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Dynamics of Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase Gene Expression in the Coccolithophorid *Coccolithus pelagicus* during a Tracer Release Experiment in the Northeast Atlantic†

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We report a pronounced diel rhythm in ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) gene expression in a natural population of the coccolithophorid *Coccolithus pelagicus* sampled during a Lagrangian experiment in the Northeast Atlantic. Our observations show that there is greater heterogeneity in the temporal regulation of RubisCO expression among planktonic chromophytes than has been reported hitherto.

Despite the importance of the oceans in the global carbon cycle, comparatively little is known of the regulation of photosynthetic carbon fixation in marine phytoplankton. As in higher plants, the bulk of CO_2 is fixed via the Calvin cycle enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) (10) in these organisms. Therefore, it is of considerable interest to understand how the environment structures the expression of this key enzyme. In the present study, we investigated the diel variability in *rbcL* mRNA abundance in a natural population of the coccolithophorid *Coccolithus pelagicus*.

Observations were made in subpolar waters to the south of Iceland within an anticyclonic eddy with a cold surface temperature anomaly (6, 12). A patch of the tracer SF_6 was deployed at the eddy center (5), and the research vessel operated in Lagrangian mode by analyzing surface SF_6 concentrations continuously (4). Seawater samples were obtained during conductivity-temperature-depth hydrocasts, and the abundance of *C. pelagicus* cells was determined by flow cytometry (13). For RNA, near-surface (~2.5-m depth) seawater samples (10 to 20 liters) were rapidly filtered (<10 min) onto 90-mm-diameter Whatman GF/C filters, and the filters were stored at −70°C in extraction buffer (15) following snap-freezing in a propan-2-ol cooling bath. RNA was isolated and prepared for Northern analysis (16) under the hybridization conditions described below.

An internal region of *rbcL* was amplified from DNA isolated from *C. pelagicus* cells collected at the study site (16) and from laboratory cultures of the haptophytes *Emiliania huxleyi* and *Pavlova salina*. The identity of the products was confirmed by comparison to previously published sequences (2, 3), and sense strand in vitro transcription products were synthesized as previously described (16). Northern slot blots were hybridized at 55°C in Easy-Hyb solution (Roche) amended with 25 ng of *C. pelagicus rbcL* probe DNA (labeled with digoxigenin-dUTP) ml^{-1}. Stringency washes were performed on the following day with 0.05× SSPE (1× SSPE is 150 μM NaCl, 10 μM Na_2HPO_4, 1 μM EDTA)–0.1% sodium dodecyl sulfate at 68°C, and hybrids were detected with alkaline phosphatase-conjugated antidigoxigenin in conjunction with the chemiluminescent substrate CDP-Star (Roche). This hybridization protocol enables the specific detection of *C. pelagicus rbcL* mRNA and was optimized empirically by testing in vitro transcription products under conditions of increasing stringency (Fig. 1). Like the *rbcL* genes of the other coccolithophorids that have been characterized to date, the nucleotide sequence of the fragment

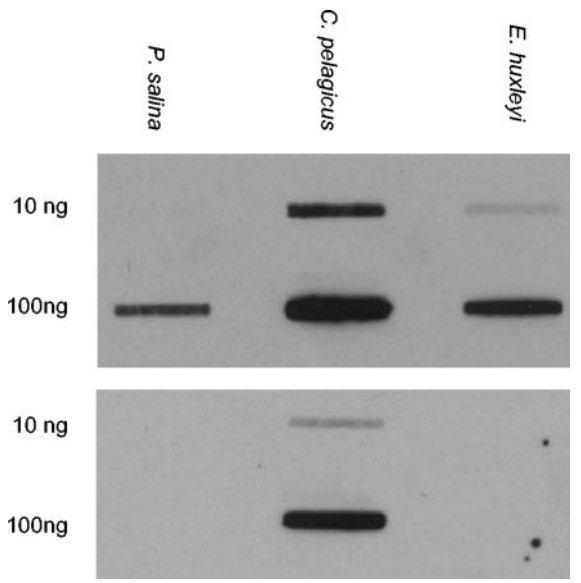

FIG. 1. Northern slot blot assays of sense strand in vitro *rbcL* transcription products probed with a digoxigenin-labeled fragment of *rbcL* from *C. pelagicus*. The upper luminograph shows the results obtained under suboptimal posthybridization wash conditions (0.2× rather than 0.05× SSPE in the stringency washes), while the lower luminograph shows the high specificity of the probe once the optimal hybridization protocol (see text) had been established.

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† Dedicated to the memory of contributing author John T. Davies, who tragically passed away in 2003 after years of struggle in an imperfect world.

