APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2009, p. 3366–3369 Vol. 75, No. 10
0099-2240/09/$08.00+0 doi:10.1128/AEM.02737-08

Light-Dependent Transcriptional Regulation of Genes of Biogeochemical Interest in the Diploid and Haploid Life Cycle Stages of *Emiliania huxleyi*▽†

Sophie Richier,[1]* Marie-Emmanuelle Kerros,[1] Colomban de Vargas,[2,3] Liti Haramaty,[3] Paul G. Falkowski,[3,4] and Jean-Pierre Gattuso[1]

Laboratoire d'Océanographie de Villefranche, CNRS-UMR 7093, Université Pierre et Marie Curie (Paris 6), 06234 Villefranche-sur-Mer Cedex, France[1]; CNRS-UMR 7144, UPMC (Paris 6), Equipe EPPO (Evolution du Plancton et Paleo-Oceans), Station Biologique de Roscoff, 29682 Roscoff, France[2]; Institute of Marine and Coastal Sciences, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08901[3]; and Department of Geological Sciences, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854[4]

Received 1 December 2008/Accepted 13 March 2009

The expression of genes of biogeochemical interest in calcifying and noncalcifying life stages of the coccolithophore *Emiliania huxleyi* was investigated. Transcripts potentially involved in calcification were tested through a light-dark cycle. These transcripts were more abundant in calcifying cells and were upregulated in the light. Their application as potential candidates for in situ biogeochemical proxies is also suggested.

Phytoplankton productivity and calcification are two major biological processes contributing to the regulation of the climate of our planet via their impact on the global carbon cycle.

Coccolithophores are planktonic unicellular microalgae which play an important role in ocean biogeochemistry (20). They are among the most productive calcifying organisms on Earth and thus are a key functional group in the marine carbon cycle.

Among modern coccolithophorids, *Emiliania huxleyi* is the most successful species, exhibiting a worldwide distribution in open and coastal oceans, except in the polar areas.

Emiliania huxleyi has been intensively studied in several contexts, but its physiology at the molecular level remains largely unknown. However, this area can now be intensively investigated thanks to the ever-growing number of analyses of expressed sequence tags (6, 10, 21, 27) and the whole-genome-annotation effort (Joint Genome Institute).

In this exploratory work, we focus on a few genes potentially involved in calcification, one of the processes which will likely be affected by current and future global environmental changes, such as ocean acidification.

Previous molecular studies of *E. huxleyi* calcification (4, 17, 21) were carried out using noncalcifying and calcifying cultures obtained by inducing phosphorus limitation. In this paper, we take advantage of the fact that the haplodiploid life cycle of *E. huxleyi* involves an alternation between calcified, coccolith-bearing diploid cells and organic scale-bearing motile haploid cells, each of which can exist independently of the other and reproduce vegetatively (8). This approach has the considerable advantage of maintaining the two life stages of *E. huxleyi* under identical culture conditions.

While the large subunit of RuBisCO (ribulose-1,5-bisphosphate carboxylase), *rbcL*, is well known for its central role in photosynthetic carbon fixation and has already been shown in a pilot field experiment to relate successfully to photosynthesis measurements (1), research aimed at identifying key genes and proteins involved in calcification is still in its infancy.

In the last few years, several novel molecular tools have been used to investigate calcification and coccolithogenesis (4, 6, 17, 21, 27). Results from these studies of coccolithophores as well as those carried out with other calcifying organisms were used to select genes. *gpa* was chosen because it codes for proteins in association with intracellular precursors of coccolith polysaccharides (2). The carbonic anhydrase (CA) proteins play an important role in the biomineralization of vertebrates and invertebrates (e.g., references 7, 9, and 25). In coccolithophores, cDNAs encoding putative γ and δ types of CA proteins have been identified (23), and a γ-*CA* cDNA was shown to be preferentially expressed in calcifying cells (6).

