REGULATION OF PHOTOSYNTHETIC CARBON FIXATION ON THE OCEAN MARGINS

Final Report of Grant

DE-FG05-93ERG6199

61699

DOE/ER/61699-T1

DOE Ocean Margins Program

John H. Paul
University of South Florida
140 Seventh Ave. S.
St. Petersburg, FL 33701
(813) 553-1168
FAX: (813) 553-3966

BACKGROUND

The US Department of energy is concerned with the fate of energy-related materials (including CO₂) in the marine environment. Regional studies along the US continental shelves have been supported by DOE, including the use of moored instrumentation, ship sampling/remote sensing, the study of the spatial and temporal concentration of chemical species, biological productivity, zooplankton grazing and respiration, ecological dynamics, and the biogeochemical flux of particles. In 1992, a plan was formulated for the study of carbon flux in the S. Atlantic Bight off Cape Hatteras. The objectives of this study were:

- (1) **Understand coastal marine biogeochemical processes and ecosystem dynamics.** This objective strives to broadly understand how the coastal ocean acts as a system, as an interface between the continental land mass, estuaries, and the open ocean.
- (2) **Quantify carbon sources and sinks at the land/ocean interface.** This objective has been broadly interpreted as defining the ocean margins as sources or sinks in global biogeochemical cycles.
- (3) **Determine whether coastal primary productivity is significant in removing atmospheric CO₂.** It is important to determine if the continental shelves are quantitatively significant in removing CO₂ from the atmosphere and isolating it by burial on the shelves or transporting it into the interior ocean.

During FY 93 DOE launched a new molecular biology initiative, the goal of which was to provide a mechanistic understanding of the complex biological processes which mediate the carbon cycle in marine systems. Specific objectives of this subprogram were to:

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1) *Understand molecular regulation of photosynthetic carbon reduction.* This succinctly describes the goals of our project.

2) *Develop molecular diagnostic markers of bacterial growth, production, and nutrient limitations to growth.*

3) *Develop molecular techniques for elucidation of metabolic pathways.*

Our project, entitled *Molecular Regulation of CO₂ fixation on the Ocean Margins*, was principally directed at answering the first major objective of the DOE OMP program, and the first molecular objective described above. Our overall goal was two-fold, using both laboratory studies with isolates and field studies with natural populations. Our objectives were:

1. To determine the mechanism of regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCase) in phytoplankton in response to changes in light fields.

2. To determine regulation of RuBPCase in response to light under nutrient deprivation.

Both of these objectives were to be performed in the laboratory of F. Robert Tabita, Co-PI, and will be discussed in the final report of his project. Our lab's objectives were:

3. To determine mechanisms of regulation of RuBPCase in natural populations of phytoplankton of the ocean margins using technology developed in objectives 1 and 2.

4. To measure regulation of RuBPCase in phytoplankton of the Hatteras System.

RESULTS AND FINDINGS

Our field studies (Objectives 3 and 4) had four components to them: 1) The study of temporal variations in regulation of carbon fixation in natural populations. These studies investigated the possibility of diel regulation and entrained rhythms in RuBPCase gene expression, done in studies with *Prochlorococcus* Pacific strain in culture, deck-top incubations of natural populations, and in Lagrangian studies. The other components included: 2) The vertical distribution of RuBPCase gene expression in stratified water columns, 3) The size distribution of the phytoplankton responsible for carbon fixation by size fractionation studies, and 4) The molecular biodiversity of organisms involved in carbon fixation of the water column.

Temporal variation in the regulation of carbon fixation. Our hypothesis for this sub-area of

the research was that phytoplankton constitutively expressed the RuBPCase gene (*rbcl*) throughout the day, and carbon fixation was controlled by light availability only. Alternatively, the gene could be transcribed in conjunction with the prime hours of sunlight for photosynthesis. To perform these studies mRNA was isolated from natural populations and probed with the *rbcl* gene probe. In initial studies, the *rbcl* gene from the cyanobacterium *Synechococcus* PCC6301 was used as a hybridization probe (cyano probe). This probe should hybridize to all cyanobacteria, green algae, and higher plants (Form IB RuBPCase). Because of molecular biodiversity in RuBPCase, in later studies we used an additional hybridization probe derived from *Cylindrotheca* species N1 (chromo probe). This probe should detect *rbcl* expression in diatoms, prymnesiophytes, pelagophytes, cryptomonads, and other chromophytic algae. In early studies when only the cyano probe was used, a consistent pattern of diel regulation of *rbcl* was found in cultures of *Prochlorococcus*, natural phytoplankton populations in deck-top incubators, or in natural populations followed in Lagrangian studies. RuBPCase gene expression was undetectable at night, and then "turned on" between 0600 and 0800 h. The maximal peak was usually 1000 h but was noted as late as 1500 h. The maximum in *rbcl* expression coincided with the time of the maximum in whole cell carbon fixation. When cultures or natural populations were incubated in continuous light, the same pattern of diel regulation was observed, indicative of endogenous rhythms in these organisms. These studies are summarized in the attached manuscript (Pichard et al., 1996) and were performed in the Gulf of Mexico.

