Office of Health and Environmental Research
Project Summary

Project Title

Regulation of Photosynthetic Carbon Fixation on the Ocean Margins
Grant DE-FG02-93ER61700

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Objectives

The overall goal of the project is to understand the regulation of photosynthetic carbon fixation on the ocean margins. Specific aims include:

1. To understand regulation of ribulose biphosphate carboxylase (RubisCO) in phytoplankton cultures in response to light regime.
2. To determine regulation of RubisCO in response to light during nutrient limitation in these cultures
3. To determine mechanisms of RubisCO regulation in natural populations of phytoplankton on the ocean margins in the Gulf of Mexico
4. To measure regulation of RubisCO in phytoplankton of the Hatteras System.

Approach

The approach involves both laboratory and field components. In laboratory studies, RubisCO genes from representative marine strains will be isolated and transcriptional regulation assessed as a function of environmental parameters known to play major influences in the marine environment. In addition, studies on the regulation of RubisCO enzyme activity will be conducted in order to discern how the function of this key enzyme of CO₂ fixation may be altered in the cell. In field studies, we will study the regulation of RubisCO in natural populations by isolating mRNA and probing with two rbcl probes that represent the major evolutionary clades of phytoplankton, the cyanobacteria/green algae (cyano probe) and the chromophytic algae (diatom probe). Areas of study include the Southeastern Gulf of Mexico (1994) and the Hatteras System (1995). Additionally, amplification, cloning and sequencing of rbcl genes from isolates and natural populations has occurred, to understand the genetic diversity of autotrophs on the ocean margins.

Results to Date

In laboratory studies, the transcriptional regulation in marine Synechococcus has been studied by isolating the RubisCO genes (rbclrbcs) from strain WH 7803. Unexpectedly analysis of the

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deduced amino acid sequence of RubisCO genes from this and related organisms indicated an unexpected homology to the RubisCO of autotrophic bacteria. Moreover, in strain WH 7803, the rbcl and rbcs gene appeared to be cotranscribed along with the ccmK gene, whose product is involved with CO₂ acquisition. The pattern of transcription reflected an interesting circadian type rhythm during the growth cycle, which appears to mimic the cyclic appearance of this organism in the ocean. Nutrient limitation also drastically affected transcription. In another organism (the marine nitrogen fixer Anabaena sp. CA), a gene (rca) encoding a protein (RubisCO activase) which modifies RubisCO activity was isolated and found to be downstream from the rbclrbcs transcripts, consistent with the role of activase in stimulating RubisCO activity. A knock-out mutation of rca yielded a strain that was severely affected in its ability to catalyze efficient RubisCO activity in situ. We have found diel regulation and entrained rhythms of rbcl transcription using a Pacific Ocean strain of Prochlorococcus (courtesy of Lisa Campbell), with highest levels of rbcl mRNA at the onset of the light period, and lowest in the dark period of growth.

In field studies, we have investigated the distribution of rbcl in stratified oligotrophic water columns of the Gulf of Mexico and the Atlantic Ocean near Bermuda, and the mixed shallow water columns of the South Atlantic Bight near Cape Hatteras. A peak in hybridizable cyano rbcl mRNA was found in the upper water column (< 60 m) at most stations, which correlated with Synechococcus direct counts (orange-fluorescing PE-containing cyanobacteria). A subsurface peak (> 80 m) was found in hybridizable diatom rbcl mRNA which corresponded to either the chlorophyll *a* maximum and/or red autofluorescing cells. The type of autotrophs in this peak is not known. We have also demonstrated clear patterns of diel regulation in the transcription of cyano rbcl which correlate with peaks in photosynthetic carbon fixation during the daylight hours. A later peak in diatom rbcl mRNA, in the late afternoon or night hours, has also been recently observed. Thus, there seems to be a spatial and temporal separation in the regulation of cyanobacteria and chromophytic carbon fixation.

We have amplified, cloned and sequenced a portion of the rbcl gene from natural phytoplankton mRNA and DNA preparations. Deduced amino acid sequence of these indicated that the types of phototrophs ranged over all known form I RubisCO's. Our work demonstrates the tremendous diversity of autotrophs involved in CO₂ fixation on the ocean margins.

Anticipated Outcomes

Deliverables

Deliverables for this project include annual progress reports, published proceedings from national and international conferences, abstracts from national meetings, and publications in the literature.

