Plant Physiol. (1997) 115: 773–781

Photoregulation of Chloroplast Gene Transcription in the Chromophytic Alga *Heterosigma carterae*[1]

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Light acts as a complex regulator of cellular development and gene expression in photoautotrophs. Although light signals are highly effective in controlling cellular division and chloroplast biogenesis in the toxic marine alga *Heterosigma carterae*, their influence on gene expression has not been well characterized. To address this need cultures of *H. carterae* synchronized by an alternating light-dark regime were sampled through 12 h of light and 12 h of dark to characterize cell division, chloroplast complement, and chloroplast RNA abundance. These studies have identified a unimodal pattern of chloroplast transcriptional activity for a suite of cellular and photosynthetic genes. To determine the alga's response to a change in photoperiod, 12-h light/12-h dark-synchronized cultures were transferred to constant light and then periodically sampled. Although cellular and chloroplast division cycles remained synchronized in constant conditions for 24 h, the transcriptional apparatus responded by increasing ctRNA abundance within 45 min of the change in photoperiod. However, the ability of the alga to mount this rapid transcriptional response was limited to the first 2 h of the putative dark period. Thus, the chloroplast transcriptional apparatus of *H. carterae* may initiate a rapid, temporally gated response to a change in photoperiod that is independent of ongoing light-entrained cellular and chloroplast division cycles.

Marine plants live in a changing and often precarious environment. To survive in the upper mixed layer of the ocean these organisms must receive a photosynthetically active spectrum of light within a limited range of intensities (Falkowski, 1992). However, the composition of a light field is neither uniform nor static. The amount of light that reaches the surface of the ocean varies both periodically (e.g. seasonally) and aperiodically (e.g. changes in cloud cover). As light enters the ocean, the optical properties of the water column create a unique spectrum of light at each depth (Kirk, 1994). Algae may be rapidly mixed through these light gradients by wind or by density-driven changes in the physical characteristics of the upper mixed layer. As a result, marine plants experience cyclic and aperiodic light fluctuations that vary on time scales from seconds to seasons (for review, see Falkowski, 1984).

Chromophytic (chlorophyll *a*- and *c*-containing) algae have adapted to life in the upper mixed layer of the ocean. Periodic variations in available light not only control their photosynthetic activity but may also entrain cellular processes (e.g. RNA transcription, photosynthetic efficiency, and cell cycle progression) to a daily photocycle (for review, see Chisholm, 1981). Although entrained by this photocycle, unicellular algae must also adapt to unexpected changes in their light field. To understand how chromophytes adapt to aperiodic fluctuations of light, it is necessary to characterize the molecular mechanisms that control their light-entrained cellular processes (LaRoche et al., 1993).

Light regulation of gene expression is well characterized in terrestrial plants and green algae. Research with chlorophytes (chlorophyll *a*- and *b*-containing plants) has shown that both light quality and quantity can affect the expression of chloroplast-encoded "photogenes" (i.e. genes regulated by light; Mullet, 1993; Mayfield et al., 1995). Light-induced developmental programs often result in transient alterations in the transcription of specific genes (Baumgartner et al., 1993). The specificity of these responses may be mediated by promoter selection (Sexton et al., 1990; Bolle et al., 1996), polymerase identity (Pfannschmidt and Link, 1994), transactivation factor interaction (Hauser et al., 1996), or transcript stability (Kim et al., 1993). Both the components of the transcriptional apparatus and their response to light fields continue to be extensively characterized in chlorophytes.

Chlorophytic and chromophytic photoautotrophs experienced an early evolutionary divergence (Douglas and Turner, 1991), and as a result, the structure of chromophytic algal genomes differ significantly from those of terrestrial plants and green algae (Valentin et al., 1992). Recently published phylogenetic analyses have demonstrated that chromophytic chloroplast genomes have unique combinations of the number, identity, and organization of their plastid-encoded genes (Kowallik et al., 1995; Reith and Munholland, 1995; Stirwalt et al., 1995). Sequence analyses of individual chromophytic genes show significant structural (e.g. no identifiable introns in *Odontella sinensis*) and functional (e.g. variations in *rbcL* catalytic sites in *Heterosigma carterae*) differences from chlorophytes

[1] This research was supported by grant no. NA36RG0071AM08 (1993–1996) from the Washington Sea Grant Program and a Plant Molecular Integration and Function predoctoral fellowship (E.D.) from the University of Washington.

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Abbreviations: L:D, a photocycle containing both light and dark periods; 24L:0D, continuous light; 12L:12D, 12 h of light followed by 12 h of dark.

(Hardison et al., 1992; Valentin et al., 1992; Kowallik et al., 1995). Although the structure of chromophytic genomes has begun to be analyzed, the mechanisms that govern the response of these unique genomes to light cues have not been well studied.

H. carterae is a well-characterized unicellular chromophyte. As an obligate phototroph, this organism is dependent on light for its survival. Studies have demonstrated that light fluctuations regulate transcriptional activity (Reynolds et al., 1992), entrain and fuel cell growth and chloroplast biogenesis (Reith and Cattolico, 1985; Satoh et al., 1987), control diurnal migration and aggregation (Watanabe et al., 1983), and mediate nutrient uptake (Tomas, 1979). When *H. carterae* blooms in estuarine waters, its toxins can cause massive, precipitous mortalities in food webs (Ward and Targett, 1989). Although there is considerable literature describing light effects on the physiology and ecology of *H. carterae*, the analysis of the alga's molecular biology is still at its inception.