The last transcript investigated in the present study is related to calmodulin. Marshall (15) and Zoccola et al. (30) have previously suggested that L-type Ca^{2+} channels, which are regulated by CaM, are involved in the calcium transport process for calcification in scleractinian corals. In addition, Li et al. (13) and Yan et al. (28) showed important roles for CaM in regulation of Ca^{+} uptake, transport, and secretion in the process of shell formation in the oyster *Pinctada fucata*. The involvement of *CaM* in calcification or calcium homeostasis of *E. huxleyi* is supported by Dyhrman et al. (4), who reported the presence of this gene in a calcifying culture of *E. huxleyi* depleted of phosphorus.

Haploid (N) and diploid (2N) cells of *Emiliania huxleyi* were grown at 17°C under a 14-h-light–10-h-dark photoperiod. Morphological differences between haploid and diploid cultures were checked and validated using microscopy analyses at the beginning and the end of the experiment. Batch cultures were grown in triplicate for each life stage. Exponentially growing cultures were collected in the dark and in the light. Total RNA was extracted with Trizol, and DNases were treated and quan-

* Corresponding author. Mailing address: Laboratoire d'Océanographie de Villefranche, CNRS-UMR 7093, Université Pierre et Marie Curie (Paris 6), 06234 Villefranche-sur-Mer Cedex, France. Phone: 33 4 93 76 38 33. Fax: 33 4 93 76 38 34. E-mail: richier@obs-vlfr.fr.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 20 March 2009.

TABLE 1. Forward and reverse primers used in real-time quantitative PCR assays

Protein	Orientation	Primer	Sequence (5′–3′)	Amplicon size (bp)
Calmodulin (CAM)	Forward	calmF	ATCGACTTCCCCGAGTTCT	151
	Reverse	calmR	CGAGGTTGGTCATGATGTG	
Carbonic anhydrase (CA)	Forward	CAF1	GCAAGAGTAGCATCGGAGAC	150
	Reverse	CAR1	CAACCACCGCAAAGTTGT	
Calcium binding protein (GPA)	Forward	gpaBRF	GTTCAGCGTGCTCTCCGAG	70
	Reverse	gpaBRR	AGGCCTTCTCCAGCATCAT	

tified. Total RNA was adjusted to a final concentration of 200 ng μl^{-1} for all samples, reverse transcribed into first-strand cDNA, and run in triplicate. Controls without reverse transcriptase were prepared simultaneously to ensure that no DNA contamination occurred. Normalization of real-time PCR data was done using equivalent amounts of DNase-treated RNA as material for cDNA synthesis (5, 24, 26). The primer sequences used for quantitative PCR in the study are listed Table 1. The methods are further detailed in the supplemental material.

Figure 1 shows a higher abundance of transcripts in the calcifying cells than in the noncalcifying cells both in the dark and in the light. The difference was far more significant for light than for darkness. The most significant difference in abundance corresponds to the *CaM* transcript in the light, with about two times more transcripts in calcifying cells than in noncalcifying cells. Fig. 2 also highlights an upregulation of all transcripts in the light in the calcifying cells.

Concerning *gpa*, the upregulation observed in calcifying cells contrasts with the downregulation reported by Quinn et al. (21). It must be pointed out, however, that in that study, calcifying cells were obtained by inducing a phosphorus limitation. In contrast, the cells used in the present study were replete with phosphorus.

