Light-Regulated Transcription of Genes Encoding Peridinin Chlorophyll *a* Proteins and the Major Intrinsic Light-Harvesting Complex Proteins in the Dinoflagellate *Amphidinium carterae* Hulburt (Dinophycae)[1]

Changes in Cytosine Methylation Accompany Photoadaptation

Michael R. ten Lohuis* and David J. Miller

Department of Biochemistry and Molecular Biology, James Cook University of North Queensland,
Townsville, Queensland 4811, Australia

In the dinoflagellate *Amphidinium carterae*, photoadaptation involves changes in the transcription of genes encoding both of the major classes of light-harvesting proteins, the peridinin chlorophyll *a* proteins (PCPs) and the major *a*/*c*-containing intrinsic light-harvesting proteins (LHCs). PCP and LHC transcript levels were increased up to 86- and 6-fold higher, respectively, under low-light conditions relative to cells grown at high illumination. These increases in transcript abundance were accompanied by decreases in the extent of methylation of CpG and CpNpG motifs within or near PCP- and LHC-coding regions. Cytosine methylation levels in *A. carterae* are therefore nonstatic and may vary with environmental conditions in a manner suggestive of involvement in the regulation of gene expression. However, chemically induced undermethylation was insufficient in activating transcription, because treatment with two methylation inhibitors had no effect on PCP mRNA or protein levels. Regulation of gene activity through changes in DNA methylation has traditionally been assumed to be restricted to higher eukaryotes (deuterostomes and green plants); however, the atypically large genomes of dinoflagellates may have generated the requirement for systems of this type in a relatively "primitive" organism. Dinoflagellates may therefore provide a unique perspective on the evolution of eukaryotic DNA-methylation systems.

In many respects dinoflagellates are an unusual group of chromophytic algae, their closest relatives being the apicomplexans (van der Peer et al., 1993). For example, uniquely among eukaryotes, their chromatin appears to be entirely devoid of histones (Rizzo, 1981, 1991) and is not organized in the normal nucleosomal structure (Herzog and Soyer, 1981). Light harvesting is mediated in dinoflagellates by two classes of proteins, the PCPs and the LHCs. PCP, a water-soluble protein containing the carotenoid peridinin, is unique to dinoflagellates and appears to be unrelated to any other light-harvesting protein (Norris and Miller, 1994), whereas the LHCs are related to the cab proteins of higher plants (Hiller et al., 1993) and their algal homologs (for review, see Grossman et al., 1990). Both PCPs and LHCs are present in multiple forms in dinoflagellates. The functional significance of this and the nature of the interaction between these two classes of light-harvesting proteins are completely unknown. Chromophyte chloroplasts appear to lack many of the mechanisms known to be central to photoadaptation in chlorophytes, such as lateral mobility of photosynthetic complexes and stacking/unstacking of thylakoids. One hypothesis is that the multiple forms of light-harvesting proteins fulfill related roles in dinoflagellates, i.e. that the synthesis of different PCP and LHC complements facilitates photoadaptation.

In *Amphibinium carterae*, both PCPs and LHCs are encoded by nuclear genes (Hiller et al., 1995; Sharples et al., 1996) and synthesized with N-terminal transit peptides directing chloroplast translocation (Norris and Miller, 1994; Hiller et al., 1995; Sharples et al., 1996). As in the case of *Euglena gracilis*, the *A. carterae* LHC mRNA encodes a polyprotein. The PCPs are encoded by discrete mRNAs, but at least some of the genes appear to be tandemly organized (Hiller et al., 1995).

At this time, almost nothing is known about the molecular basis of photoadaptation in dinoflagellates. PCP may account for up to 95% of the total cellular-soluble protein under low-light conditions (Prezelin, 1987), and there is some indirect evidence that PCP synthesis is light regulated at the level of transcription in *Glenodinium* sp. (Roman et al., 1988). As stated above, the LHCs are the dinoflagellate homologs of the cab proteins and, by analogy with higher plants (for review, see Anderson, 1986) and green algae (Kindle, 1986), light regulation is likely.

In green plants light-regulated transcription of the cab genes is mediated via phytochrome; response elements

* Corresponding author; e-mail michael.tenlohuis@jcu.edu.au; fax 61–77–25–1394.