Recent studies of *H. carterae* have begun to document how a chromophyte may regulate the expression of its chloroplast-encoded genes. Variations in chloroplast mRNA transcript abundance throughout a growth cycle (e.g. 12L:12D) are controlled principally by transcriptional activity (Reynolds et al., 1992). Unlike terrestrial plants, no alternative promoter-usage or posttranscriptional-processing events have been reported for this alga. Rather, there appears to be a simple oscillating pattern of chloroplast gene transcription over a diel cycle. However, Reynolds et al. (1992) analyzed only a limited number of genes and did not investigate the regulatory mechanisms that control RNA transcription. The purpose of the present study was to expand the number of genes analyzed, identify the response of the chloroplast transcriptional apparatus to changes in photoperiod, and investigate the relationship between chloroplast transcriptional activity and cell cycle events.

MATERIALS AND METHODS

Culture Conditions

Axenic cultures of *Heterosigma carterae* (Hulbert) Taylor (formerly *Heterosigma akashiwo*; Taylor, 1992) were grown in 2.8-L Fernbach flasks containing 1 L of 0–3 medium (McIntosh and Cattolico, 1978). Batch cultures were shaken continuously (orbital; 60 rpm) and maintained at 20°C on a 12L:12D (cool-white fluorescent bulbs; 40 μmol photons m^{-2} s^{-1}) regime. Changes in light availability were initiated and terminated as step functions. For each experiment cellular growth was monitored daily using a Coulter counter equipped with a 100-μm aperture (ZBI, Coulter Electronics, Hialeah, FL).

Chloroplast Enumeration

The number of chloroplasts per cell was determined as previously described (Cattolico et al., 1976). Briefly, cells were pelleted in a clinical centrifuge at 3000*g* for 90 s, the supernatant was poured off, and the cells were resuspended in approximately 0.1 mL of 0–3 medium remaining with the pellet. An aliquot (20 μL) of concentrated cells was placed on a standard light microscope slide, a coverslip was applied, and then the sample was allowed to dry at room temperature. As the sample became desiccated, cells flattened into a single plane, which allowed chloroplasts per cell to be easily counted. For each time point, approximately 100 cells were analyzed at 400× magnification using a light microscope. Differences in the frequency distribution of chloroplasts per cell between cultures were analyzed using a Kolmogorov-Smirnov test (version 5.2, SYSTAT, Evanston, IL).

DNA Isolation

Total DNA was extracted by pelleting approximately 10^3 cells at 3000*g* for 4 min at 4°C and then resuspending them in 500 μL of 100 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0), 500 mM NaCl, 10 mM β-mercaptoethanol, and 1% (w/v) SDS in a microfuge tube. This mixture was incubated at 65°C for 10 min and then mixed with 25 μL of 5 M potassium acetate and chilled at 0°C. The tubes were spun in a microcentrifuge for 10 min at top speed, and then the supernatant was extracted and mixed with 640 μL of isopropanol and 60 μL of 3 M sodium acetate. After 15 min at −20°C the tubes were microcentrifuged for 30 min, the supernatant was discarded, and the pelleted DNA was resuspended in 200 μL of 50 mM TE (0.01 M Tris and 0.001 M EDTA). The extracted DNA was mixed with 1.5 mL of ethanol and 20 μL of 3 M sodium acetate and stored at −20°C until needed.

Homologous Probe Labeling

PCR primers specific to *H. carterae* were used to produce digoxigenin-labeled internal fragments for a suite of photosynthetic, regulatory, and metabolic genes (Table I). The PCR reaction mixture contained 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 2 mM $MgCl_2$, 0.001% (w/v) BSA, 0.2 mM deoxyribonucleotide triphosphates, 4 mM each primer, 5 ng of *H. carterae* total DNA, and 2 units of *Taq* polymerase (Stratagene). The samples were "hot started" at 94°C for 1 min and then incubated in 30 cycles for 1 min at 94°C, for 2 min at 65°C, and for 3 min at 72°C (Innis et al., 1994). The specificity of these reactions was verified by running the PCR products on 1% (w/v) agarose gels stained with ethidium bromide and identifying the appropriately sized DNA fragments (Maniatis et al., 1982). Templates were purified by excising the band of interest, eluting the DNA, and precipitating the product with ethanol (Maniatis et al., 1982). Approximately 50 ng of each purified template was then added to a single PCR reaction mixture made with digoxigenin-labeled deoxyribonucleotide triphosphates. These PCR reactions were thermocycled as described above to create a suite of gene-specific, digoxigenin-labeled probes. The probes were then mixed with hybridization fluid containing 50% (v/v) deionized formamide, 5× SSC (150 mM NaCl and 15 mM sodium citrate), 0.1% (w/v) sodium lauryl sarcosine, 0.02% (w/v) SDS, and 2% (w/v) blocking reagent (Boehringer Mannheim) and stored at −20°C. The specificity of these probes was optimized by

Table I. *PCR primers and fragments used to generate H. carterae ctDNA probes*

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Fragment Size
			kb
clpC	GCTTTTGAGTGTCTCTTGCG	CTGGGGTTAGGAAAACGG	1.4
petB/D	CGATTCCAATCCTAGTTGTAAG	CCTTCTTAATTTATCGAATTG	2.0
psbA	ATGACTGCAACTTTAGAA	AATATGAAATTGAATCTG	1.4
rbcL	ATGTCTAACAACGTATAC	TGGGTTTGATGTAGCAGT	1.2
rbcS	GTGAGACTTACACAAGGAGC	AATATCGTCAGGTAC	1.2
rpoB	GGGTCTTCCATCTCTCG	GTAGGATCAAAAATTCG	0.9
rRNA 16S	GCTCAGGATGAACGCTGG	GTGATCCAGCCGCACCTT	1.4

analyzing their binding to template and foreign DNA under various temperature and washing regimes.