The γ-*EhCA2* preponderance in calcifying cells supports the

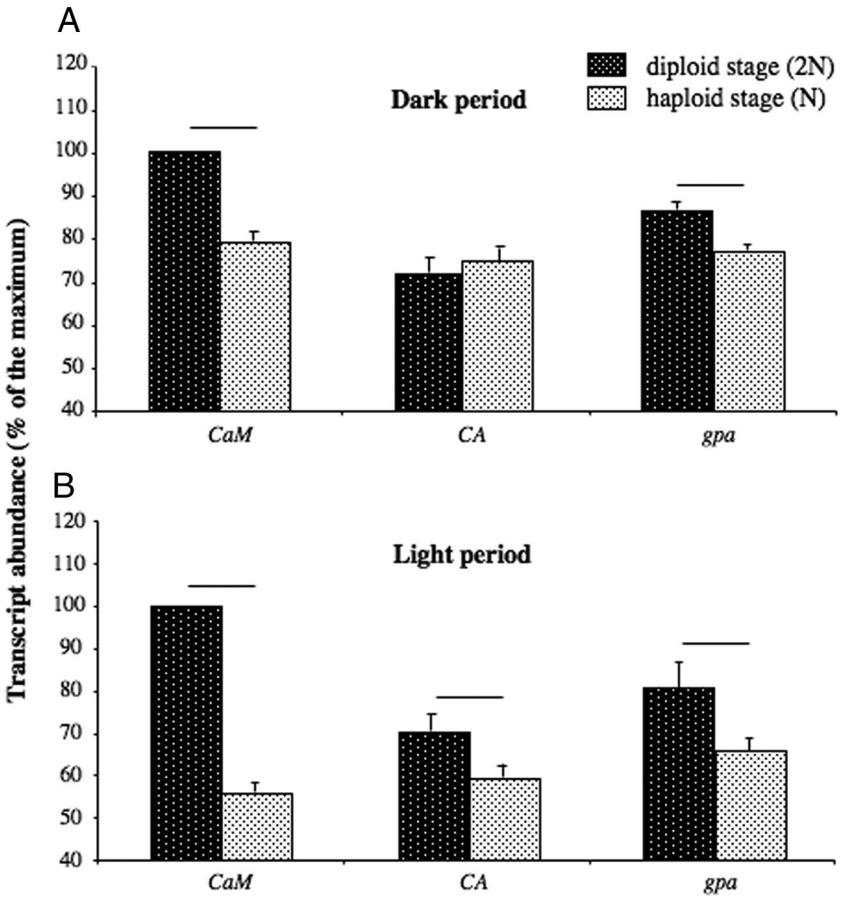

FIG. 1. Relative abundances of *CaM*, *CA*, and *gpa* transcripts in the dark (A) and light (B) periods in the diploid (dotted black bars) and haploid (dotted white bars) stages in *E. huxleyi* (strains RCC1216 and RCC1217). Data are presented as means ± standard deviations for three independent cultures. Bars indicate significant differences between diploid and haploid cells (Student's *t* test; $P < 0.05$). Note that the *x* axes start at 40%.