Abbreviations: 5-MeC, 5-methylcytosine; LHC(s), chlorophyll *a*/*c*-containing intrinsic light-harvesting protein(s); PCP(s), peridinin chlorophyll *a* protein(s).

have been identified in the promoters of the cab genes (Terzaghi and Cashmore, 1995). However, phytochrome has been detected only in chlorophytes, implying that the regulation of synthesis of light-harvesting proteins may be achieved via alternative mechanisms in other algal groups. In the chrysophyte *Giraudyopsis stellifer*, synthesis of the chlorophyll *a/c* protein is light regulated at the level of transcription (Passaquet and Lichtl, 1995), whereas in *E. gracilis*, LHC synthesis is apparently regulated posttranscriptionally (for review, see Houlne and Schanz, 1993). How these effects are mediated in the apparent absence of the phytochrome system is unknown at this time.

Preliminary studies in our laboratory suggested the presence of significant levels of 5-MeC at CpG motifs in genomic DNA from the dinoflagellate *A. carterae*. Many studies suggest that a major function of this type of methylation in eukaryotes is in the regulation of gene expression (for review, see Lewis and Bird, 1991). Transcriptionally inactive regions of chromatin are generally highly methylated at CpG motifs (Keshet et al., 1986; for review, see Lewis and Bird, 1991), whereas active chromatin is often undermethylated (Antequera et al., 1989; Kochanek et al., 1993). Moreover, for many genes CpG methylation status reflects transcriptional activity.

Although it is not clear whether methylation is a cause or a consequence of alterations in gene activity, a number of lines of evidence suggest that the effects of methylation may be mediated via modifications of chromatin structure. For example, introduced DNAs generally adopt chromatin structures specified by their methylation status (Keshet et al., 1986; Buschhausen et al., 1987). Methylation may affect chromatin structure by modifying nucleosome assembly through the action of methylcytosine-binding proteins (Nan et al., 1997) or by a combination of processes. Regions containing 5-MeC may preferentially bind histone H1 (Ball et al., 1983; Jost and Hofsteenge, 1992) and associate with histones H3 and H4 mainly in their nonacetylated forms (Tazi and Bird, 1990), presumably promoting more condensed forms of chromatin. Methylation-specific binding proteins (Meehan et al., 1989; Lewis et al., 1992) can also mediate changes in chromatin structure.

In summary, the evolution and roles of eukaryotic DNA-methylation systems and the interactions between three processes, methylation, alterations of chromatin structure, and changes in gene activity, are not well understood. In this respect, dinoflagellates may be highly informative because, although otherwise typical eukaryotes, they are unique in posessing permanently condensed chromosomes and in lacking histones (Rizzo, 1981, 1991) and nucleosomes (Herzog and Soyer, 1981).

To investigate the possible involvement of DNA methylation in the regulation of gene expression in dinoflagellates, we examined the methylation status of *A. carterae* PCP and LHC genes under different light conditions. Photoadaptation involved major changes in the levels of transcripts encoding both of these classes of light-harvesting proteins. DNA from *A. carterae* was normally highly methylated at CpG motifs and significantly methylated at CpNpG motifs. However, when grown under reduced light conditions, elevated mRNA levels were accompanied by decreases in the methylation of CpG and CpNpG motifs within or near PCP- and LHC-coding regions. Therefore, cytosine-methylation levels are nonstatic in *A. carterae* and may be influenced by environmental factors. However, chemically induced undermethylation was found to be insufficient for gene activation. These results provide the first evidence to our knowledge that the relationship between cytosine methylation and transcriptional activity, which has been observed for a large number of genes in a wide range of eukaryotes, does not require histones and nucleosomes.

MATERIALS AND METHODS

Source and Maintenance of Cultures

A unialgal culture of *Amphidinium carterae* (Cs-21) was obtained from the Microalgae Culture Collection (Commonwealth Scientific and Industrial Research Organization, Division of Fisheries, Hobart, Tasmania). Cells were grown axenically in (0.4 μm) filtered seawater (Millipore) supplemented with Provasoli's enrichment solution (Sigma). Generally, cultures (500 mL) were grown at 18°C in 1-L Erlenmeyer flasks without agitation until the density reached 0.5 to 3.0 × 10^6 cells mL^{-1}. Cells were normally grown on a 14-h/10-h light-dark cycle at 80 to 100 μmol m^{-2} s^{-1} (two white, two warm, and two Grow-Lux tubes [Sylvania]), referred to here as high-light conditions. For low-light treatments, cells were grown under high-light conditions for 1 week before being transferred to either 18 to 20 μmol m^{-2} s^{-1} (moderate-light cultures; two Grow-Lux fluorescent tubes only) or < 2 μmol m^{-2} s^{-1} (low-light cultures) for 14 d.