When the chromo probe was used, a different pattern of regulation was observed. The chromo probe hybridized most strongly with samples taken in the late afternoon or early evening, usually lagging 6 to 8 hr behind the peak in cyano *rbcl* expression. This was demonstrated in the Cape Hatteras system on two cruises, the first in a decktop incubator study of water taken from the central mooring of the mooring array, and the second during a Lagrangian study during the R/V Seward Johnson cruise of July, 1996. The latter was part of the DOE field experiment. Sampling occurred every 12 h between 0800 and 1000 and every evening between 1900 and 2100 h. Cyano *rbcl* expression was maximal during the morning sampling while chromo *rbcl* peaked during the evening sampling (Paul et al., in preparation). The chromo *rbcl* peak undoubtedly was the result of diatom carbon fixation gene expression, as diatoms are major contributors to carbon cycling in these waters. These results for the first time demonstrate the temporal separation in the expression of carbon fixation genes between these two broad evolutionary groups of phytoplankton.

The vertical distribution of rbcl gene expression in stratified water columns. These studies were aimed at deciphering the vertical distribution of evolutionary clades of phytoplankton in the water column, and were performed both in the Gulf of Mexico and in the Hatteras System. In both areas, maximal expression of cyano-like *rbcl* genes occurred in the upper water column (0-60 m) and correlated with *Synechococcus* and *Prochlorococcus* counts determined by flow cytometry. The base of the euphotic zone (68-100 m) was characterized by a peak in the chromo *rbcl* hybridization that coincided with the

subsurface chlorophyll a maximum (SCM) and picoeucaryotes as determined by flow cytometry. The picoeucaryotes included diatoms, prymnesiophytes and pelagophytes as determined by HPLC pigment analysis. In the DOE study site we also found a predominance of cyano *rbcL* mRNA in the upper water column (0-10 m) and a dominance of chromo *rbcL* RNA (presumably diatoms) at the base of the water column (~35 m). These results show that chromophytes, and diatoms in particular, are well adapted to carbon fixation in dimly lit subsurface waters, where they may participate in the removal of CO₂ by sedimentation and burial on the shelves (Paul et al, in preparation).

The size distribution of autotrophs. In coastal waters, primary producers are generally larger in size than in offshore waters. Chromo *rbcL* expression was found in the > 5 µm fraction while the cyano mRNA was found usually both in the < 1 µm fraction and the > 5 µm fraction. Cyano *rbcL* mRNA was exclusively found in the < 1 µm fraction of offshore stations. These results indicate that these two evolutionary clades of carbon fixing organisms can be effectively separated by size fractionation. It is as if the niches for these organisms were separated by size.

The molecular biodiversity of organisms involved in carbon fixation in the water column. This area was not formally proposed in our project but we believe it is one of the most important issues in controlling carbon fixation on the continental margins. These studies looked at how genetically diverse the components involved in photosynthetic carbon fixation are in these environments. We have isolated the genes of carbon fixing organisms by two approaches: DNA amplification and RNA amplification (RT-PCR). The former should detect all organisms present (rather those hit with our PCR primers) while the latter determines the transcriptionally active phototrophs. Both approaches have met with success and our efforts yielded 8 such sequences. The results indicate that diversity of carbon fixers is great in the oceans and on the continental margins, because the 8 sequences obtained spanned the whole range of previously described RuBPCase sequences.