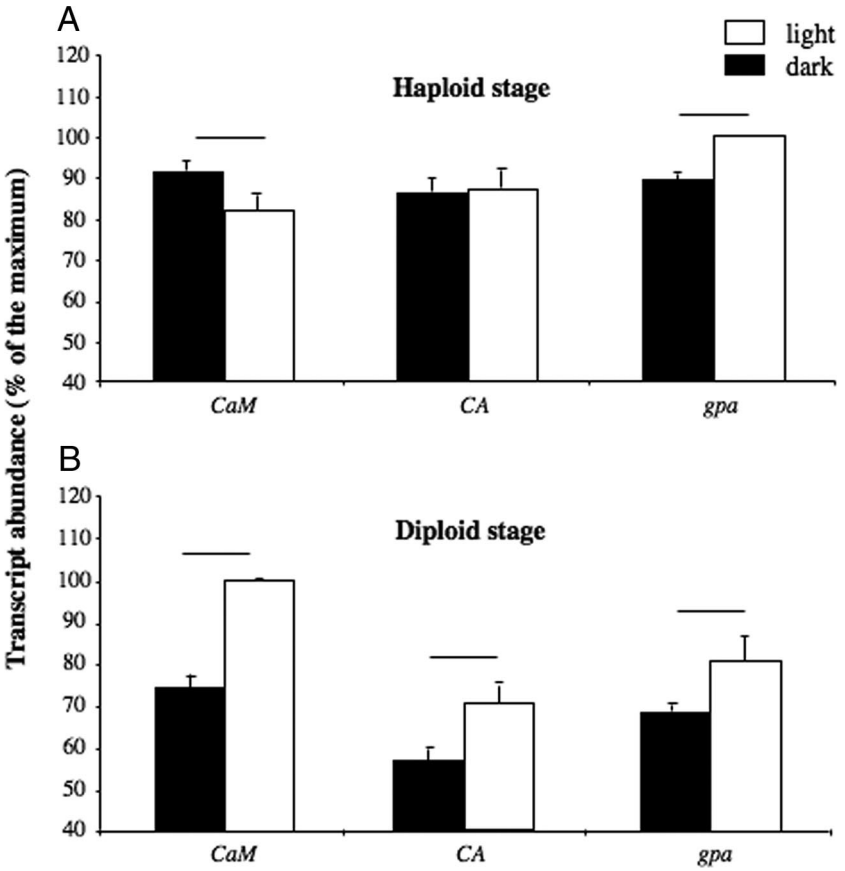

FIG. 2. Relative abundances of *CaM*, *CA*, and *gpa* transcripts in the haploid (A) and diploid (B) stages in *E. huxleyi* (strains RCC1216 and RCC1217) during the dark (black bar) and light (white bar) periods. Data are presented as means ± standard deviations for three independent cultures. The bars indicate significant differences between results for dark and light conditions (Student's *t* test; $P < 0.05$). Note that the *x* axes start at 40%.

results obtained by Fujiwara et al. (6). The upregulation of the *CA* transcript by light in calcifying cells is supported by Soto et al. (23) and contradicted by other results obtained for calcifying organisms, such as scleractinian corals, where *CA* expression decreases in the light (16). However, a recent review of carbonic anhydrases reported five CA proteins (α, β, γ, δ, and ζ), and while the functions of most of the classes have been well described, the physiological roles of the γ class are not well documented (29). Soto et al. (23) already suggested that isoforms have different roles in inorganic carbon metabolism.

CaM is by far the target most sensitive to light, presenting the largest differences in abundance between calcifying and noncalcifying cells. In the oyster *Pinctada fucata*, the CAM protein was shown to modify the morphology of the calcite through regulation of its growth (28).

Monitoring gene expression in the haploid and diploid life stages of *E. huxleyi* demonstrates that all transcripts investigated were more abundant in calcifying cells and that their expression is upregulated in light. These molecular results are consistent with physiological data obtained using tracers such as ^{14}C (3) and ^{45}Ca (14). These studies unequivocally demonstrated that coccolith formation in *E. huxleyi* is strongly light dependent. The fact that *gpa*, *CA*, and *CaM* are also expressed in darkness is also in agreement with multiple sources of evidence showing that *E. huxleyi* can form coccoliths in the dark, albeit at a lower rate than in the light (18, 22).

These results open the way for investigating (i) the regulation of calcification by other environmental changes (e.g., elevated temperature and/or partial CO_2 pressure) and (ii) the potential use of selected genes as molecular proxies for biogeochemical processes in the field. In fact, a relatively large number of studies have already analyzed photosynthesis through *rbcL* expression, and an intercalibration of biogeochemical fluxes and gene expression measurement has already been suggested (12). Also, further studies are required to identify additional genes involved in calcification.

Thanks are due to J. Kegel, K. Bidle, L. Kerkorff, I. Probert, B. Read, B. Warwrik, Swati Naravan-Yadav, and K. Wyman for their valuable assistance. B. Read and J. Kegel kindly provided sequences and primers.

This work was supported by the BOOM (Biodiversity of Open Ocean Microcalcifiers) project, funded by the Agence Nationale de la Recherche.

