M. pyrifera* (PEPFRAG). The amino acids are designated by the one-letter code and have been aligned with introduced gaps (–) to maximize similarity. Only amino acid residues that are different from those of cDNA clone pFcpA are shown. *Underlined regions* correspond to potential membrane spanning regions. The *arrow* under position 40 indicates the N-terminus of the mature polypeptides from *P. tricornutum, Nannochloropsis* sp. and *A. carterae* and the presumed N-terminus for *M. pyrifera* Fcps. **B** Alignments of conserved regions of Fcp and chlorophyll *a*/*b* binding (Cab) polypeptides corresponding to the first (I) and third (III) membrane spanning regions. Sequences shown are *M. pyrifera* FcpB (MFcpB I, III), *P. tricornutum* Fcp3 (DFcp3; Grossman et al. 1990) and tomato Cab 3C (TCab3C I, III; Pichersky et al. 1985) and 6A (TCab6A I, III; Hoffman et al. 1987). Amino acids marked by *stars* are 100% conserved in all Fcp and Cab polypeptides. *Dots* include conservative substitutions

tricornutum Fcp polyclonal antibody. From over 100 immunologically positive clones, 5 were purified to homogeneity. Clone pFcpF, containing the largest insert, was used as a DNA probe to rescreen the cDNA library and obtain 20 additional clones. Based on restriction endonuclease maps (Fig. 2), the clones were divided into six groups. The cDNA clone from each group that contained the largest insert was sequenced. Additional clones from each of the six groups were partially sequenced to confirm their identity. The inserts from the six groups of clones were of two size classes. Four of the clones (pFcpA, pFcpB, pFcpC, pFcpD) had inserts of approximately 1.2 kb, while two of these clones (pFcpE, pFcpF) had inserts of approximately 1.6 kb (Fig. 2).

The nucleotide sequences of the six cDNA clones are presented in Fig. 3. Three clones were predicted to have inserts with complete open reading frames (ORFs) of 654 (pFcpA, pFcpB) or 651 (pFcpC) bases. Three other clones (pFcpD, pFcpE, pFcpF) were missing short regions of the 5' ends. The clones containing the complete coding region predict polypeptides of 217 (pFcpA, pFcpB) or 216 (pFcpC) amino acids with molecular masses of approximately 23 kDa and a predicted pI of 5.0–5.5. The 3' untranslated regions from the different genes vary in size from approximately 300 bp to 1000 bp (Fig. 3). No recognizable sequence homology or secondary structure patterns were evident in this region among the different genes. Examination of multiple cDNAs of each gene suggests variation in the length of the 3' untranslated region, with some clones being over 100 bases shorter at the position of poly(A) attachment (i.e. pFcpA, data not shown). The 3' untranslated regions occasionally exhibit G, C, T or A rich sequences with as many as 12 consecutive bases of a single nucleotide.

The predicted amino acid sequences of five of the *M. pyrifera* Fcp clones (pFcpA, pFcpB, pFcpC, pFcpD, pFcpF) are over 90% identical (Fig. 4). FcpE is least similar to the others with 80% identity. Two Fcp peptide fragments from *L. saccharina* are also 90% identical to corresponding regions of *M. pyrifera* Fcps. *P. tricornutum* Fcp3 is 54% identical to the predicted amino acid sequence of *M. pyrifera* clone pFcpA. The *M. pyrifera* Fcps are 12 amino acids longer at the C-terminus than *P. tricornutum* Fcps. Many of the amino acid differences between *P. tricornutum* and *M. pyrifera* Fcps represent conservative substitutions. A peptide fragment isolated after Lys-C protease digestion of purified *M. pyrifera* Fcp is virtually identical to the C-terminal sequences of FcpA-D (Fig. 4A). All six ORFs have a strong codon bias (Table 1); 57% of the third codon positions are C, 33% are G, and the remainder are either T (8%) or A (2%). Ten codons containing A or T in the third position are not utilized.