DNA Extraction, Restriction, and Southern Analysis

To avoid possible interference by intrinsic circadian rhythms, cells were always harvested by centrifugation 6 h into the light phase of growth. Pellets were ground in liquid N$_2$, and genomic DNA was isolated using the standard high-salt method (Dellaporta, 1983). Purified DNA was restricted and fractionated on 0.8 to 1.2% agarose gels. After Southern transfer to Hybond-N membranes (Amersham), blots were hybridized with ^{32}P-labeled (Feinberg and Vogelstein, 1983) DNA probes. Bacteriophage λDNA was used as an internal control in restriction digests. Homologous probes for the LHC (pRGH201; Hiller et al., 1995) or the main form of the PCP genes (see figure 6 of Sharples et al., 1996) were kindly provided by R. Hiller (Macquarie University, Sydney, Australia). 16S-Like rDNA probes were generated from *A. carterae* DNA using universal PCR primers (Medlin et al., 1988). Heterologous hybridization probes (from the coral *Acropora millepora*) for the highly conserved ubiquitin CEP52 (*ub52*; Berghammer et al., 1996) and elongation factor 1α (*Ef 1α*) genes were provided by H. Berghammer (James Cook University, Townsville, Australia).

RNA Extraction, Transfer, and Hybridization

Poly(A$^+$) RNA was extracted from approximately 10^7 cells with an mRNA purification kit (QuickPrep, Pharmacia), using the manufacturer's recommended protocol. Precipitated RNA was resuspended in Tris-EDTA buffer, quantified spectrophotometrically, and stored at $-70°C$. Aliquots of mRNA (0.25–1.0 μg) were applied to nylon membranes through a slotted template following the supplier's (Schleicher & Schuell) instructions. For the preparation of northern blots, 2.0-μg aliquots of mRNA were subjected to electrophoresis in 1% agarose/formaldehyde gels prior to transfer to nylon membranes in 20× SSC. Hybridizations (6× SSC, 0.1% SDS, and 2× Denhardt's solution at 60°C for 24 h) and three washes (0.5× SSC, and 0.1% SDS at 60°C for 20 min) were conducted as described by Sambrook et al. (1989).

RESULTS

PCP and LHC mRNA Levels Increase under Low-Light Conditions

Northern analysis (Fig. 1A) and slot-blotting experiments (Fig. 1B) clearly demonstrated that the abundance of PCP and LHC mRNAs changed substantially in response to light conditions, whereas the level of the mRNAs corresponding to the housekeeping genes *ub52* and *EF1α* did not vary significantly.

Quantitative analyses indicated that the abundance of the PCP transcript was increased approximately 33- and 86-fold in cells grown in moderate- and low-light conditions, respectively, relative to cells grown under high-light conditions (Fig. 1B). Low-light conditions resulted in both quantitative and qualitative changes in the LHC mRNA population (Fig. 1A; right panel). Total LHC mRNA was approximately one order of magnitude more abundant in cells grown under lower illumination compared with cells grown under high-light conditions. Under lower illumination, two distinct LHC transcripts, approximately 3 and 6 kb in size, were detected, whereas the larger of these was not detected under high-light conditions.

In addition, significant differences in cell pigmentation were observed in response to light levels. *A. carterae* cells grown under full-light conditions were rather pale, whereas cultures grown under reduced illumination were intensely dark-brown pigmented. Four days after transfer from high-light conditions to either moderate- or low-light conditions, PCP protein levels increased from 2 to 50% of total soluble protein (data not shown).

PCP and LHC Loci Are Normally Hypermethylated at CpG Motifs and Their Methylation Status Is Modified by Light Conditions

Preliminary experiments indicated a substantial level of cytosine methylation in the genome of *A. carterae*. DNA prepared by Dellaporta's (1983) method was digestible by a range of methylation-insensitive restriction endonucleases, including *Mva*I and *Hae*III. However, the same DNA
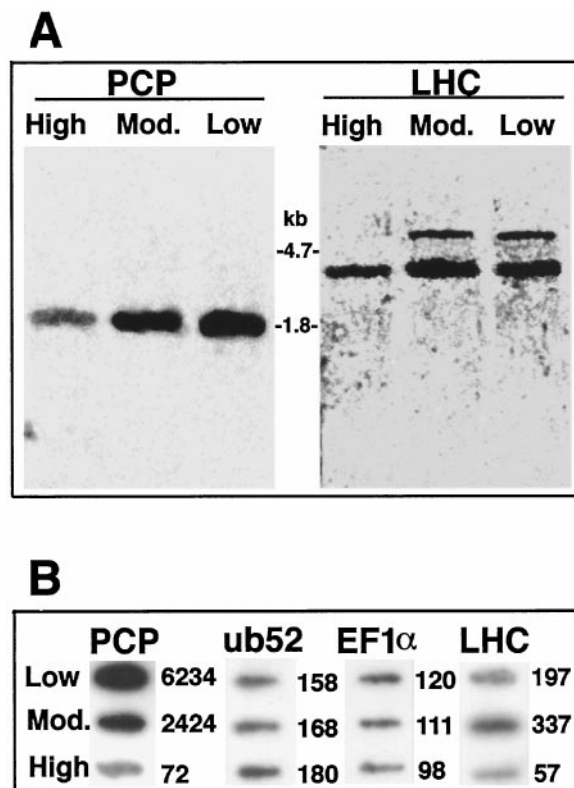