REFERENCES

1. **Corredor, J. E., B. Wawrik, J. H. Paul, H. Tran, L. Kerkhof, J. M. Lopez, A. Dieppa, and O. Cardenas.** 2004. Geochemical rate-RNA integration study: ribulose-1,5-bisphosphate carboxylase/oxygenase gene transcription and photosynthetic capacity of planktonic photoautotrophs. Appl. Environ. Microbiol. **70:**5459–5468.

2. **Corstjens, P. L. A. M., A. van der Kooij, C. Linschooten, G.-J. Brouwers, P. Westbroek, and E. W. de Vrind-de Jong.** 1998. GPA, a calcium-binding protein in the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae). J. Phycol. **34:**622–630.
3. **Dong, L. F., N. A. Nimer, E. Okus, and M. J. Merret.** 1993. Dissolved inorganic carbon utilization in relation to calcite production in *Emiliania huxleyi* (Lohmann) Kampter. New Physiol. **123:**679–684.
4. **Dyhrman, S. T., S. T. Haley, S. R. Birkeland, L. L. Wurch, M. J. Cipriano, and A. G. McArthur.** 2006. Long serial analysis of gene expression for gene discovery and transcriptome profiling in the widespread marine coccolithophore *Emiliania huxleyi*. Appl. Environ. Microbiol. **72:**252–260.
5. **Frigeri, L. G., T. R. Radabaugh, P. A. Haynes, and M. Hildebrand.** 2006. Identification of proteins from a cell fraction of the diatom *Thalassiosira pseudonana*: insights into silica structure formation. Mol. Cell. Proteomics **5:**182–193.
6. **Fujiwara, S., Y. Hirokawa, Y. Takatsuka, K. Suda, E. Asamizu, T. Takayanagi, D. Shibata, S. Tabata, and M. Tsuzuki.** 2007. Gene expression profiling of coccolith-bearing cells and naked cells in haptophyte *Pleurochrysis haptonemofera* with a cDNA macroarray system. Mar. Biotechnol. (New York) **8:**1–11.
7. **Goreau, T. F.** 1959. The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. Biol. Bull. **116:**59–75.
8. **Green, J. C., P. A. Course, and G. A. Tarran.** 1996. The life cycle of *Emiliania huxleyi*: a brief review and a study of ploidy levels analysed by flow cytometry. J. Mar. Syst. **9:**33–44.
9. **Kakei, M., and H. Nakahara.** 1996. Aspects of carbonic anhydrase and carbonate content during mineralization of the rat enamel. Biochim. Biophys. Acta **1289:**226–230.
10. **Kegel, J., M. J. Allen, K. Metfies, W. H. Wilson, D. Wolf-Gladrow, and K. Valentin.** 2007. Pilot study of an EST appproach of the coccolithophorid *Emiliania huxleyi* during a virus infection. Gene **406:**209–216.
11. Reference deleted.
12. **Kerkhof, L., J. Corredor, J. Paul, D. Bronk, J. Lopez, and J. Cherrier.** 2003. Experiment explores intercalibration of biogeochemical flux and nucleic acid measurements. Eos Trans. Am. Geophys. Union **84:**167.
13. **Li, S., L. Xie, C. Zhang, Y. Zhang, M. Gu, and R. Zhang.** 2004. Cloning and expression of a pivotal calcium metabolism regulator: calmodulin involved in shell formation from pearl oyster (*Pinctada fucata*). Comp. Biochem. Physiol. B **138:**235–243.
14. **Linschooten, C., J. D. L. van Blejswijk, P. R. van Emburg, J. P. M. De Vrind, E. S. Kempers, P. Westbroek, and E. W. De Vrind-De Jong.** 1991. Role of the light-dark cycle and medium composition on the production of coccoliths by *Emiliania huxleyi* (Haptophyceae). J. Phycol. **27:**82–86.
15. **Marshall, A. T.** 1996. Calcification in hermatypic and ahermatypic corals. Science **271:**637–639.