A hydropathy plot of *M. pyrifera* FcpB amino acid sequence (Fig. 5) predicts four membrane spanning regions (designated Pre, I, II, and III in Figs. 4 and 5). The regions of highest amino acid homology between *M.*

Table 1 Combined *M. pyrifera* Fcp codon usage

TTT	Phe	2	CTT	Leu	10	ATT	Ile	9	GTT	Val	4
TTC	Phe	69	CTC	Leu	88	ATC	Ile	55	GTC	Val	10
TTA	Leu	0	CTA	Leu	0	ATA	Ile	0	GTA	Val	3
TTG	Leu	5	CTG	Leu	24	ATG	Met	61	GTG	Val	33
TCT	Ser	3	CCT	Pro	1	ACT	Thr	1	GCT	Ala	27
TCC	Ser	53	CCC	Pro	45	ACC	Thr	27	GCC	Ala	81
TCA	Ser	0	CCA	Pro	5	ACA	Thr	0	GCA	Ala	8
TCG	Ser	36	CCG	Pro	14	ACG	Thr	6	GCG	Ala	58
TAT	Tyr	0	CAT	His	0	AAT	Gln	1	GAT	Asn	2
TAC	Tyr	18	CAC	His	18	AAC	Gln	69	GAC	Asn	43
TAA	*	3	CAA	Gln	1	AAA	Lys	0	GAA	Glu	2
TAG	*	0	CAG	Gln	47	AAG	Lys	43	GAG	Glu	68
TGT	Cys	1	CGT	Arg	4	AGT	Ser	0	GGT	Gly	14
TGC	Cys	7	CGC	Arg	35	AGC	Ser	5	GGC	Gly	90
TGA	*	3	CGA	Arg	1	AGA	Arg	0	GGA	Gly	10
TGG	Trp	12	CGG	Arg	5	AGG	Arg	1	GGG	Gly	8

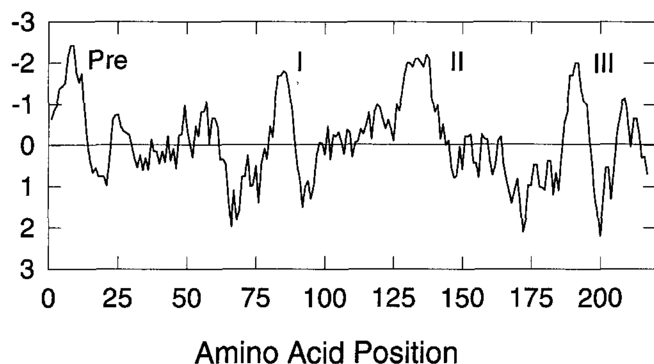


Fig. 5 Hydropathy plot of the derived amino acid sequence of Fcp cDNA clone pFcpB. The range of the scale on the y-axis is −3 (most hydrophobic) to 3 (most hydrophilic). Peaks Pre, I, II and III represent hydrophobic regions that potentially span the membrane

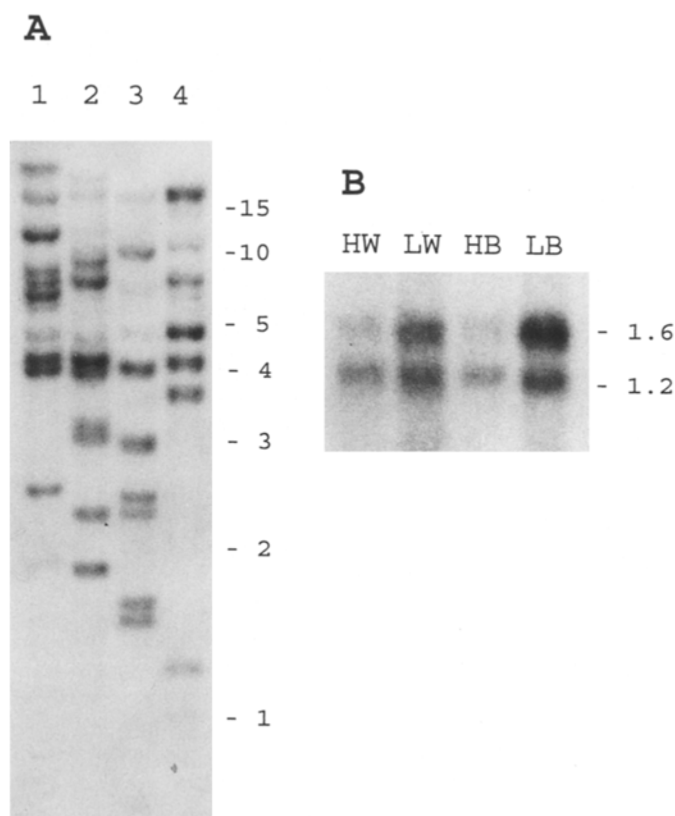


Fig. 6A, B Southern and Northern analyses. **A** Southern blot of total genomic DNA isolated from *M. pyrifera*, female gametophyte, digested with *Eco*RI (lane 1), *Pst*I (lane 2), *Sac*I (lane 3), and *Sal*I (lane 4) and hybridized with the coding region of Fcp cDNA clone pFcpB (0.6 kb *Sac*I fragment). Size markers are in kilobase pairs. **B** Northern blot of total RNA isolated from *M. pyrifera* hybridized using the same probe as for the Southern blot. Both hybridizations were at low stringency (52°C). Total RNA isolated from thalli grown in high white light (HW), low white light (LW), high blue light (HB), low blue light (LB). Light conditions as described in Materials and methods. Size markers are in kilobases