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.
2. Li, L-A., and F.R. Tabita. 1994. Transcription control of ribulose biphosphate carboxylase/oxygenase and adjacent genes in Anabaena species. *J. Bacteriol.* 177: 6697.
3. Gibson, J.L., Y. Qian, G.C. Paoli, J.N. Dubbs, H.H. Xu, H.V. Modak, K.M. Horken, T.M. Wahlund, G.M.F. Watson, and F.R. Tabita. 1996. Molecular control of CO₂ fixation in photosynthetic bacteria. In: *Microbial Growth on C₁ Compounds* (M.E. Lidstrom and F.R. Tabita, eds.) Kluwer Acad. Publ. (in press).
4. 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.
5. Watson, G.M.F. and Tabita, F.R. 1995. Organization and regulation of carbon fixation genes in the marine cyanobacterium Synechococcus sp WH 7803 (to be submitted).
6. Paul, J.H. and S.L. Pichard. Molecular approaches to studying natural communities of autotrophs. In: Tabita, F.R. and M. Lidstrom. *C1 Metabolism*. In review.
7. Paul, J.H. Carbon Cycling: Molecular regulation of photosynthetic carbon fixation. Submitted to *Microbial Ecology*
8. Pichard, S.L. and J.H. Paul. Extraction of microbial RNA from aquatic sources: marine environments. In: A.D. L. Akkermans, J.D. Van Elsas, and F.J. de Bruijn, *Molecular Microbial Ecology Manual*, ed. Kluwer Academic Publishers, Wageningen, in review.
9. Paul, J.H. Extraction of microbial DNA from aquatic sources: Marine environments. In: A.D. L. Akkermans, J.D. Van Elsas, and F.J. de Bruijn, *Molecular Microbial Ecology Manual*, ed. Kluwer Academic Publishers, Wageningen, in review.
10. Watson, G.M.F. and Tabita, F.R. 1995. Organization and regulation of CO₂ fixation genes in the marine cyanobacterium Synechococcus sp WH 7803. Abst. Vth Cyanobacterial Molecular Biology Workshop.
11. Tabita, F.F. 1995. Molecular regulation and biochemistry of RubisCO in cyanobacteria. Abst. Vth Cyanobacterial Molecular Biology Workshop.
12. J.H. Paul. 1994. Microbial gene expression in the marine environment: Who, what, where, and so what? University of North Carolina, Greensboro, NC Feb 2, 1994.
13. J.H. Paul. Carbon cycling: Molecular regulation and biodiversity of ribulose biphosphate carboxylase in the marine environment. Workshop "Microbial Diversity and Cycling of Bioelements". October 26, Tokyo, Japan
14. J.H. Paul. 1994. C-cycling: Rubisco studies. 94th General Meeting of the American Society for Microbiology, Las Vegas, Nevada. May 23-27.
15. S.L. Pichard and J.H. Paul. 1994. Diel variation in Rubisco mRNA levels in open ocean phytoplankton communities: Evidence for transcriptional regulation in carbon fixation. 1994 Ocean Sciences Meeting, AGU/ASLO, Sand Diego, CA abst. 012L-11
16. J.H. Paul. 1994. Molecular regulation of carbon fixation on the ocean margins. DOE Ocean Margins Program PI Meeting, Woods Hole

Oceanographic Institution, September 18-20, 1994.

17. G. Watson and F.R. Tabita 1994. RubisCO transcription in marine cyanobacteria. Studies in Anabaena CA and Synechococcus sp WH7803. DOE Ocean Margins Program PI Meeting, Woods Hole Oceanographic Institution, September 18-20, 1994.

18. S. Pichard, J. Brown, and J.H. Paul. 1995. RubisCO gene expression in marine phytoplankton communities: A taxonomic perspective. DOE Ocean Margins Program Conference, Sept. 25-27, Savannah, GA

19. G. Watson and F. R. Tabita. 1995. Organization and regulation of carbon fixation genes in the marine cyanobacterium, Synechococcus sp. WH 7803. DOE Ocean Margins Program Conference, Sept. 25-27, Savannah, GA

Project and Success Measures

1. New genes and gene products identified for marine CO₂ fixation research.

2. New isolates from phytoplankton for study of regulation of carbon fixation have been obtained.

3. Newly identified genotypes involved in carbon fixation have been found in natural populations of phytoplankton.

4. For long term success, a melding of molecular-based laboratory studies and its correlation with field-based investigations has been achieved.

5. An understanding of the regulation of photosynthetic carbon fixation and its role in sequestering or transport of atmospheric CO₂ on the ocean margins.

Impact and External Outreach

Anticipated Project Impact

This study will provide useful information to understand, at the molecular level, how productivity may fluctuate in the oceans. This study will also give information on the factors that control CO₂ removal by phytoplankton on the ocean margins.

Collaborative projects integral to the research of this project

Overall Scope and plans for the collaboration.

This is a collaborative investigation, using both laboratory and field expertise, of groups at The Ohio State University and the University of South Florida. Reagents, samples, expertise, and personnel resources have been shared by the two groups, with the result that the total project has been enhanced to an extent not possible on an individual basis.

Other collaborations or dependencies

Lisa Campbell of the University of Hawaii has interacted with us by sharing her Prochlorococcus culture with us. Ken Carder has assisted us with pigment analysis from environmental samples.

Completed Technology Transfer

None

U.S. DEPARTMENT OF ENERGY CHICAGO OPERATIONS OFFICE

DVORSCAK
<i>Penelope</i>

PATENT CLEARANCE FORM

1 TO: Office of Intellectual Property Law Date: 2/9/98

FROM: Jenny Obbish ACQ SD 1836
SPECIALIST BRANCH EXTENSION

SUBJECT: PATENT CLOSEOUT CLEARANCE REVIEW

DE-FG02-93ER61700 Ohio State Univ
AWARD NUMBER AWARDEE

A copy of the final technical report for this award is attached. I would appreciate receiving your recommendations regarding closeout of this award insofar as Patent aspects are concerned.

Enclosure:

1. Final Report No. IPL has FTR & Patent Clearance
- cc: OSTI, Oak Ridge, TN (THRU OFFICE OF INTELLECTUAL PROPERTY LAW) with enclosure (1 copy)
Form 1332.1 and OSTI Closeout Clearance (CH Form 305b).

2 TO: Date: _____

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IS THIS REPORT
SATISFACTORY FROM
A PROGRAMMATIC
INTEREST?

IT DOES NOT APPEAR
TO HAVE ANY RELEVANCE
TO "REGULATION OF
PHOTOSYNTHETIC CARBON
METABOLISM ON THE
OCEAN MARGINS etc."

THE DOE CONTRACT NUMBER
IS NOT REFERENCED.

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matters are concerned, to the release
ard.

Deputy Chief Counsel
Office of Intellectual Property Law