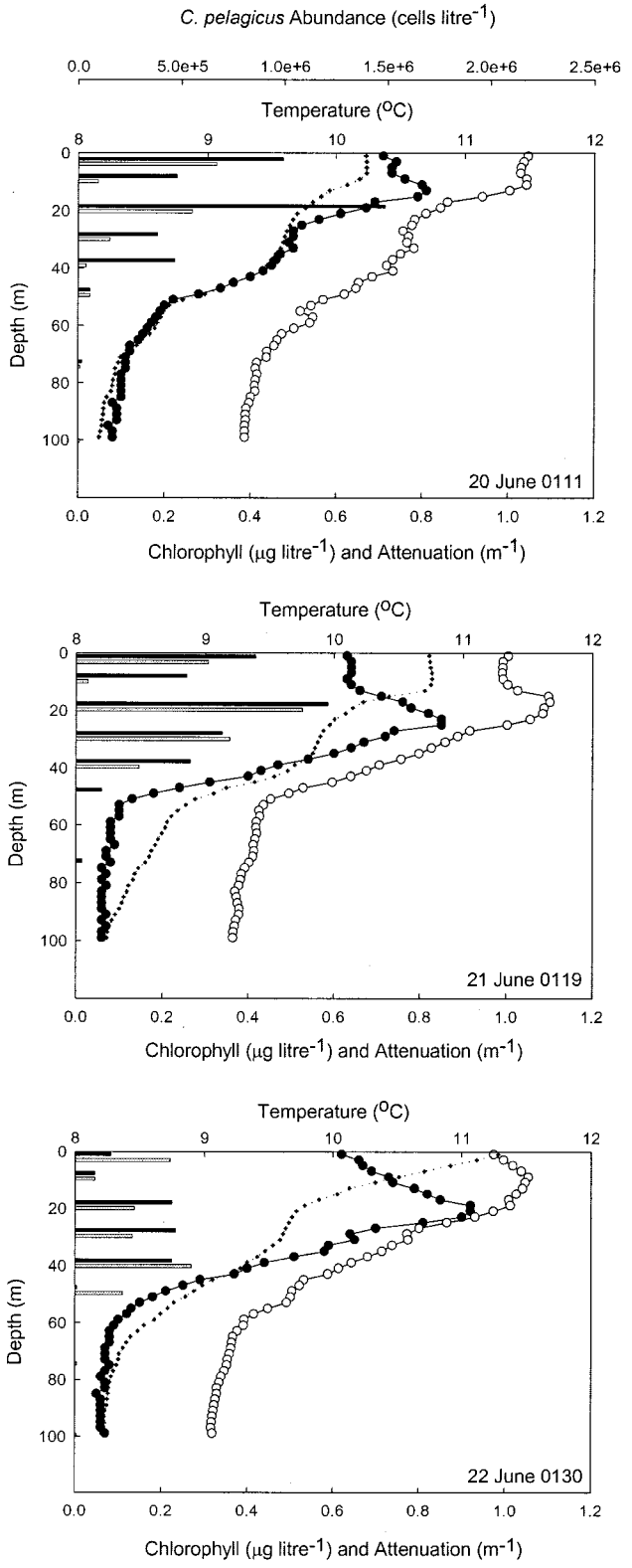

FIG. 2. Depth profiles of temperature (◆), chlorophyll concentrations (●), and beam attenuation (○) through the upper 100 m of the water column at the eddy center on 20, 21, and 22 June 1996. The histograms show the vertical distribution of *C. pelagicus* biomass observed at discrete depths sampled during the midday (gray bars) and midnight (black bars) hydrocasts. Greenwich mean times are shown.