pyrifera Fcps and *P. tricornutum* Fcps are in the regions corresponding to or near the membrane spanning regions. Segments of membrane spanning regions I and III are homologous to corresponding regions of member polypeptides of the Cab family, with considerable amino acid conservation at specific positions (Fig. 4B).

The N-terminal sequence of *Nannochloropsis* sp. and *Amphidinium carterae* chlorophyll *c* containing LHC polypeptides and the *P. tricornutum* Fcps can be aligned with the N-terminus (arrow in Fig. 4A) of the *M. pyrifera* Fcps suggesting the position of the N-terminus of the mature protein. Attempts to sequence the N-terminus of *M. pyrifera* Fcp polypeptides were unsuccessful. The presumed presequence of the brown algal Fcp has a positively charged amino acid following the initiator methionine, which in turn is followed by a hydrophobic region of approximately 14 amino acids (membrane spanning region Pre in Figs. 4A and 5). The C-terminus of the presequence is more hydrophilic. These characteristics are typical of an ER signal sequence.

Total genomic DNA isolated from *M. pyrifera* and probed with a DNA fragment specific to the *fcp* coding region (clone pFcpB, 0.6 kb *Sac*I fragment) hybridized to more than six distinct DNA fragments after digestion with a variety of restriction endonucleases (Fig. 6A) that do not cut within the coding regions of the characterized genes. This suggests that there may be more than six *fcp* genes or that some of the genomic sequences have introns that contain sites for some of the restriction enzymes used in this analysis. The lane with *Sal*I-digested DNA (Fig. 6A, lane 4) had only five major hybridizing bands, leaving open the possibility that some of the

genes are clustered on the brown algal genome, as they are on the diatom genome (Bhaya and Grossman 1993).

Hybridization of total RNA with a coding region specific probe (clone pFcpB, 0.6 kb *Sac*I fragment) detects two broad signals at approximately 1.2 and 1.6 kb (Fig. 6B). Gene-specific probes that hybridize to the 3' untranslated region of the RNAs placed the different genes into one of two groups. One group (*fcpA*, *fcpB*, *fcpC*, *fcpD*) encodes transcripts of 1.2 kb, while the other group (*fcpE*, *fcpF*) encodes transcripts of 1.6 kb. The size of the hybridizing transcript corresponds to the size of its cognate cDNA. Different lengths of the 3' untranslated regions of the transcripts account for the two different transcript classes. Longer exposures reveal a third transcript of very low abundance present at approximately 3.0 kb that may represent unprocessed mRNA (data not shown).

To examine the responses of the *fcp* genes to different environmental conditions, duplicate cultures were grown at different light intensities and qualities and in medium limited for specific nutrients. Total RNA was isolated from the cultures and hybridized with probes for the *fcp* coding region or specific 3' sequences (Figs. 6, 7, 8). Thalli grown in high white or blue light contain transcripts of both 1.2 kb, and 1.6 kb (Fig. 6B, HW and HB); the former being much more abundant. Thalli grown in low white or blue light exhibit a dramatic increase in the level of the 1.6 kb transcript (Fig. 6B, LW and LB). Furthermore, thalli grown under low white or blue light consistently contained approximately twofold more of most of the individual *fcp* transcripts when compared with high intensity white or blue light (Figs. 7, 8). An exception was the *fcpD* gene which had approximately equal transcript levels under all conditions of illumination, except in the dark. The transcript