Figure 1. PCP and LHC mRNA levels are increased under reduced light conditions. A, Northern analysis of mRNA (2 μg/lane) from *A. carterae* grown under various light conditions and probed with either the insert in pRGH201 (Hiller et al., 1995) for LHC or the part of the MFPCP gene encoding the mature protein (generated by PCR; see figure 6A of Sharples et al., 1996). B, Slot blots of mRNA from *A. carterae* grown under various light conditions (High, moderate [Mod.], or Low) probed with probes for PCP or LHC (as described in A) or for the housekeeping genes *ub52* and *EF1α*. Numbers to the right of the blots are the corresponding standardized photon equivalents, determined by quantitative phosphor imaging.

preparations were highly resistant to a range of CpG-methylation-sensitive restriction endonucleases, including *Hpa*II and *Hha*I (Fig. 2). The presence of diffusible inhibitors of digestion in the DNA preparations was excluded by the use of bacteriophage λDNA as an internal control (see Fig. 2A, right panel). These results therefore imply that *A. carterae* DNA is hypermethylated at CpG motifs, as has been reported for a few higher plants (Gruenbaum et al., 1981) and algae (Jarvis et al. 1992).

To establish the extent of cytosine methylation at PCP and LHC loci, and to determine whether methylation levels varied under different light regimes, we hybridized Southern blots of restricted DNA from cells grown under various levels of illumination using homologous probes. High-light-grown *A. carterae* cells exhibited high levels of methylation at CpG motifs within *Hpa*II restriction fragments homologous to either PCP, LHC, or rDNA probes (Fig. 3). When grown under different light intensities, the methylation status of both PCP and LHC loci was variable at some CpG motifs (Fig. 3A, left and center panels). By contrast, we found no evidence for changes in the methylation status

Figure 2. DNA from *A. carterae* is highly resistant to digestion by restriction enzymes that are sensitive to CpG methylation. Aliquots (10 µg) of genomic DNA prepared from cells grown under high-light conditions were digested with a 6-fold excess of the restriction enzymes indicated and subjected to electrophoresis on 1% (w/v) agarose, and the gels were stained with ethidium bromide. Control lanes (C) were treated exactly as digests, except that restriction enzymes were not added. Lanes λH and λM correspond to restriction digests of *A. carterae* DNA to which 3 µg of bacteriophage λDNA was added. C, Methylation sensitivities and recognition specificities of the endonucleases used. "+" indicates that digestion with the restriction endonuclease is inhibited by a 5-MeC at that position. "o" indicates that digestion with the restriction endonuclease was not inhibited by a 5-MeC at that position.
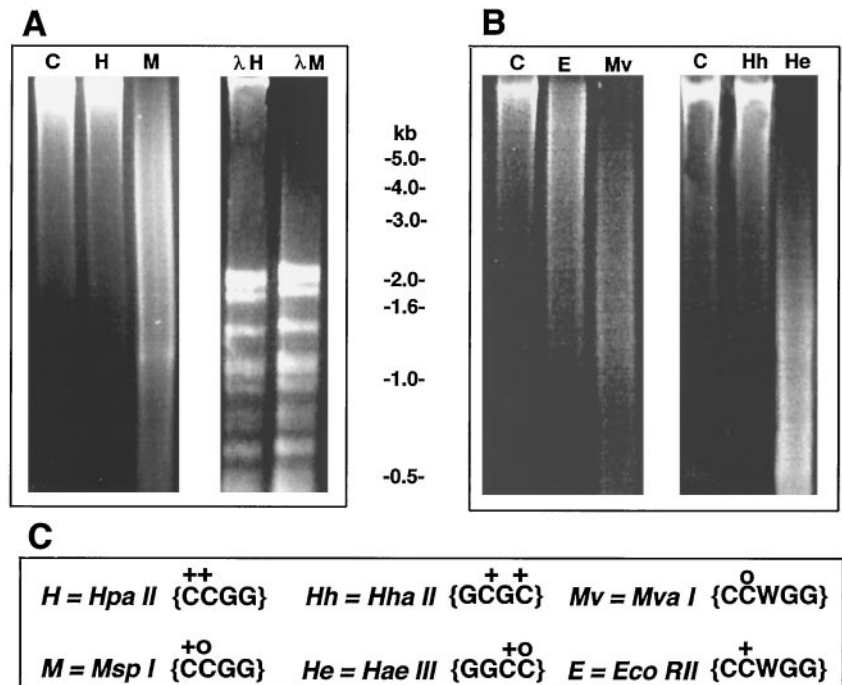
of the 16S-like rDNA in response to light levels (Fig. 3A, right panel).