RNA Extraction and Analysis

Replicate *H. carterae* cultures were grown to logarithmic phase (6×10^4-8×10^4 cells/mL) with one flask designated for each time point. At the time of harvest an aliquot containing 5×10^7 cells was recovered by centrifugation at 3000*g* for 4 min at 4°C. Cells were lysed in 1 mL of guanidine isothiocyanate solution (4 M guanidine, 25 mM sodium citrate, 0.5% [w/v] sarcosyl, 0.1 M 2-mercaptoethanol) and then stored at −20°C. Total RNA was extracted and purified from each point at the completion of the experiment (Maniatis et al., 1982; Chomczynski and Sacchi, 1987). Approximately 15 μg of the purified RNA was denatured for 20 min at 68°C in a solution that contained 50% (v/v) formamide, 7% (v/v) formaldehyde, and 1× SSC. The RNA was cooled to 0°C, mixed with 2 volumes of 20× SSC, blotted onto nitrocellulose paper (S & S Minifold II, Schleicher & Schuell), and then cross-linked with UV light (UV Cross-Linker, Stratalinker, Stratagene).

Protocols and reagents for the chemiluminescent identification of RNA were supplied as components of the Genius system (Boehringer Mannheim). The blots were prehybridized for 1.5 h in hybridization solution (as previously described) at room temperature and then hybridized approximately 18 h with chemiluminescence-labeled homologous gene probes. The blots were washed twice for 15 min in 2× SSC that contained 0.1% (w/v) SDS and twice for 5 min in 0.5× SSC that contained 0.1% (w/v) SDS. After the blots were exposed to a blocking solution for 1 h, anti-digoxigenin antibodies were added to the solution and incubated for 30 min The blots were washed twice for 15 min in 0.1 M maleic acid and 0.15 M NaCl (pH. 7.5), rinsed for 30 s in 0.1 M Tris-HCl (pH. 9.5), 0.1 M NaCl, and 50 mM $MgCl_2$, and incubated with the chemiluminescent substrate (Lumiphos 530). The labeled blots were exposed to x-ray film (Kodak X-Omat AR) for 1 to 12 h. The developed films were digitized using a flatbed scanner and the resulting image was analyzed with National Institutes of Health Image software. This software was used to identify individual bands and to analyze the intensity of the chemiluminescent signal for each gene at every time.

In Vivo Fluorescence

The functioning of the photosynthetic electron transport pathway was monitored by measuring active fluorescence (Falkowski and Kolber, 1993). For each time point, replicate 5-mL samples were removed from each culture and dark-adapted for 20 min. Next, 4 mM 3-(3,4-dichlorophenyl)-1,1-dimethylurea was added to one of the samples (to determine maximum fluorescence), and the second sample was unamended. Both samples were then analyzed using a fluorometer (Turner Designs, Sunnyvale, CA). The fluorometer was configured with a fluorescent lamp (F4T5), blue excitation filter (CS 5–60, Corning, Corning, NY), and a red emission filter (CS 2–64, Corning) to measure chlorophyll fluorescence (Cleveland and Perry, 1987). These analyses were done in triplicate for each time point. PSII photochemistry was assumed to be proportional to the difference between maximal and initial fluorescence (i.e. variable fluorescence) scaled to the maximal fluorescence (Van Kooten and Snell, 1990).

RESULTS

Alternating Light Regimes Control the Timing of Cell and Chloroplast Division

As an obligate autotroph, *H. carterae* requires a finite complement of functional chloroplasts to survive. For this reason, chloroplast and cell division must be temporally constrained to ensure that adequate numbers of daughter plastids are present in each cell during rapid growth. When cellular and chloroplast division were monitored during a 12L:12D photocycle, the data in Figure 1 were obtained. As shown, cell and chloroplast division occurred during a restricted period of the 12L:12D dark cycle (4 h of dark to 1 h of L and 8 h of L to 8 h of D, respectively). To determine whether these division cycles would respond to an extended light period, the patterns of cell and chloroplast division were compared between experimental (24L:0D) and control (12L:12D) cultures. No significant differences in the phase, period, or amplitude of either cell or chloroplast division were observed (Fig. 1). When the pattern of chloroplast division was analyzed in control (12L:12D) and experimental (24L:0D) cultures, both the means and the frequency distributions of chloroplasts per cell showed no significant statistical difference at any time examined.