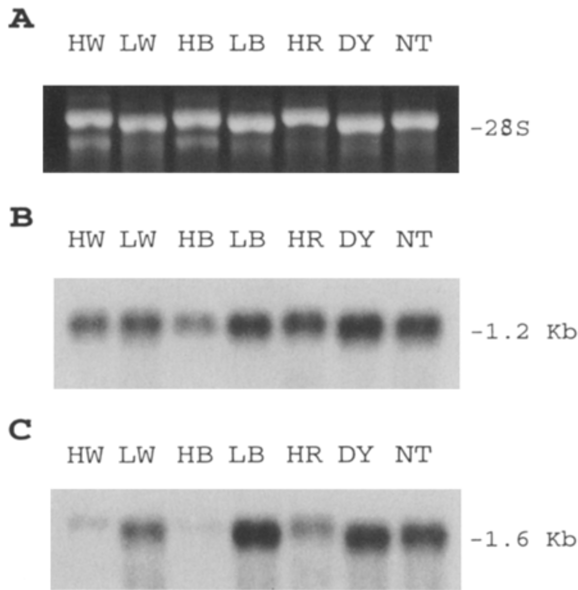
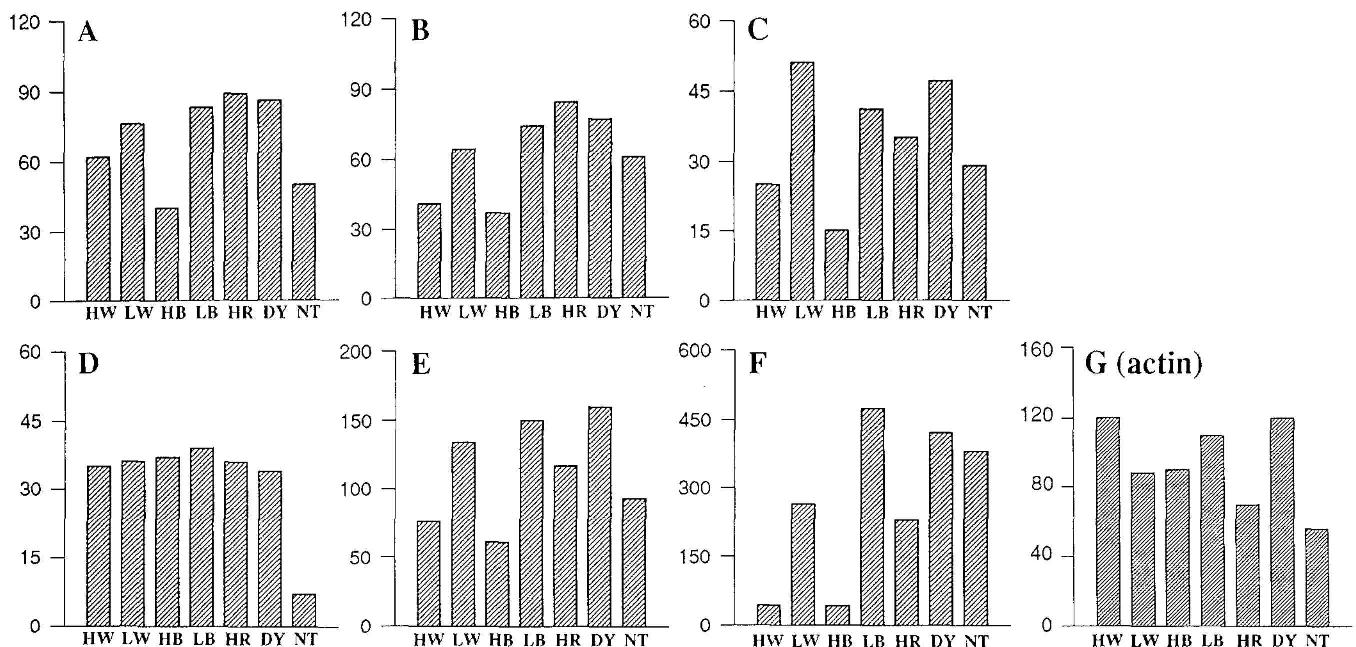


Fig. 7A–C Northern blots of total RNA isolated from *M. pyrifera*, female gametophytes grown under different light conditions as described in Materials and methods. **A** 28S RNA band stained with ethidium bromide (EtBr). **B** Hybridized with a gene-specific DNA probe to cDNA clone pFcpB (0.3 kb *Apa*I-*Sma*I fragment). **C** Hybridized with gene-specific DNA probe to cDNA clone pFcpF (0.5 kb *Hind*III-*Pst*I fragment). Lane HW, high white light; lane LW, low white light; lane HB, high blue light; lane LB, low blue light; lane HR, high red light; lane DY, medium blue light, middle of day cycle; lane NT, medium blue light, middle of night cycle. The sizes of the transcripts were determined as in Fig. 6