Under high-light conditions, all PCP and LHC loci appear to be fully methylated at *Hpa*II sites (CCGG); the single high-M_r hybridization signal indicates that 5-MeC methylation occurs at every *Hpa*II site, in all homologous loci, and in every cell present in the culture. Under lower light conditions, the presence of low-M_r (< 1000 bp) hybridizing fragments implied significant undermethylation at these same sites in both PCP and LHC loci, but not in rDNA loci. These patterns suggest a homogeneous and complete demethylation event, which is likely to involve restriction sites near or within the corresponding coding regions. However, the remaining strong hybridization signals in the high-M_r (> 10.0 kb) range suggest that either only a few members of the total cell population or a limited subset of PCP and LHC genes undergo light-related demethylation at the CpG motifs analyzed.

Heterogenous DNA-Methylation Changes at CpNpG Motifs

The complex and variable band patterns given by *Msp*I digests (see Fig. 3, left and center panel) implied significant methylation at CpNpG as well as at CpG motifs in *A. carterae*. Although *Msp*I is insensitive to methylation at the second C in its recognition sequence (CCGG), it is sensitive to methylation at the first C. Whereas CpG methylation at PCP and LHC loci appears to be relatively homogenous (see above), the wide range of restriction fragment sizes (10.0–0.3 kb) detected in high-light-grown *A. carterae* implied that CpNpG methylation was considerably more heterogeneous, either among loci or among cells. Light-related changes in CpNpG methylation in *Msp*I sites at PCP and

LHC loci were pronounced and appeared to affect the majority of cells and homologous loci.

To further investigate CpNpG methylation levels, we probed Southern blots generated using a second pair of restriction enzymes, *Eco*RII and *Mva*I, which have the same recognition sequence (CCWGG), but differ in that the former is sensitive to CpNpG methylation, whereas the latter is not. In this case, changes in *Eco*RII-specific CpNpG-methylation were observed for LHC loci only (Fig. 4).

Chemically Induced Undermethylation Does Not Alter PCP Transcript Levels

Since demethylation events near or within the PCP- and LHC-coding regions correlated with increases in the abundance of the corresponding transcripts, it was of immediate interest to determine if transcription patterns could be altered by artificial manipulation of 5-MeC levels. 5-MeC undermethylation was induced by treating cells with 5-azacytidine or ethionine. 5-Azacytidine is a base analog that is both an inhibitor of many methylases and, once incorporated, cannot be methylated at the 5-position. Ethionine is a Met analog that inhibits Met-adenosyltransferase. Culturing *A. carterae* under high-light conditions in the presence of either of these inhibitors considerably increased the susceptibility of DNA to cleavage by *Hpa*II (Fig. 5A), confirming that cytosine methylation was responsible for the observed inhibition of restriction digestion.