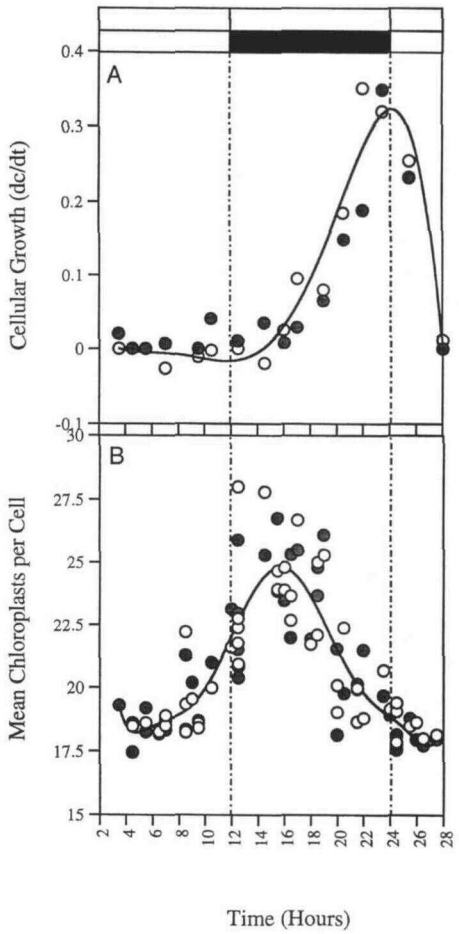

Figure 1. Cell and chloroplast division in 24L:0D and 12L:12D regimes. Replicate cultures were grown to logarithmic phase under an alternating 12L:12D regime and then either transferred to 24L:0D (○) or maintained in 12L:12D (●). Data in each graph represent either a change in cell division per unit time (A) or variations in the mean number of chloroplasts per cell (B). Data representing one (A) to four (B) individual experiments are presented. White bar, Light; black bar, darkness.

These data suggest that cell and chloroplast division cycles are strongly entrained by an alternating L:D regime.

Light Regimes Determine the Pattern of CtRNA Abundance in a Diel (12L:12D) Cycle

Transcript abundance levels were analyzed for a suite of photosynthetic and metabolic genes to identify the pattern of chloroplast mRNA abundance that occurred in a 12L:12D cycle. RNA was isolated from the same number of cells approximately every 2 h, purified, and hybridized with homologous chemiluminescent gene probes. These probes were derived from *clpC* (putative *clp* protease ATP-binding subunit), *petB/D* (Cyt *b6/f*), *psbA* (PSII reaction center D1 protein), *rbcL* and *rbcS* (Rubisco large and small subunits, respectively), *rpoB* (RNA polymerase B subunit), and rRNA *16S* (rRNA) genes. rRNA transcript abundance did not vary over the L:D cycle and, therefore, acted as an internal control. The chemiluminescent signal for each gene was scaled first to the rRNA signal (which was invariant) and then as a fraction of the gene's highest signal. These studies showed a single oscillating-abundance profile that increased before the onset of light, peaked early in the light period, decreased before the onset of dark, and reached a minimum at the beginning of the dark period, after which mRNA again began to slowly accumulate (Fig. 2).

The Pattern of CtRNA Abundance Responds to a Change in Photoperiod

To determine whether the oscillating pattern of ctRNA abundance shown in Figure 2 is temporally fixed or can be altered by a change in light regime, the following experiment was performed. RNA abundances for a suite of genes were analyzed in cultures that were given a second 12 h of light rather than 12 h of dark. As shown in Figure 3, the pattern of *psbA* RNA abundance differed significantly between the 12L:12D and 24L:0D cultures despite the entrainment of their cellular and chloroplast division responses. Similar to *psbA*, *clpC* and *petB/D* RNA abundances increased significantly (Fig. 4A), whereas *rbcL* and *rbcS* RNA abundances remained relatively constant after exposure to the second 12 h of light (Fig. 4B). These results demonstrate that the oscillations in mRNA abundance respond directly to changes in the light field.

Chloroplast Transcriptional Responses to a Change in Photoperiod Are Rapid and Temporally Gated

Although *H. carterae* appears to have the ability to respond to an alteration in photoperiod, the experimental results presented in Figure 4 did not provide sufficient resolution to define the time required to mount this response. To address this problem, the rapidity of the chlo-

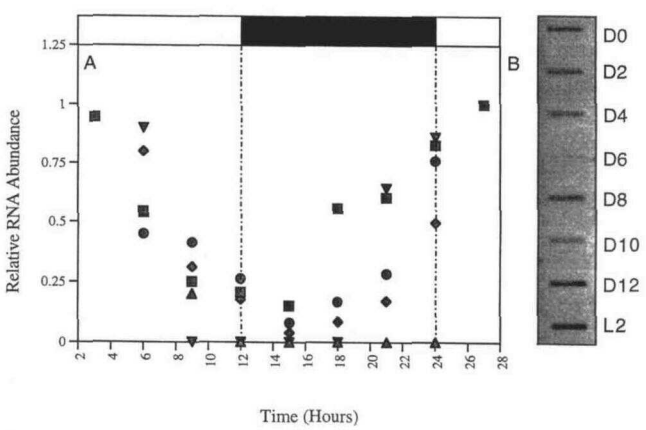

Figure 2. Relative chloroplast transcript abundance of *H. carterae* cells maintained in a 12L:12D regime. The chemiluminescent signal from each gene in A was normalized to its highest signal. Genes plotted include *clpC* (■), *rbcL* (◆), *rbcS* (▲), *psbA* (●), and *rpoB* (▼). Abundance of 16S RNA transcripts did not vary over the photocycle and therefore is not shown. B shows a representative digitized chemiluminescent blot of *petB/D* RNA abundance extracted from the same number of cells every 2 h from darkness (D) at h 0 to light (L) at h 2 in a 12L:12D photocycle. White bar, Light; black bar, darkness.