Fig. 8A–G Relative abundance of *M. pyrifera fcp* and actin transcripts under different light conditions. **A** *fcpA*, **B** *fcpB*, **C** *fcpC*, **D** *fcpD*, **E** *fcpE*, **F** *fcpF*, **G** actin. Levels of hybridization were quantified on a PhosphoImager (Molecular Dynamics) and are the average of two independent experiments. Quantitation of the data in **A–G** are in arbitrary units. Light conditions are described in Materials and methods. Lane HW, high white light; lane LW, low white light; lane HB, high blue light; lane LB, low blue light; lane HR, high red light; lane DY, medium blue light, middle of day cycle; lane NT, medium blue light, middle of night cycle



levels from *fcpF* appeared to be the most responsive to the light conditions; transcripts increased fivefold in low intensity white light relative to high intensity white light and tenfold in low intensity blue light relative to high intensity blue light (Figs. 7C, 8F). Thalli grown in high intensity red light consistently maintained higher transcript levels than those grown under similar levels of blue light. For example, the level of transcript from *fcpF* was fivefold higher in high red light then in equivalent levels (100 μE m^2 per second) of blue light. When mRNA was isolated from tissue grown in low intensity red light it was found to be consistently degraded and transcript levels could not be reliably determined. Transcript levels of actin mRNA did not show any consistent trend under the different light conditions, except for a 50% decline during the dark period (Fig. 8G).

The levels of transcripts from the three genes present in plasmids pFcpA, pFcpB, pFcpC were also determined under conditions in which they were limited for nitrogen and phosphate. The level of transcripts from each of these three different genes declined by a factor of five (data not shown).

Discussion

The genes encoding Fcps from *M. pyrifera* are nuclear encoded and the poly(A)$^+$ *fcp* transcripts are very abundant. When a reticulocyte lysate in vitro translation system is primed with *M. pyrifera* poly(A)$^+$ RNA, approximately 10% of the primary translation products synthesized are Fcp polypeptides. This indicates that the Fcps are the dominant species being synthesized in the cytoplasm of the cell. The Fcp primary translation product is approximately 3 kDa larger than the mature polypeptide, indicating that a post-translation cleavage of a presequence probably occurs during the maturation of the protein. These results are comparable to those observed for the diatom *P. tricornutum* (Grossman et al. 1990)

Genes encoding Fcp polypeptides from *M. pyrifera* comprise a multigene family of at least six members, as indicated by the isolation of six different Fcp cDNA clones and analysis by Southern blot hybridization. The predicted sequence similarities between the Fcps of *P. tricornutum*, *L. saccharina* peptide fragments and *M. pyrifera* are high (>50% identity) suggesting a close evolutionary relationship between the brown alga and diatom *fcp* genes. Phylogenetic analysis of 5S RNA (van de Peer et al. 1990) and 18S RNA (Medlin et al. 1988; Bhattacharya et al. 1992) sequences have also indicated that brown algae and diatoms are closely related evolutionarily. However, brown algal Fcps differ from those of diatoms by having a 12 amino acid extension at the C-terminus and a longer N-terminal presequence.

Like the diatom Fcps, those of *M. pyrifera* have three predicted membrane spanning regions in the mature polypeptide. The first and the third membrane spanning regions have similarity to corresponding regions of Cab polypeptides from higher plants and green algae, and contain residues (arginine, asparate) that are absolutely conserved among all of the Cab polypeptides. These conserved residues have been identified as important for stabilizing the tertiary structure of the protein and for chlorophyll binding (Kühlbrant and Wang 1991; Kühlbrant et al. 1994). The data further support the proposal that Fcps, Cabs and probably other LHC type polypeptides are functionally related and share a common ancestor (reviewed in Green and Pichersky 1994).

The primary translation product of the *M. pyrifera* Fcps is 3–4 kDa larger than the mature protein. The exact location of the N-terminus of the mature protein is not known, but it probably begins at amino acid 40, which corresponds to the N-termini of Fcps from *P. tricornutum* (Grossman et al. 1990) and analogous LHC polypeptides from *Nannochloropsis* sp. (Sukenik et al. 1992) and *A. carterae* (Hiller et al. 1993). A methionine immediately precedes the final cleavage site of all *P. tricornutum* Fcps. *M. pyrifera* Fcps have a methionine in a similar position relative to the conserved presequence of the mature protein, suggesting that a methionine at the −1 position may be an important functional requirement for proper processing.