After growth in the presence of 5-azacytidine, significant reductions in CpG and CpNpG methylation were observed (data for PCP loci are shown in Fig. 5B). However, levels of the corresponding mRNAs did not differ significantly between controls and inhibitor treatments (Fig. 5C).
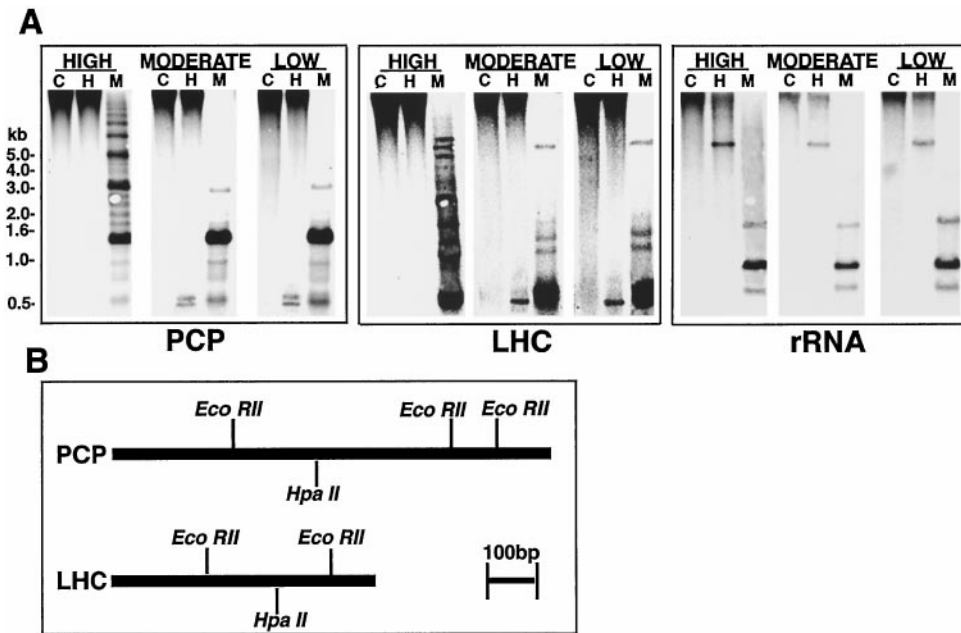
Figure 3. *A. carterae* PCP and LHC loci are normally hypermethylated at CpG motifs and their methylation status is modified by light conditions. A, Southern analysis of PCP, LHC, and rDNA loci in cells grown under different light conditions using the restriction enzymes *Hpa*II (H) and *Msp*I (M); C, controls. The sizes indicated to the left of the figure correspond to positions of DNA size standards. Note that the apparently repeating pattern seen for the *Msp*I digest of high-light-grown cells (A, left panel) is consistent with the hypothesis that several of the PCP loci are tandemly organized (Sharples et al., 1996). B, Restriction maps of single *A. carterae* PCP (based on figure 6A of Sharples et al., 1996; bp 35–1110)- and LHC (based on figure 1B of Hiller et al., 1995, bp 10–600)-coding sequences that were used as probes.

The use of azacytidine and ethionine treatment in methylation studies has sometimes been criticized on the grounds that these agents can cause pathological (i.e. unrepresentative) changes in some organisms (e.g. Juetterman et al., 1994). However, although exposure to either inhibitor reduced the growth rate of *A. carterae* by around 35 to 50% (data not shown), there appeared to be no adverse effects on morphology or development. Motility appeared to be unaltered in the presence of inhibitors, and effects on growth rates were fully reversible; transfer of azacytidine-treated *A. carterae* back into normal medium resulted in an immediate return to normal growth rates. Treated and untreated cells were indistinguishable in terms of pigmentation, and levels of both total mRNA and soluble proteins (data not shown) were shown not to differ significantly

between treatments. In summary, although we cannot completely exclude chemically induced artifacts, our results strongly suggest that simply reducing the extent of 5-MeC within or near PCP genes does not appear to affect transcription.

DISCUSSION

Light-Regulated Transcription of PCP and LHC Genes

Transcription of LHC and PCP genes is light regulated in *A. carterae*, and it appears likely that this is the major means by which levels of the corresponding proteins are controlled. This is consistent with indirect evidence from in vitro translation studies, which implied that changes in
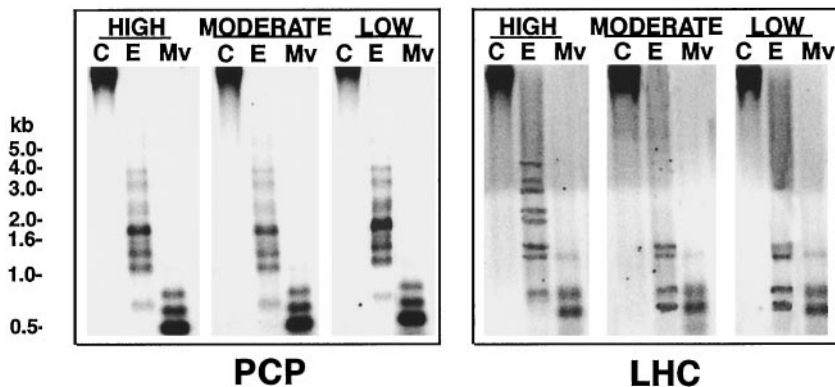