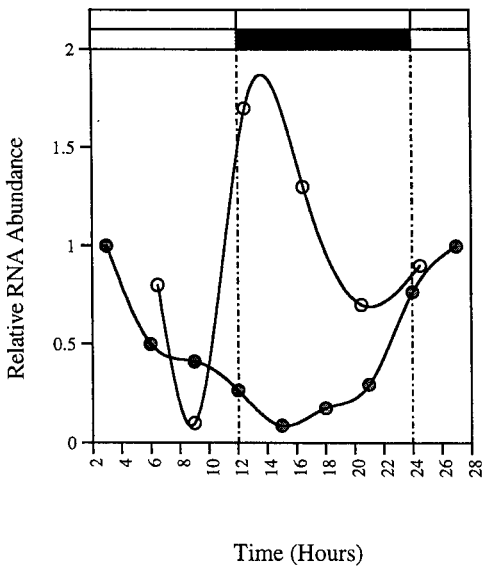

Figure 3. *psbA* transcript abundance in 24L:0D and 12L:12D regimes. The pattern of *psbA* RNA abundance in 12L:12D (●) and 24L:0D (○) cycles is shown. White bar, Light; black bar, darkness.

roplast transcriptional response was characterized by comparing RNA abundance in cultures as they progressed through a light-to-dark or light-to-light transition. Sampling every 20 min from L10 to putative D2 showed that an increase in RNA abundance occurred only in the 24L:0D cultures. *ClpC*, *petB/D*, and *psbA* RNA abundances increase, levels of *rbcL* and *rbcS* transcripts stabilize, and mRNA levels in all control cultures decline within 45 min of the transition between light and dark periods (Fig. 5). These results show that the chloroplast transcriptional apparatus in *H. carterae* has the ability to rapidly respond to a change in photoperiod.

A series of light "step up" experiments were designed to determine whether *H. carterae* chloroplasts can initiate a change in mRNA abundance throughout the dark period. Replicate cultures were exposed to dark periods for varying durations, returned to the light, and sampled for RNA abundance. Cultures exposed to only 2 h of dark before being returned to light showed a distinct increase in *psbA* RNA (Fig. 6A). However, those cultures exposed to 4 or 6 h of dark before returning to the light did not show an increase in *psbA* mRNA (Fig. 6, B and C). All three cultures showed a slight increase in mRNA abundance at the end of the putative dark period similar to the control cultures (Fig. 2). These data suggest that the ability of the chloroplast transcriptional apparatus to respond to a change in photoperiod is temporally regulated.

Alternating Light Regimes Establish an Ordered Progression of Cellular Growth.

In unicellular algae variations in the number of chloroplasts per cell are dependent on the synthesis of new chloroplast components, the packaging of functional chloroplasts, and the distribution of the chloroplasts into the daughter cells. However, the ability of the alga to change its chloroplast complement is dependent on the amount of energy that the cell can harvest from its environment. Therefore, a set of active fluorescence experiments was designed to characterize the pattern of photosynthetic efficiency over an alternating photocycle in *H. carterae*. As shown in Figure 7, the abundance of chloroplast-encoded mRNAs parallels oscillations in photosynthetic efficiency. Following the increase in mRNA abundance there was a rapid decrease in photosynthetic efficiency, followed by sequential increases in chloroplast and cellular replication (Fig. 7). Thus, in *H. carterae* there is an ordered progression of ctRNA accumulation, chloroplast division, and cellular division when cultures are synchronized by a 12L:12D photocycle.

DISCUSSION

Chloroplast-Encoded Gene Transcription

An increased number of available chloroplast-encoded gene sequences have created new opportunities to study the photoregulation of *H. carterae* chloroplast gene expression. To analyze the regulation of chloroplast-encoded genes, Reynolds et al. (1992) developed the only available

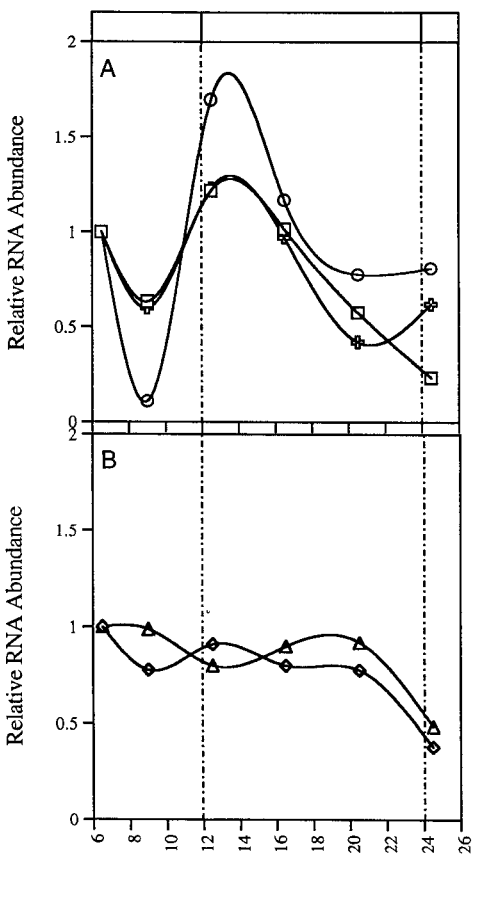

Figure 4. Chloroplast-encoded transcript abundance in a 24L:0D photocycle. The abundance pattern of *clpC* (□), *petB/D* (✠), *psbA* (○), *rbcL* (◇), and *rbcS* (△) in a 24L:0D photocycle is presented.