Also like diatoms, the *M. pyrifera* Fcps possess a presequence with similarities to ER signal sequences (see Bhaya and Grossman 1991; Apt et al. 1994, for detailed discussion). Brown algae, diatoms and other chromophytes have plastids that are completely surrounded by a second set of membranes referred to as the plastid ER (Gibbs 1981). Nuclear-encoded plastid proteins must first transverse the plastid ER prior to import into the organelle. These proteins are probably synthesized on ribosomes bound to the plastid ER and the signal sequence facilitates the passage of the proteins into the ER lumen. Following this initial targeting event a second signal, presumably located in the second half of the presequence, facilitates the movement of the proteins across the plastid envelope. The second half of the presequence has a high proportion of serine and/or threonine and a low proportion of acidic amino acids. These characteristics are similar to those of chloroplast transit peptides of terrestrial plants (Keegstra 1989; De Boer and Weisbeck 1991). Hence, the presequence of the Fcps from *M. pyrifera* is probably bipartite: the first portion functions as a signal sequence while the second is analogous to a transit peptide. A bipartite presequence also appears to be present in nuclear-encoded plastid genes in diatoms, although the domain that may be involved in the passage of proteins across the plastid envelope is reduced in diatom Fcps (Bhaya and Grossman 1993; Apt et al. 1994).

The *M. pyrifera* gametophyte *fcp* transcripts are of two sizes, 1.2 and 1.6 kb. The size difference is accounted for by the length of the 3' untranslated region, which can be up to 1000 bases. There is no similarity in the primary structure of the 3' regions in the six *fcp* genes described here. Furthermore, there are no recognizable secondary structure similarities among the 3' untranslated regions of the different mRNA species. The 3' untranslated region may control transcript stability or in-

fluence the translation efficiency of the mRNA, as has been observed in other eukaryotic organisms (reviewed in Jackson and Standart 1990). cDNA clones encoding actin from *M. pyrifera* (B. Goodner, personal communication) and tubulin from the brown alga *Ectocarpus variabilis* (Mackay 1991) also have long 3' untranslated regions. These regions may be characteristic of many brown algal genes. In contrast, *fcp* transcripts from the diatom *P. tricornutum* have short 3' untranslated regions (Grossman et al. 1990).

The abundance of *fcp* transcripts from *M. pyrifera* gametophytes is inversely correlated with light quantity. Transcript levels of individual genes in the 1.2 kb size class increase approximately twofold when thalli are transferred from high to low light. The greatest increase was for the 1.6 kb transcript from *fcpF* which increased tenfold in low light. Elevated levels of transcripts from this specific gene would contribute significantly to the large increase observed in the 1.6 kb transcript size class under low light conditions. Transcripts of the 1.6 kb size class change from a minor component to over 50% of the total *fcp* message when cells are transferred from high to low light. Changes in the relative proportion of individual *fcp* transcripts presumably reflect changes in the proportion of the Fcp proteins themselves. Furthermore, the two members of the 1.6 kb size class (*fcpE* and *fcpF*) are the most divergent of the Fcps that have been characterized. What advantage a particular Fcp might have under specific light conditions is not known, although it might bind more pigment molecules and assemble into a complex that is more efficient in light harvesting.

The mechanisms that control transcript levels are not known. It is possible that the level of transcript is controlled by changes in the cellular energy balance (photosynthesis) that result from changes in the rate of photosynthetic electron transport. This mode of control is believed to occur in a number of photosynthetic organisms (Melis et al. 1985; Kindle 1987; LaRoche et al. 1991).

The large changes observed in the level of the *fcpF* transcript, particularly in blue light, suggest another level of control besides energy balance. This is suggested by the finding that high intensity red light is not nearly as effective in suppressing *fcp* transcript accumulation as high intensity blue light. This is particularly apparent in the analysis of *fcpF* mRNA levels. Numerous blue light responses have been described for brown algae, including some for *Macrocystis* (reviewed in Dring 1987). For example, gametogenesis of *M. pyrifera* is known to be dependent on blue light (Lüning and Neushul 1978). Additional research will help in evaluating the nature of blue light receptors involved in developmental processes in brown algae.

Blue light regulated developmental processes have been extensively studied in higher plants (reviewed in Jenkins et al. 1993; Senger 1984) and several nuclear genes encoding plastid proteins (i.e. *cab*) have been shown to be regulated by blue light (Marrs and Kaufman 1989). A number of different blue light photoreceptors that control a variety of processes have been postulated to exist in higher plants (Kaufman 1993). Recently, a putative blue light photoreceptor (*hy4*) has been cloned from *Arabidopsis* (Ahmad and Cashmore 1993). It would be interesting to define the relationship of blue light receptors in brown algae to those in higher plants.

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