Figure 4. CpNpG methylation at LHC loci is variable in *A. carterae*. The figure shows Southern analysis of DNA from cells grown under various light conditions digested with either *Eco*RII (E; sensitive to CpNpG methylation) or *Mva*I (Mv; an *Eco*RII isoschizomer insensitive to CpNpG methylation) hybridized with the corresponding probe. Lanes C, Controls.
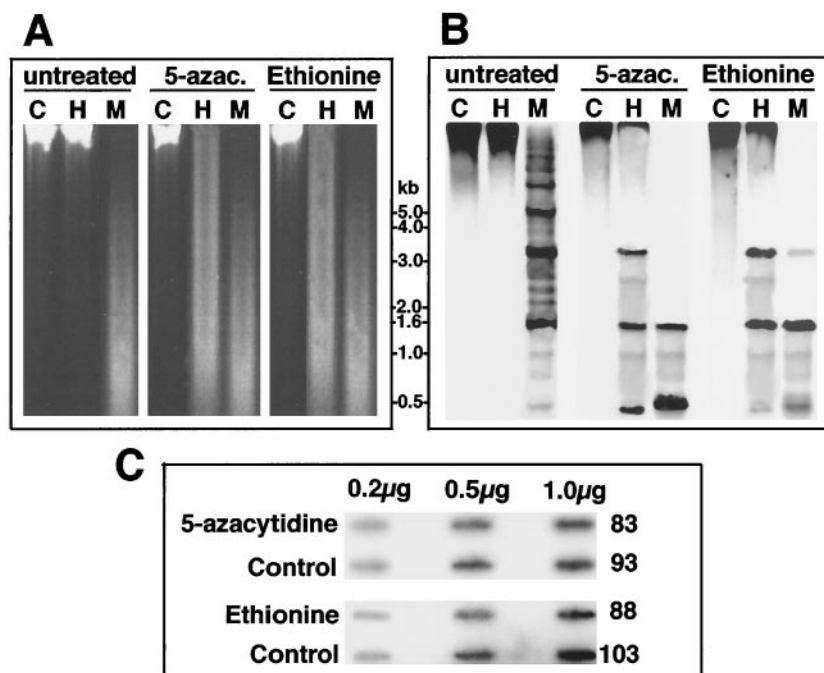
Figure 5. Chemically induced undermethylation is not sufficient to activate transcription of PCP genes in *A. carterae*. *A. carterae* cultures in the exponential phase of growth were transferred to medium supplemented with either 5-azacytidine (200 μM) or ethionine (300 μM), and grown under high-light conditions for 6 weeks, after which DNA was extracted and digested with *Hpa*II (H) or *Msp*I (M) prior to electrophoresis (A), and blotting and hybridization with the PCP probe (B). Lanes C, Controls. C, Slot blots of mRNA prepared from cells treated in the same manner and hybridized with the PCP probe. Numbers to the right of the blots are the corresponding standardized photon equivalents determined by quantitative phosphor imaging. Note that exposure of *A. carterae* to these compounds resulted in reductions in growth rates of around 35 to 50%, but appeared to otherwise have no adverse effects on morphology or development of the organisms (data not shown). Transfer of treated cells back into normal medium resulted in an immediate return to normal growth rates.

PCP protein levels correlated with mRNA abundance in *Glenodinium* sp. (Roman et al., 1988).

The size of the PCP transcript was consistent with a previous estimate (Hiller et al., 1995). The larger (6 kb) form of the *A. carterae* LHC mRNA detected here corresponds to the previously estimated size (Hiller et al., 1995). Although lower-M_r forms of the LHC mRNA have previously been observed (Hiller et al., 1995), these appeared to be very minor components. The *A. carterae* LHC-coding sequences are relatively heterogeneous (Hiller et al., 1993, 1995), and the use of different LHC probes in the previous and present studies may account for this difference; the probes have approximately 70% identity, which, under the conditions employed, may have had a significant effect on the results of northern analysis. The functional significance of the qualitative shift in the LHC mRNA population with light intensity is presently unclear. The LHC mRNA encodes a polyprotein, with the 6.1-kb form encoding possibly up to 10 LHC protein units (Hiller et al., 1995). If there is only one LHC locus, we might predict that the proteins encoded by the 3′ end of the mRNA might be required only under reduced illumination. Alternatively, multiple loci under independent regulation may encode the 3- and 6-kb forms of the LHC mRNA. Analyses of the genomic loci will be required to resolve these alternatives.

CpG and CpNpG Methylation Patterns in *A. carterae* May Be Influenced by Environmental Factors

Although no significant differences were observed between cells grown under moderate- and low-light conditions, when *A. carterae* cells grown under high-light conditions were shifted to lower light intensities, demethylation occurred at CpG and CpNpG motifs within or proximal to the LHC- and PCP-coding regions. CpG demethylation appears to be partial, affecting a limited number of methylatable sites or loci, a minor proportion of cells, or a combination of both. By contrast, light-induced CpNpG demethylation events appear to be more pronounced and uniform. Consideration of the available sequence data for *A. carterae* PCP (Sharples et al., 1996) and LHC (Hiller et al., 1995) implies that whereas changes in the methylation status of CpG motifs may be localized at or near the coding sequence, CpNpG methylation changes are likely to occur over larger regions, affecting sequences distant to those transcribed or occurring in tandemly arranged multiple copy loci. The existence of tandemly organized PCP loci has been recently documented for *Gonyaulax polyedra* (Le et al., 1997) and has been previously proposed for *A. carterae* (Sharples et al., 1996). Our apparently repeating pattern seen for the *Msp*I digest of high-light-grown cells (Fig. 3A, left panel) is consistent with this proposal.