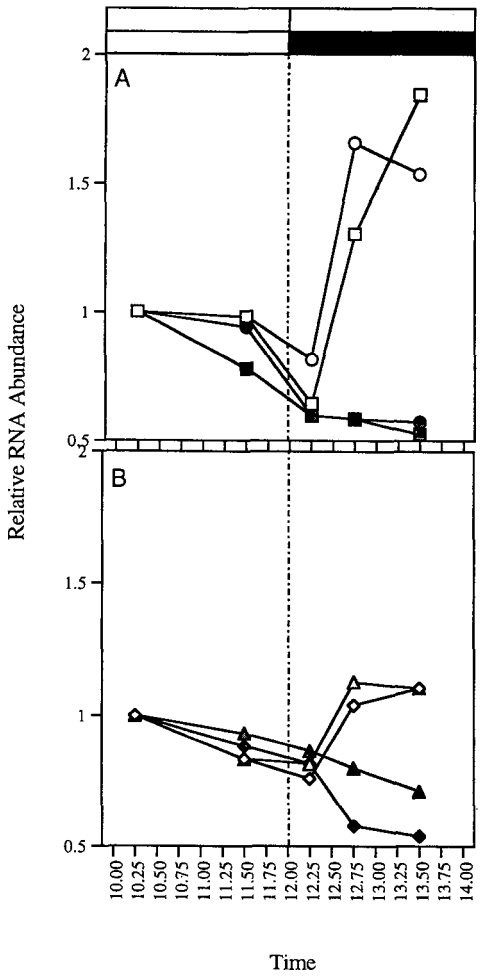

Figure 5. Comparison of chloroplast-encoded transcript abundance during a light-to-dark or light-to-light transition. The pattern of *clpC* (□), *psbA* (○, ●), *rbcL* (◇, ◆), and *rbcS* (△, ▲) in 24L:0D and 12L:12D cycles, respectively, is shown. White bar, Light; black bar, darkness.

run-on transcription system for chromophytic plants. Using this assay, these investigators demonstrated that *rbcL*, *psbA*, and rRNA abundances were controlled by transcript initiation during a 12L:12D cycle (Reynolds et al., 1992). Both transcript initiation and abundance for *rbcL* and *psbA* oscillated over the L:D cycle, whereas rRNA abundances remained constant. By using a larger array of genes, we have shown that the abundance profiles of all of the *H. carterae* chloroplast genes studied oscillate in parallel with *psbA* and *rbcL*. We hypothesize that *H. carterae* regulates chloroplast transcript abundance predominantly over a diel cycle coordinately through variations in transcript initiation rather than by impacting posttranscriptional processing or modifying RNA stability.

Patterns of transcript initiation in *Chlamydomonas reinhardtii* chloroplast-encoded genes oscillate over a daily cycle in a manner similar to that observed in *H. carterae*. Transcript initiation of *atpA, atpB, psaA, psaB, psbD,* and *tufA* increases before the onset of the light, peaks in the first few hours of the light period, and then rapidly decreases to reach a minimum early in the dark period (Leu et al., 1990; Hwang et al., 1996). However, unlike *H. carterae*, transcript abundance in chlorophytes results from changes not only in transcript initiation but also in splicing efficiency and RNA stability (Mullet and Klein, 1987; Gruissem, 1989; Adamska et al., 1991; Bringloe et al., 1995). Thus, in *C. reinhardtii* the abundance of each chloroplast mRNA has a characteristic pattern over an L:D cycle (Leu et al., 1990; Salvador et al., 1993). Transcript levels of *atpA, atpB,* and *tufA* oscillate over the whole photoperiod, *psaB* abundance increases only in the light period, and the number of *16s rRNA, rbcL,* and *psbA* transcripts remains constant throughout the L:D cycle (Salvador et al., 1993). Unlike chloroplast photogenes in chlorophytes, *H. carterae* chloroplast mRNA abundances are not unique to individual genes and appear to be primarily regulated by transcript initiation.

Alternating light cues regulate the timing and the pattern of chloroplast transcript initiation in *H. carterae* and *C.*