16. **Moya, A., S. Tambutté, A. Bertucci, E. Tambutté, S. Lotto, D. Vullo, C. T. Supuran, D. Allemand, and Z. Zoccola.** 2008. Carbonic anhydrase in the scleractinian coral *Stylophora pistillata*: characterization, localization, and role in biomineralization. J. Biol. Chem. **283:**25475–25484.
17. **Nguyen, B., R. M. Bowers, T. M. Wahlund, and B. A. Read.** 2005. Suppressive subtractive hybridization of and differences in gene expression content of calcifying and noncalcifying cultures of *Emiliania huxleyi* strain 1516. Appl. Environ. Microbiol. **71:**2564–2575.
18. **Paasche, E.** 1966. Adjustment to light and dark rates of coccolith formation. Physiol. Plant. **19:**271–278.
19. Reference deleted.
20. **Paasche, E.** 2002. A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. Phycologia **40:**503–529.
21. **Quinn, P., R. M. Bowers, X. Zhang, T. M. Wahlund, M. A. Fanelli, D. Olszova, and B. A. Read.** 2006. cDNA microarrays as a tool for identification of biomineralization proteins in the coccolithophorid *Emiliania huxleyi* (Haptophyta). Appl. Environ. Microbiol. **72:**5512–5526.
22. **Skattebol, S.** 1995. Coccolith formation in the light and in the dark in *Emiliania huxleyi* (Lohmann) Hay et Mohler. M.S. thesis. University of Oslo, Oslo, Norway.
23. **Soto, A. R., H. Zheng, D. Shoemaker, J. Rodriguez, B. A. Read, and T. M. Wahlund.** 2006. Identification and preliminary characterization of two cDNAs encoding unique carbonic anhydrases from the marine alga *Emiliania huxleyi*. Appl. Environ. Microbiol. **72:**5500–5511.
24. **Thamatrakoln, K., and M. Hildebrand.** 2007. Analysis of *Thalassiosira pseudonana* silicon transporters indicates distinct regulatory levels and transport activity through the cell cycle. Eukaryot. Cell **6:**271–279.
25. **Tohse, H., E. Murayama, T. Ohira, Y. Takagi, and H. Nagasawa.** 2006. Localization and diurnal variations of carbonic anhydrase mRNA expression in the inner ear of the rainbow trout *Oncorhynchus mykiss*. Comp. Biochem. Physiol. B **145:**257–264.
26. **Tricarico, C., P. Pinzani, S. Bianchi, M. Paglierani, V. Distante, M. Pazzagli, S. A. Bustin, and C. Orlando.** 2002. Quantitative real-time reverse transcription polymerase chain reaction: normalization to rRNA or single housekeeping genes is inappropriate for human tissue biopsies. Anal. Biochem. **309:**293–300.
27. **Wahlund, T. M., A. R. Hadaegh, R. Clark, B. Nguyen, M. Fanelli, and B. A. Read.** 2004. Analysis of expressed sequence tags from calcifying cells of marine coccolithophorid (*Emiliania huxleyi*). Mar. Biotechnol. (New York) **6:**278–290.
28. **Yan, Z., Z. Fang, Z. Ma, J. Deng, S. Li, L. Xie, and R. Zhang.** 2007. Biomineralization: functions of calmodulin-like protein in the shell formation of pearl oyster. Biochim. Biophys. Acta **1770:**1338–1344.
29. **Zimmerman, S. A., and J. G. Ferry.** 2008. The beta and gamma classes of carbonic anhydrase. Curr. Pharm. Des. **14:**716–721.
30. **Zoccola, D., E. Tambutte, F. Senegas-Balas, J. F. Michiels, J. P. Failla, J. Jaubert, and D. Allemand.** 1999. Cloning of a calcium channel alpha1 subunit from the reef-building coral, *Stylophora pistillata*. Gene **227:**157–167.