There are many precedents for environmentally influenced changes in methylation patterns for endogenous genes (e.g. Burn et al., 1993; Galaud, 1993) or transgenes (e.g. Meyer et al., 1992); for example, light-induced demethylation of distant regulatory sites correlated with increased levels of PEPCase mRNA levels in *Zea mays* (Langdale et al., 1991). However, we were unable to find precedents for methylation changes affecting endogenous genes involved in light harvesting.

Decreased Methylation Is Not Sufficient to Activate Transcription of PCP and LHC Genes

The question of whether DNA methylation states are required for gene activity or represent consequences of genomic changes involved in the activation of chromatin is controversial. Although treatment with 5-azacytidine or ethionine reduced the extent of methylation of PCP genes, these changes were not accompanied by increased transcript levels, nor did the corresponding protein levels increase (data not shown). This indicates that simply reducing overall levels of methylation is not sufficient to activate PCP transcription in *A. carterae*. As in our study, 5-azacytidine treatment did not reactivate methylated regions in *Z. mays* (Brown, 1989); however, there are numerous reports of the opposite effect, 5-azacytidine-treatment-mediated reactivation of T-DNA in plants (Klaas et al., 1989; Kilby et al., 1992) and of a hepatitis-C cDNA transgene in mice (Kato et al., 1996) and chemically mediated demethylation initiating flowering in *Arabidopsis thaliana* and *Thlaspi arvense* (Burn et al., 1993).

There are a number of possible explanations for the failure of chemically induced undermethylation to activate *A. carterae* PCP genes. Transcription may require demethylation of specific CpG motifs that are not sufficiently affected by azacytidine or ethionine treatment. Alternatively, demethylation may be necessary but not sufficient for transcription activation, or demethylation may be a consequence rather than a cause of gene activity (Selker, 1990).

The Evolution and Possible Roles of Dinoflagellate DNA-Methylation Systems

Our studies establish the presence of significant and variable levels of CpG and CpNpG methylation in dinoflagellates, suggesting the presence of a higher-plant-like methyltransferase(s) (Theiss et al., 1987). Our data are consistent with the hypothesis that methylation systems may have arisen as a means of noise reduction in organisms with large genomes (Bestor, 1990; Bird, 1995; Jablonka and Regev, 1995). Dinoflagellates have atypically large genomes, ranging from 2 to 3 pg in *A. carterae* to > 200 pg in *G. polyedra* (Holm-Hansen, 1969; Rizzo, 1987), compared with other algae at the same apparent level of complexity (e.g. < 0.2 pg for the green algae *Chlamydomonas* and *Chlorella*; Cattolico and Gibbs, 1975; Holm-Hansen, 1969). This suggests that major amounts of heterochromatin may be present in dinoflagellates, perhaps generating a requirement for methylation systems to maintain this in a transcriptionally inactive state.

Although, in principle, dinoflagellates are attractive model organisms to study the relationship between cytosine methylation and gene activity, there are a number of major hurdles that must be overcome before their full potential can be realized. All dinoflagellate genes characterized to date are members of complex families. In addition to the multiplicity of PCPs and LHCs that have been documented for a number of dinoflagellates, there are thought to be at least 1000 copies of the gene encoding the luciferin-binding protein in *G. polyedra* (Machabee et al., 1994), and certainly tens and probably hundreds of copies of the *rbcL* gene in *Symbiodinium microadriaticum* (R. Rowan, personal communication). None of these complex multigene families has been fully characterized, and in every case probes for single genes are likely to cross-hybridize. Thus, it is not possible to relate demethylation events to active copies of genes.

Changes in the DNA-methylation status of 5'-regulatory sequences are also likely to be involved in regulation (e.g. Langdale et al., 1991); however, with the exception of one copy (of the approximately 1000 copies) of a *G. polyedra* LBP gene (Lee et al., 1993) and one copy (of the suggested 5000 copies) of a *G. polyedra* PCP gene (Le et al., 1997), sequence data are not available for any dinoflagellate promoters. Further progress in understanding the significance of DNA methylation in dinoflagellate gene regulation requires the characterization of genes or gene families and the corresponding promoters to a level that will permit the development of highly specific probes for single loci.

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