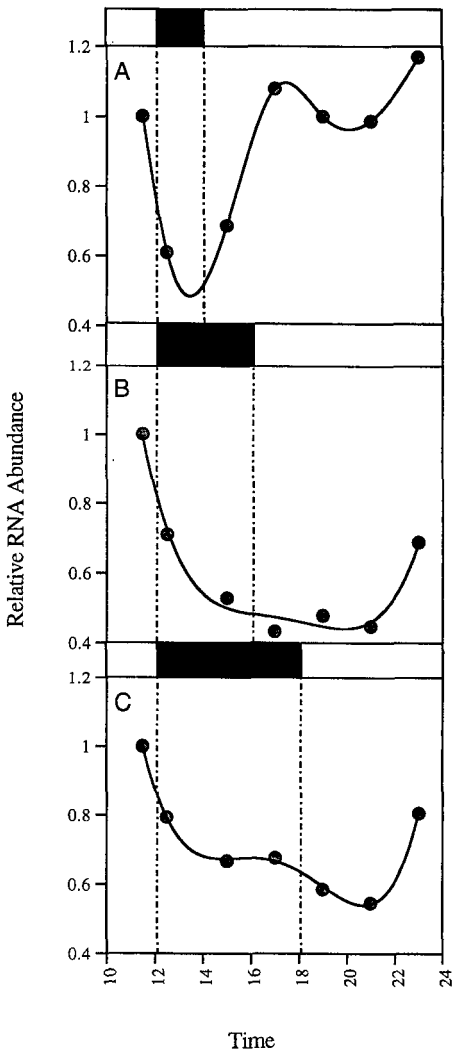

Figure 6. *psbA* Transcript abundance in truncated dark periods. The change in *psbA* transcript abundance occurs when cells are exposed to 2 (A), 4 (B), or 6 (C) h of darkness before transfer to light. White bar, Light; black bar, darkness.

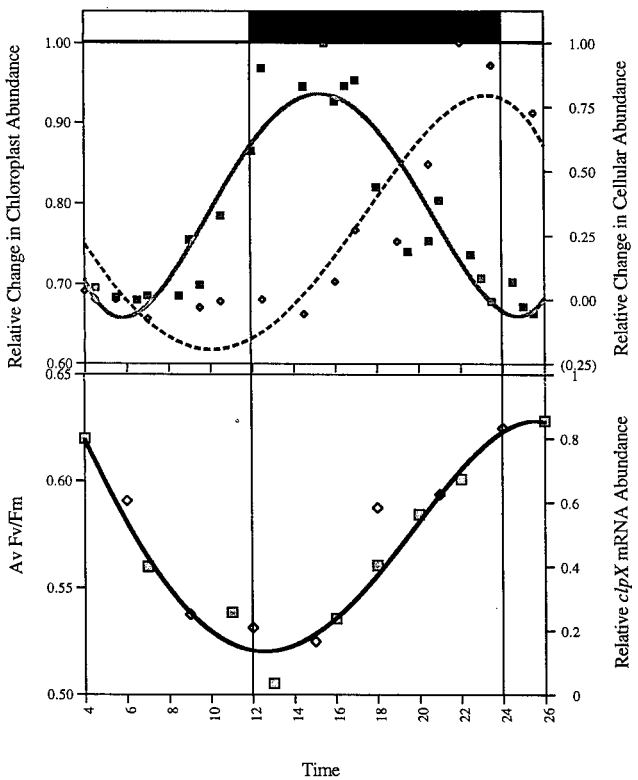

Figure 7. Variations in *clpC* RNA abundance, photosynthetic efficiency, chloroplast complement, and cell division in a 12L:12D cycle. Top, The progression of chloroplast complement (■ and solid line) and cell division (◆ and dashed line) under a 12L:12D photocycle. Bottom, *clpC* RNA abundance (◇) and active fluorescence (■) in a 12L:12D cycle. Active fluorescence (Av) was calculated as the difference between maximal (Fm) and initial fluorescence scaled to maximal fluorescence (Fv). These analyses were performed in triplicate (coefficient of variance <4.5%). White bar, Light; black bar, darkness.

reinhardtii during an L:D cycle (Reynolds et al., 1992; Hwang et al., 1996). Data suggest that the transcription of certain *C. reinhardtii* genes is controlled by a circadian oscillator. When light-entrained *C. reinhardtii* cells were transferred to constant light or dark conditions, there was no significant effect on the phase or amplitude of *atpA*, *atpB*, and *tufA* transcription for at least 48 h (Hwang et al., 1996). The pattern of *H. carterae* chloroplast transcript abundance oscillates over the daily cycle in the same phase as *C. reinhardtii*. However, unlike the chlorophytic alga the pattern of chloroplast gene transcription in *H. carterae* did not continue when light-entrained cultures were transferred to constant light. *H. carterae* responded rapidly to a sudden change in its photoperiod with a significant increase in ctRNA abundance. Thus, although the transcription of *H. carterae* chloroplast photogenes varies over a daily photocycle in the same pattern as selected *C. reinhardtii* genes, *H. carterae* chloroplast transcription does not appear to be regulated by a circadian oscillator.

We suggest that *H. carterae* chloroplast transcription is regulated by a noncircadian, endogenous oscillator. The transcription of all of the chloroplast-encoded genes studied varies over an L:D cycle in a predictable uniform oscillation. However, the alga did not maintain this pattern of transcription under conditions of constant light (24L:0D). Rather, *H. carterae* responded to a sudden change in photoperiod through the rapid accumulation of chloroplast-encoded mRNAs. Johnson and Kondo (1992) demonstrated that the ability of a particular light cue to induce a response in an endogenous oscillator is dependent on both the timing and the magnitude of the cue. *H. carterae* was able to sense and respond to the change in its light field only during the first few hours of the putative dark period. It appears that the pattern of transcript abundance in *H. carterae* is regulated by an oscillator that has a temporally limited ability to sense and respond to changes in the light field. How changes in light intensity during the light phase of the photocycle affect the transcriptional oscillator remains to be studied.