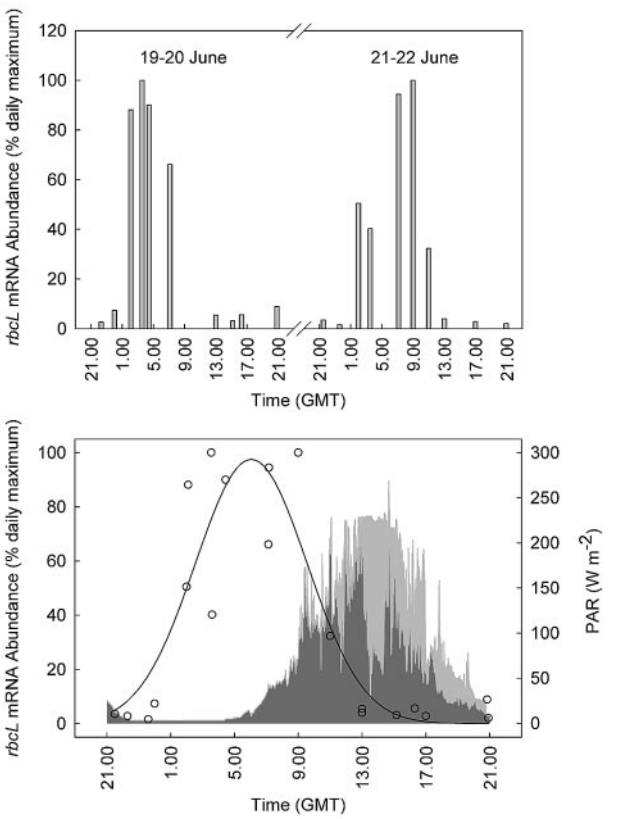

FIG. 3. Diel variability in the abundance of *C. pelagicus rbcL* mRNA normalized to the maximum hybridization signal recorded during each diel cycle (top panel). The bottom panel shows a three-parameter Gaussian regression (SigmaPlot for Windows, version 8.02) of the combined data set from both diel cycles and the temporal variability in photosynthetically active radiation (PAR) in the 24-h period between 2100 on 19 June and 2100 on 20 June (light gray) and the 24-h period between 2100 on 21 June and 2100 on 22 June (dark gray). The temporal resolution of the irradiance data is 1 min and was logged with a 4π collector mounted on the unshaded upper deck of the research vessel. GMT, Greenwich mean time.

from *E. huxleyi* is 88 to 89% identical to that of *C. pelagicus* whereas *rbcL* from noncalcifying *P. salina* is less closely related (86% identical).

Field observations and the sampling program for this study were confined to the first 5 days after the tracer release. During this time, the SF_6 patch remained coherent and the surface waters within the eddy retained their identity (6). *C. pelagicus* accounted for approximately 30% of the phytoplankton biomass within the eddy but was absent from surrounding waters (13), highlighting not only its distinct biological signature but also its physical resolution. Two distinct *C. pelagicus* population maxima were evident: a near-surface maximum and a somewhat deeper population at ~20 m that extended to a depth of 40 to 50 m (Fig. 2).

Considerable variability was observed in the abundance of *C. pelagicus rbcL* mRNA over the course of both diel cycles (Fig. 3, top panel) that was unrelated to temporal changes in the size of the *C. pelagicus* population, which generally increased at night (Fig. 2). RubisCO expression was greatest at around daybreak but declined by at least an order of magni-

tude during the latter half of the day and into the early nighttime period. Because of gaps in the sampling regimen when the research vessel was surveying waters outside of the SF_6 patch (particularly on the morning of 20 June), the two data sets were combined (Fig. 3, bottom panel) and analyzed by Rayleigh's test (1). The mean vector determined for those samples ($n = 8$), when the mRNA abundance was ≥40% of the daily maximum, was found to be 4:48 a.m. ($r = 0.812$, $0.002 < P < 0.005$), indicating that there was significant clustering around the daily peak in gene expression during both diel cycles. The excellent reproducibility of the rhythm between days was confirmed also by nonlinear regression analysis ($r^2 = 0.805$, $P < 0.0001$).

Previous studies have examined temporal variability in *rbcL* mRNA abundance in mixed communities of marine phytoplankton (8, 9, 15, 16), but this is the first report of species- rather than group-specific determinations of RubisCO gene expression in a natural population. Intriguingly, the diel rhythm in *C. pelagicus rbcL* mRNA abundance found was quite distinct from that observed in the haptophyte *Pavlova gyrans* or in natural phytoplankton populations at lower latitudes with a chromophyte-specific *rbcL* probe (7, 8). In both cases, *rbcL* mRNA abundance maxima were observed toward the end of the diurnal period whereas in *Heterosigma carterae* and the diatom *Phaeodactylum triconutum*, a broad mid-morning maximum in *rbcL* mRNA abundance has been reported (11, 14). Like the natural population of *C. pelagicus* studied here, *rbcL* mRNA began to accumulate in the dark prior to the onset of illumination. No evidence of a nighttime increase in transcriptional activity was found in *P. gyrans* (7) or the natural communities of diatoms and picoeukaryotes investigated previously (8).

Evidently, there is considerable heterogeneity in the diel regulation of RubisCO gene expression between and within different taxonomic classes of marine chromophytic algae. To what extent this reflects differences in genetic controls, the life cycle of individual species, or environmental variability is worthy of further investigation to establish its ecological significance. It has been proposed, for instance, that differences in the diel pattern of RubisCO expression between chromophytes and the picocyanobacteria indicate that they occupy different temporal niches (8). This study has shown that this may not always be the case since the diel rhythm in *C. pelagicus* RubisCO expression reported here is very similar to that of natural populations of *Synechococcus* spp. from the same oceanic region (15).

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