APPLICATION TO DOE OMP OBJECTIVES

As mentioned above, our results are particularly applicable to the first objective, to understand coastal marine biogeochemical processes and ecosystem dynamics. The diel regulation of RuBPCase in natural phytoplankton populations had not been known prior to our work. The fact that the two evolutionary clades (cyano and chromo) showed a temporal separation in expression was not known, either. That these large evolutionary groups were separated in expression of carbon fixation genes by cell size, position in the water column, and time of day was not known. When one studies carbon fixation, in a whole water sample, as a function of time of day, or at various depths in the water column, the result that is obtained is a one-dimensional picture of the carbon assimilation process. For example, in a whole water sample, a total net rate of carbon fixation, per volume of

sample is obtained, without knowledge of the various components that contribute to this fixation. Size-fractionation has enabled us to ascertain that it is the result of picocyanobacteria and larger chromophytic eucaryotes. In fact, these two groups are apparently under different regulatory controls that may respond to different environmental stimuli. Thus, when one measures carbon fixation every hour during the day and finds one peak at midday, this is in fact the result of different groups of organisms which turn on and off their carbon fixation genes at different times of the day. When carbon fixation in the water column is measured and a broad peak occurs from 25 to 75 m, this is in fact the net result of two different evolutionary groups, with procaryotes dominating the upper portion and chromophytic picoeucaryotes dominating the lower part of this peak. Working in concert, an impression is given that a homogeneous group of autotrophs are responsible for carbon fixation. Thus, our work has helped resolve the complexity of the fundamental biogeochemical process of photosynthetic carbon fixation on the continental margins.

The application of our results to the question of sources and sinks for CO₂ on the continental margins or is burial and/or transport offshore of fixed carbon is this. When models are made to describe the carbon fixation process on the shelf and they are based only upon diatom fixation and biomass, such models will underestimate the true CO₂ sequestering by the phytoplankton. They must include the smaller-sized picocyanobacteria which are abundant in the small size fractions and in the upper water column. If a vertical profile is measured to estimate carbon fixation in the water column and the time of day sampled is not given proper attention, the true rate of CO₂ removal may be underestimated or overestimated, as differing phytoplankton groups express their carbon fixation genes at different times of the day. These results should be helpful in designing future field experiments and probing the vital question of the biogeochemical cycling of CO₂ on the ocean's margins.

PUBLICATIONS RESULTING FROM THE AWARD

- ✓ 1. Pichard, S.L., M.E. Frischer, and J.H. Paul. 1993. Ribulose biphosphate carboxylase gene expression in subtropical marine phytoplankton populations. Mar. Ecol. Progr. Ser. 101: 55-65. *Reprint - separate cycling*
- ✓ 2. Paul, J.H. and S.L. Pichard. 1995. Extraction of DNA and RNA from Aquatic Environments. In: Nucleic Acids in the Environment: Methods and Applications, by J.T. Trevors and J.D. Van Elsas, ed., Springer-Verlag, pp 153-177. *Reprint - separate cycling*
- ✓ 3. Paul, J.H. 1996. Carbon Cycling: Molecular regulation of photosynthetic carbon fixation. Microbial Ecology 32:231-245 *Reprint - separate cycling*
- ✓ 4. Paul, J.H. and S.L. Pichard. 1996. Molecular approaches to studying natural

communities of autotrophs. In: M. Lidstrom and F.R. Tabita. Microbial Growth on C1 Compounds. pp. 301-309. *Reprint*

- ✓ 5. Pichard, S.L., J.B. Brown, L. Campbell, F.R. Tabita, and J.H. Paul. 1996. Regulation of ribulose biphosphate carboxylase gene expression in natural phytoplankton communities. I) Diel rhythms. Marine Ecology Progress Series 139:257-265. *Reprint*

- ✓ 6. Pichard, S.L., L. Campbell, K. Carder, J.B. Kang, J. Patch, F.R. Tabita, and J.H. Paul. 1997. Analysis of ribulose biphosphate carboxylase gene expression in natural phytoplankton communities by group-specific gene probing. Mar. Ecol. Progr. Ser. (In press). ~~*Reprint*~~ *Reprint*

- ✓ 7. Pichard, S.L., L. Campbell, and J.H. Paul. Diversity of Ribulose biphosphate carboxylase/oxygenase form I gene (*rbcl*) in natural phytoplankton communities. Submitted to Applied and Environmental Microbiology *Reprint*

- ✓ 8. Paul, J.H. and S. L. Pichard. Phytoplankton activity through the measurement of ribulose biphosphate carboxylase gene expression [RuBisCO]. In: K. Cooksey, ed. Molecular Approaches to the Study of the Oceans. Chapman and Hall, in review. *Reprint*

STUDENTS SUPPORTED BY THE AWARD

1. Scott L. Pichard, PhD student, is in the process of writing his dissertation and should graduate this summer.
2. Jordan Brown Kang, MS student, is in the midst of his thesis research.