We offer two hypotheses to explain the response of the chloroplast transcriptional apparatus to a sudden change in photoperiod. Researchers have recently proposed that the expression of genes necessary for photosynthetic function can be regulated by the activity of the photosynthetic apparatus (LaRoche et al., 1991; Pearson et al., 1993; Danon and Mayfield, 1994; Maxwell et al., 1995). For example, *Lhc* transcription in *Dunaliella teriolecta* and *psbA* stability in the cyanobacterium *Synechocystis* are both controlled by the activity of the photosynthetic electron transport chain (Mohamed and Jansson, 1991; Escoubas et al., 1995). As shown in Figure 7, an alternating light regime not only controls *H. carterae* ctRNA transcription but also phases oscillations of photosynthetic efficiency. If a causal relationship between the activity of the photosynthetic apparatus and transcript initiation exists, then the rapid increase in transcript abundance may reflect an increase in photosynthetic electron transport during a significant step up in available light. Alternatively, the oscillations of photosynthetic and transcriptional activity may be simply correlative. Earlier research in terrestrial plants and algae have demonstrated that light cues can reset the phase of endogenous oscillators (Aoki et al., 1995; Kolar et al., 1995). Therefore, the response of the *H. carterae* chloroplast transcriptional apparatus may represent the "resetting" of the phase of the transcriptional oscillator by an altered photoperiod. The rapid increase in chloroplast-encoded mRNA abundance observed during the second 12-h light period may reflect a reinitiation of the chloroplast transcriptional program.

Chloroplast Growth and Division

The chloroplast growth cycle is well characterized in *H. carterae*. Chloroplast-encoded gene transcription begins at the end of the dark phase soon after the cells finish dividing. Chloroplast-encoded mRNAs begin to accumulate before the onset of the light phase, peak early in the light period, and decrease as the plastids begin to divide. Unlike chloroplast transcription, mRNA translation appears to be light dependent in this alga (Reith and Cattolico, 1985). As a result, there is a linear increase in chloroplast- and nuclear-encoded proteins per cell only during the light period. Similar photoregulation of transcription and trans-

lation has also been identified in cyanobacteria. Liu et al. (1995) observed daily oscillations of transcription and light-dependent translation when they fused a *luxAB* reporter gene to the *psbAI* promoter in *Synechococcus. Heterosigma* pigment synthesis (i.e. chlorophylls *a* and *c*, fucoxanthin, β-carotene, and violaxanthin) increases linearly throughout the L:D cycle (Latasa et al., 1992). After accumulating chloroplast-encoded mRNAs, proteins, and photosynthetic pigments, the chloroplasts begin to divide.

The newly synthesized chloroplast components must be packaged into individually functioning chloroplasts. Satoh et al. (1987) demonstrated that when *H. carterae* is entrained by an alternating photocycle, the onset of chloroplast division is determined by the termination of the previous light period. Our data demonstrate that once the chloroplast division cycle is entrained by an L:D cycle, its phase is not affected by a change in photoperiod for at least 20 h. If the onset of chloroplast division is timed by the previous photocycle, we would predict that a change in the light field would not alter the timing of chloroplast division until the next division cycle. Although the phase of the ongoing chloroplast division cycle would not be affected by a change in available light, there may be a variation in the amplitude (i.e. the number of cells dividing) or the period (i.e. the amount of time chloroplasts are dividing) of chloroplast division (R.A. Cattolico and K. Goodham, unpublished data). Therefore, although the timing of chloroplast division is controlled by the photoperiod and the distribution to daughter cells is stochastic, it appears that *H. carterae* has a compensatory mechanism that regulates the number of chloroplasts that are permitted to progress through division.

Our studies have demonstrated that newly generated plastids are distributed into daughter cells during a defined portion of the photoperiod. Field and laboratory studies have analyzed light-synchronized cell division in both phytoplankton communities and axenic cultures (Cattolico et al., 1976; Prezelin, 1992; Vaulot et al., 1995). Cell division in cultures of *C. reinhardtii* and *Euglena* sp. appear to be regulated by a circadian oscillator (Edmunds, 1988; Goto and Johnson, 1995). When light-synchronized cultures of *H. carterae* were transferred into constant light conditions, there was no significant response in the phase or amplitude of cell division. It appears that once an *H. carterae* cell has progressed through a suitable light period (i.e. one of appropriate spectrum, intensity, and duration), it is committed to completing cytokinesis during the dark phase. However, it is not presently known whether there are distinct transition points during the light phase that commit the cell to divide or whether a circadian oscillator controls cell division in *H. carterae* .

Preliminary experiments suggest that alterations in the duration and intensity of the light phase in a diel cycle affect both plastid and cell division in *H. carterae*. Unlike *Chlorella* sp., *C. reinhardtii*, and *Euglena* sp., this alga displays a temporal separation between cytokinesis and plastokinesis. When *H. carterae* is grown on an alternating photocycle, the onset of cellular division is set by the terminus of the dark period, whereas chloroplast division is timed by the end of the light phase (Satoh et al., 1987). However, chloroplast division cycles appear to be much more sensitive than cell division cycles to changes in the light field. To identify how *H. carterae* responds to a change in light cues, we have identified the events necessary for chloroplast growth and division during a normal growth cycle. The mechanisms that control the initiation and completion of events necessary for chloroplast biogenesis are presently under investigation.

ACKNOWLEDGMENTS

We thank Virginia Armbrust, William Hatheway, Estella Leopold, and Mary Jane Perry for their comments concerning this manuscript and M. Kay Suiter for manuscript preparation.

Received February 7, 1997; accepted June 24, 1997.
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